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Influence of *Spirulina* Supplementation with Mulberry Leaf on Reproductive Potentiality in Silk Moth *Bombyx mori* L.

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Research Article

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ABSTRACT

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 reproductive potentiality of female moth *B. mori* L. The ovariole length reached the maximum of 139.64 mm at 5%. The egg hatchability was 83.6%. Hence, it can be concluded that *Spirulina* induces an increased energy metabolism which supports gonadal growth and embryo development of the silkworm. The study indicated that *Spirulina* can be utilized in sericulture for improving reproduction and egg quality of the silkworm, *B. mori* L.

Keywords: *Bombyx mori*; Mulberry leaf; *Spirulina*; Supplementation; Reproductive potential

INTRODUCTION

Silk, the finest and longest among natural fibres is still enjoying the exclusive status of "Queen of Textiles" due to its excellent serviceability as fashionable clothing with harmony of various properties graceful lustre, rich dyeing capacity, moderate elasticity as well as elegancy in wear ability [1].

Sericulture in India is practiced predominantly in tropical environmental regions such as Karnataka, Tamil Nadu, Andhra Pradesh, and West Bengal and to a limited extent in the temperate environment of Jammu and Kashmir. It is the biggest village industry after handloom and khadi, providing full or partial employment to about 6.5 million people in India. The silk industry plays an important role in the Indian rural economy, so research on silkworm and mulberry crop enhancement is of high importance. The mulberry silkworm, *Bombyx mori* L. is reckoned to be one of the commercial insects to produce the best quality of fibre among the different varieties of silk produced in the country. The mulberry silkworm *Bombyx mori* belongs to family *Bombycidae*. 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 [2].

Mulberry (*Morus* species) leaf is the solo food and source of nutrition for the silkworm, *Bombyx mori* L. due to the presence of morin. Nutrients in the mulberry leaves like sugars, ascorbic acid, phytosterol, such and others were found phygostimulative action for silkworm, *Bombyx mori* (L) Investigation on silkworm nutrition may form active area of research to enhance silk production. The supplementation and fortification of mulberry leaves is a recent technique in sericulture research. Mulberry leaves have been supplemented with various nutrients for silkworm feeding to promote silk quality and also quantity.

The successful egg deposition by female silk moth is depends on factors including hormonal, chemical, environmental, physical, behavioural aspects etc. have been significant in oviposition behaviour. Also the reproductive physiology, as mating, vitellogenesis, ovulation, oviposition, weight of pupae and pharate adult, surface texture and inclination also are important factors for viable egg de-position by adult female moth. Photoperiod in insects serves as a clock indicating the seasonal changes and influences their life cycle, distribution and abundance. Egg laying capacity of various breeds of silkworm, *Bombyx mori* L. have been reported to vary even under identical nutritional, atmospheric, mating and laying conditions. Hatching of silkworm is predominantly diurnal. It seems to be under the circadian control [3].

The better growth and development of silkworm larvae as well as good quality cocoons depend on feed with nutritionally enriched leaves. Hence, in the Indian scenario, improvement of silk production is by enriching the nutrition of silkworm through fortification of mulberry leaves which has given high returns to Indian sericulturists. In recent years attempts have made in sericulture with nutrient such as proteins, carbohydrates, amino acids, vitamins, sterols, hormones, antibiotics etc. for better performance and get higher yield, quantity and quality cocoons. *Spirulina*, blue-green algae contains 18 amino acids *viz.*, glutamine, glycine, histidine, lysine, methionine, creatine, cysteine, phenylalanine, serine, proline, tryptophan, asparagine, pyruvic acid and vital vitamins like biotin, tocopherol, thiamine, riboflavin, niacin, folic acid, pyrodozoic acid, beta-carotene and vitamin B12. Therefore, the study intended to observe the influence of *Spirulina* supplementation with Mulberry leaf as a feed on reproductive potentiality of female moth [4].

MATERIALS AND METHODS

Mulberry leaves procurement

The mulberries were procured from mulberry plantation at Tamil Nadu sericulture training centre, Nanjikkottai, Thanjavur

is being well established with MR2 variety of mulberry (Figure 1), maintenance of which is through standard horticulture techniques [5].

Figure 1. Mulberry leaves.

Experimental animals

The egg cards of silkworm *B. mori* (cross breed: Local, a multivoltine × NB_4D_2, a bivoltine) were obtained from State Grainage centre, Trichirappalli and silkworms were reared under standard conditions at 26°C ± 2°C in Tamil Nadu sericulture training centre, Nanjikkottai, Thanjavur, India. The mulberry leaves harvested at the irrigated mulberry garden were used as food for the silkworm: Larvae were reared in plastic trays (70 larvae/tray) and were exclusively fed with mulberry leaves (Figure 2). Fresh mulberry leaves (MR2) 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 at regular interval (6.30 h, 11.30 h, 16.00 h and 22.00 h) [6].

Figure 2. *Bombyx mori* L.

Experimental procedures

Spirulina platensis (*Arthrospira platensis*) powder was purchased from PARRY nutraceuticals (Division of EID parry (India) Ltd., at Pannangudi, Pudukkottai Dist., Tamil Nadu, India. In this experiment, three concentrations of Spirulina were supplemented to the silk worm. *Spirulina* were dissolved in distilled water and mulberry leaves were dipped in each concentrastion 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 to examine the reproductive character and related parameters [7].

Experimental design: The fifth instars of *B. mori* larvae were used in this study and animals were grouped as the following (Figures 3-5).

Group I (Control): Larvae supplied with fresh mulberry leaves

Group II: Larvae supplied with 1% *Spirulina*, supplemented mulberry leaves.

Group III: Larvae supplied with 3% *Spirulina*, supplemented mulberry leaves.

Group IV: Larvae supplied with 5% *Spirulina*, supplemented mulberry leaves.

Figure 3. *Spirulina platensis*.

Figure 4. Preparation of different concentrations of *Spirulina*.

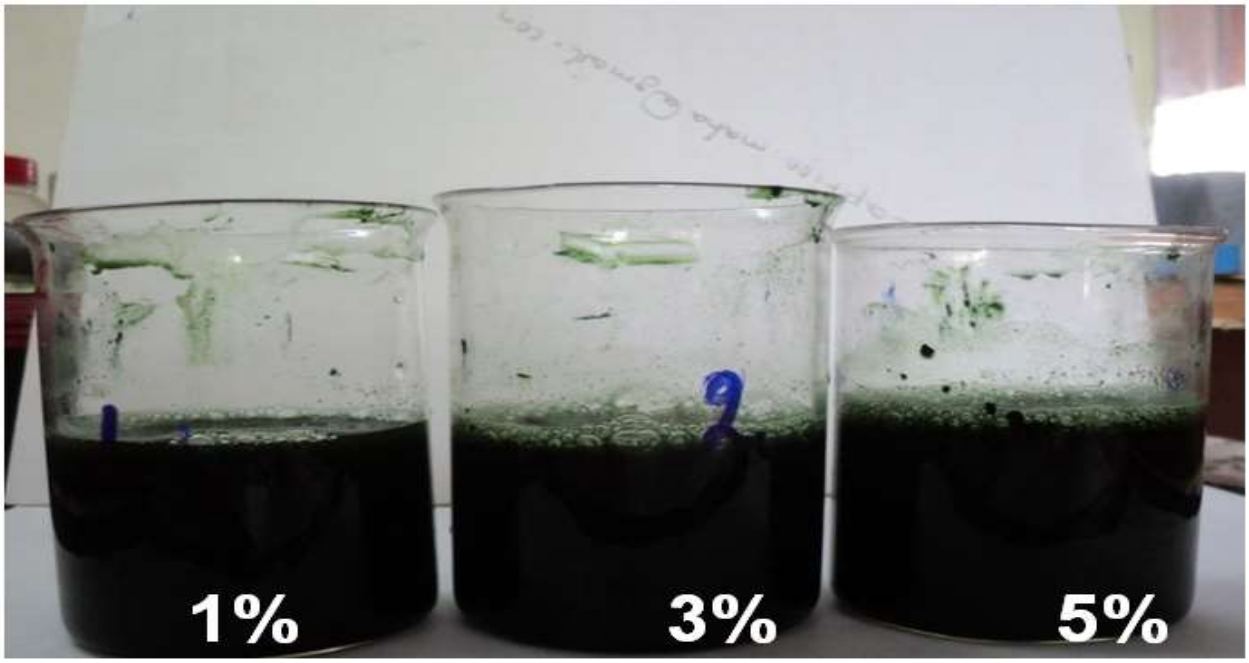


Figure 5. Experimental design.

Reproductive characters

The female moth, emerged from experimental and control groups, were allowed to lay eggs after mating and their fecundity was counted separately. The hatching percentage was calculated in both supplemental and control groups. To determine the length of the ovariole, freshly emerged female of both experimental and control groups were anesthetized. The abdomen was dissected out and the length of each ovariole was measured. The testes were isolated and weighed. The testes and ovaries were homogenized with 0.1 M Tris Hcl buffer (pH 7.4) for ovariole length, testis weight, fecundity, egg hatchability and organic constituent changes of testes and ovaries etc,.

RESULTS AND DISCUSSION

Ovariole length

The ovariole length in the freshly emerged female moth was found to increase in the supplemented groups compared with the control. The freshly emerged adult female moth of control has the ovariole length was 128.87 mm. In female, obtained from larvae fed with 1 per cent *Spirulina* the ovariole length increased to 131.64 mm (Table 1 and Figure 6). The ovariole length reached the maximum of 139.64 mm at 5.00 per cent levels. A positive correlation was noticed between the ovariole lengths with different concentrations of *Spirulina* [8].

Testis weight

The testis weight of freshly emerged male was found to increase in the *Spirulina* supplemented groups than the control. The freshly emerged adult male of control has 5.24 mm of weight. In male, obtained from larvae fed with 1% *Spirulina* the testis weight increased to 5.57 mm. The testis weight reached the maximum of 6.29 mm at 5% levels. A positive correlation was noticed between the testis weight and different concentrations of *Spirulina* [9].

Fecundity

In the control groups, the female moth laid 406 eggs/female. 1% percent *Spirulina* supplemented female moth, showed fecundity increased to 430 eggs/female. The fecundity was a maximum of 498 eggs/female at 5% *Spirulina*. A positive linear regression was observed between different concentrations of *Spirulina* and the fecundity. The computation of data on one way ANOVA revealed that the fecundity length was significantly influenced by different concentrations of *Spirulina*.

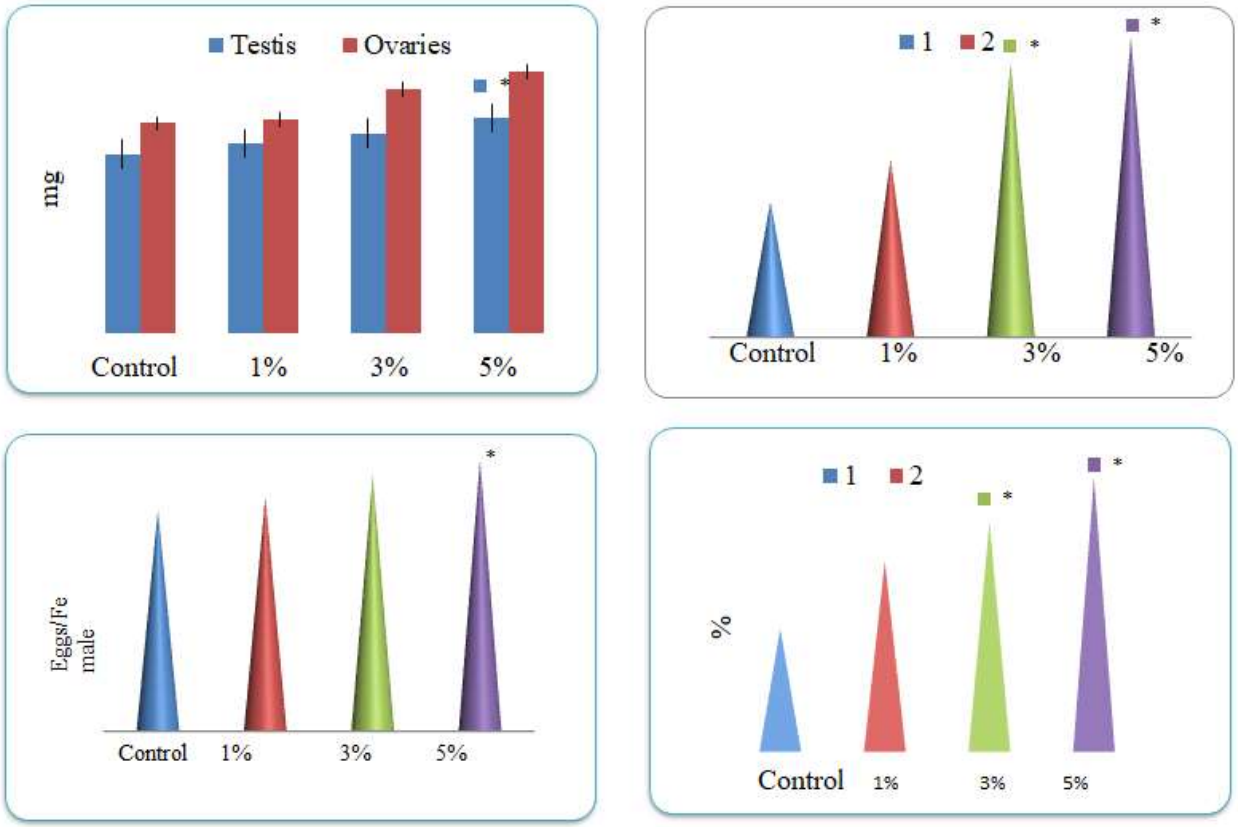
Egg hatchability

In control group, the egg hatchability was 70.6 percent. 1% *Spirulina* supplementation was increased to 76.4 percent. Maximum of 83.6 percent hatchability was observed at 5% *Spirulina* (Table 1 and Figure 6). A highly significant positive correlation was obtained on relating *Spirulina* to egg hatchability up to the optimal dose [10].

Table 1. Effect of *Spirulina* supplemented with mulberry leaves on testis weight, length and ovaries weight, length, fecundity and egg hatchability 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%)	
	Testis	Ovaries	Testis	Ovaries	Testis	Ovaries	Testis	Ovaries
Weight (mg)	5.24 ± 0.49	6.13 ± 0.53	5.57 ± 0.48	6.24 ± 0.57	5.84 ± 0.48	7.13 ± 0.74	6.29 ± 0.51*	7.62 ± 0.64
Length (mm)	-	128.8 ± 1.01	-	131.6 ± 1.11	-	137.9 ± 1.46*	-	139.6 ± 1.8*
Fecundity (Eggs/Female)	-	406	-	430	-	469	-	498*
Egg hatchability (%)	-	70.6	-	76.4	-	79.8*	-	83.6*
Note: *Significantly different from control P<0.05								

Figure 6. Effect of *Spirulina* supplemented with mulberry leaves on testis weight, length and ovaries weight, length, fecundity and egg hatchability of silkworm, *Bombyx mori* L.



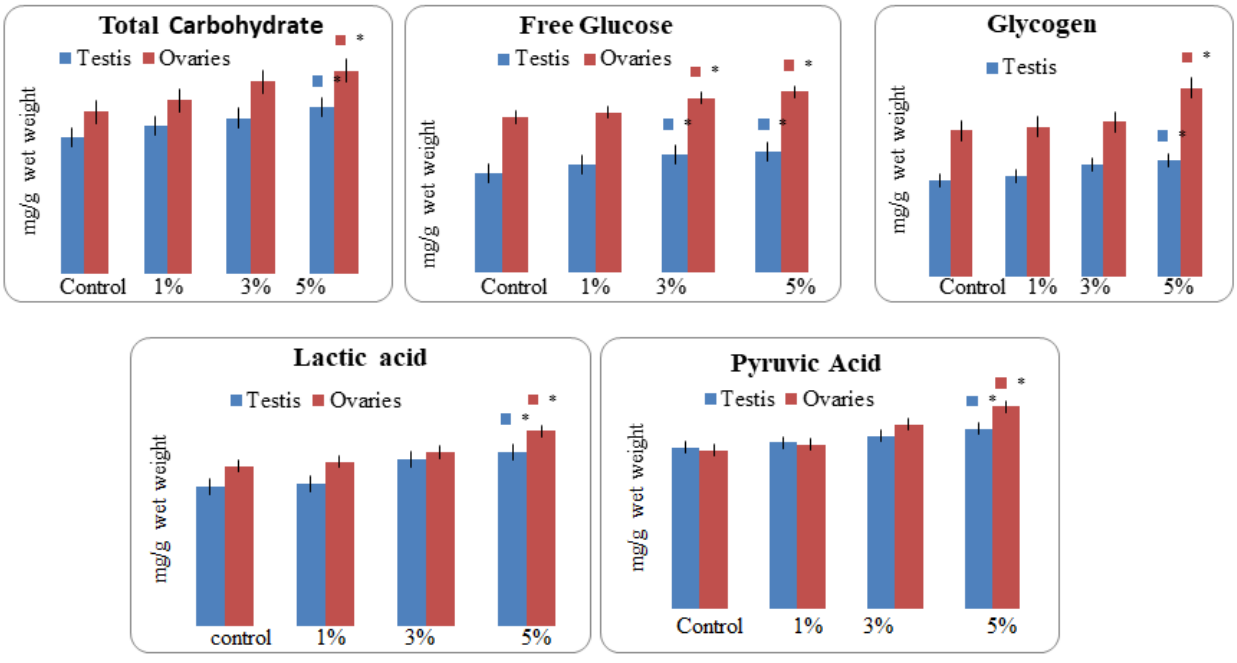
Organic constituent changes of testes and ovaries

The total carbohydrates in testes and ovaries were increase. The content of free glucose, glycogen, lactic acid and pyruvic acid in both testes and ovaries increased (P<0.05) in different dosage of *Spirulina* treated silkworm larvae, and the 5% *Spirulina* increased on these biochemical constituents in the ovaries, compared to the testes (Table 2 and Figure 7).

Table 2. Effect of *Spirulina* supplemented with mulberry leaves on total carbohydrate, free glucose, glycogen, lactic acid and pyruvic acid contents in testis and ovaries 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%)	
	Testis	Ovaries	Testis	Ovaries	Testis	Ovaries	Testis	Ovaries
Total carbohydrate (mg/g wet weight)	7.23 ± 0.50	8.56 ± 0.59	7.81 ± 0.468	9.17 ± 0.55	8.25 ± 0.569	10.18 ± 0.702	8.81 ± 0.599*	10.7 ± 0.73*
Free glucose (mg/g wet weight)	0.51 ± 0.03	0.80 ± 0.056	0.56 ± 0.033	0.82 ± 0.049	0.61 ± 0.042*	0.9 ± 0.062*	0.62 ± 0.042*	0.93 ± 0.06*
Glycogen (mg/g wet weight)	0.28 ± 0.02	0.438 ± 0.03	0.30 ± 0.018	0.44 ± 0.018	0.336 ± 0.023	0.465 ± 0.032	0.34 ± 0.023*	0.56 ± 0.03*
Lactic acid (mg/g wet weight)	0.71 ± 0.04	0.81 ± 0.057	0.72 ± 0.043	0.84 ± 0.043	0.852 ± 0.058	0.890 ± 0.061	0.88 ± 0.06*	0.99 ± 0.06*
Pyruvic acid (mg/g wet weight)	0.29 ± 0.02	0.28 ± 0.019	0.29 ± 0.017	0.29 ± 0.017	0.311 ± 0.021	0.331 ± 0.022	0.32 ± 0.022*	0.36 ± 0.02*
Note: *Significantly different from control P<0.05								

Figure 7. Effect of *Spirulina* supplemented with mulberry leaves on total carbohydrate, free glucose, glycogen, lactic acid and pyruvic acid contents in testis and ovaries of silkworm, *Bombyx mori* L.



Effect of different dosage of *Spirulina* on the aldolase, acid phosphatae and LDH activity of testes and ovaries

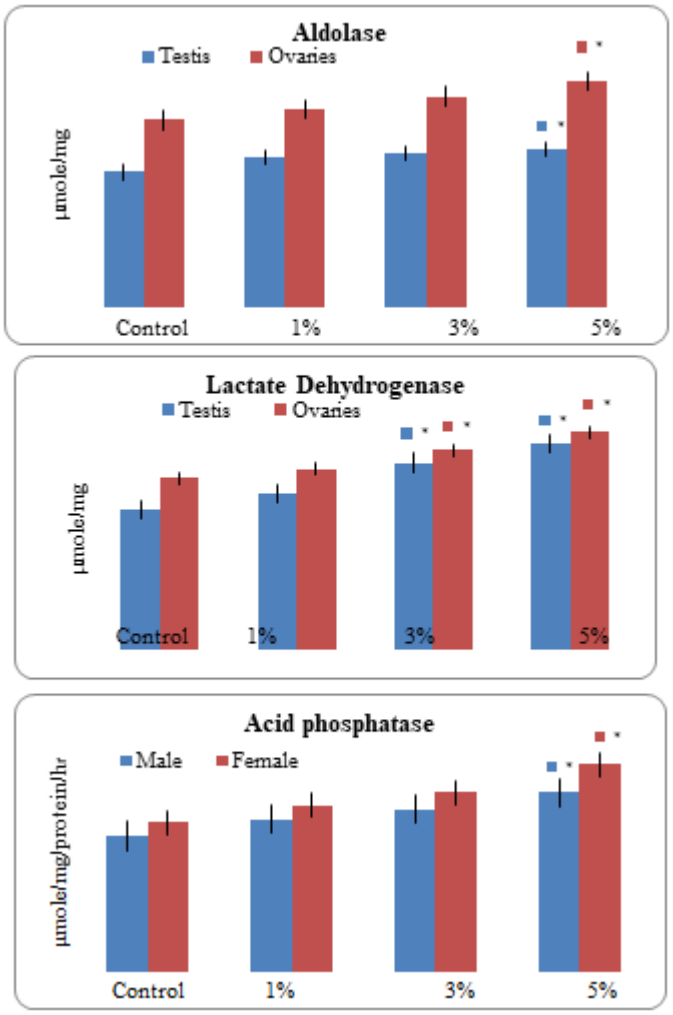
The effect of different dosages of *Spirulina* on aldolase, acid phosphatase and LDH activities were examined in testes and ovaries of the silkworm. Aldolase and acid phosphatase activity of the gonads were increased (P<0.05) for the larvae treated with different dosages of *Spirulina*. The aldolase activity of the testes and ovaries were high in 5% *Spirulina*. The LDH activity was also increased of the testes and ovaries than the control (Table 3 and Figure 8).

Table 3. Effect of *Spirulina* supplemented with mulberry leaves on aldolase, acid phophatase and LDH activity in testis and ovaries 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%)	
	Testis	Ovaries	Testis	Ovaries	Testis	Ovaries	Testis	Ovaries
Aldolase (µmol/mg protein/h)	1.02 ± 0.07	1.4 ± 0. 09	1.13 ± 0.06	1.49 ± 0.08	1.16 ± 0.08	1.59 ± 0.109	1.19 ± 0.08*	1.7 ± 0.11*
Lactate dehydrogenase (LDH) (µmol/mg protein/h)	0.46 ± 0.03	0.5 ± 0.03	0.51 ± 0.03	0.59 ± 0.03	0.6 ± 0.01*	0.65 ± 0.04*	0.67 ± 0.04*	0.7 ± 0.04*
Acid phosphatase (µmole/mg/protein/hr)	5.65 ± 0.38	6.21 ± 0.35	6.34 ± 0.34	6.93 ± 0.46	6.75 ± 0.46	7.45 ± 0.51	7.44 ± 0.56*	8.6 ± 0.51*

Note: *Significantly different from control P<0.05

Figure 8. Effect of *Spirulina* supplemented with mulberry leaves on aldolase, acid phophatase and LDH activity in testis and ovaries of silkworm *B. mori* L.



Effect of *Spirulina* on Antioxidant enzymes in silkworm larvae

SOD and catalase activity were increased (P<0.05) for the larvae treated with different dosages of *Spirulina*. The increase in SOD and catalase activities was high in 5% *Spirulina* as compared to control (Table 4 and Figure 9) [11].

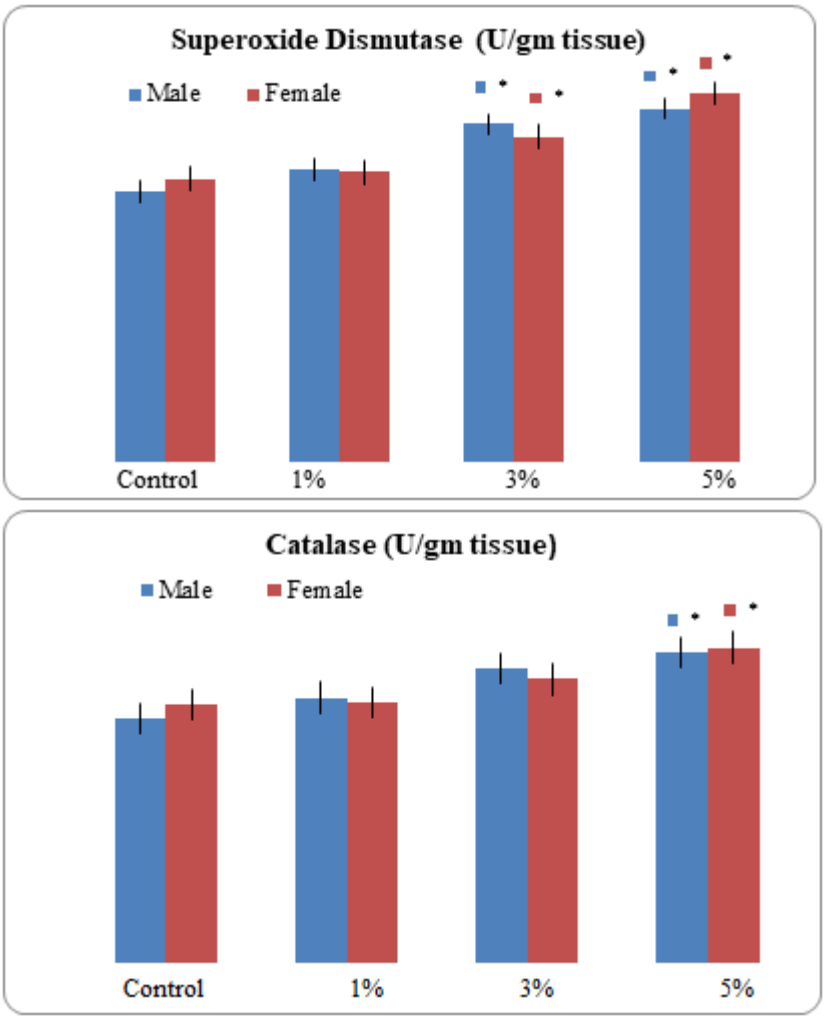
Previous studies have shown that the ovaries which produce diapause eggs store much more carbohydrates and lipids than non-diapause ovaries of the silkworm, *B. mori*. These carbohydrates and lipids would be consumed during the

diapause stage. This is suggests that there is a close relationship between energy metabolism and diapause and embryo development.

Table 4. Effects of *Spirulina* on antioxidant defence.

Parameters	Control		Group I (1%)		Group II (3%)		Group III (5%)	
	Male	Female	Male	Female	Male	Female	Male	Female
Superoxide dismutase (U/mg protein in tissues)	2.31 ± 0.16	2.42 ± 0.16	2.50 ± 0.17	2.48 ± 0.17	2.89 ± 0.2*	2.78 ± 0.19*	3.02 ± 0.21*	3.15 ± 0.22*
Catalase (U/mg protein in tissues)	3.24 ± 0.22	3.42 ± 0.23	3.51 ± 0.24	3.45 ± 0.24	3.8 ± 0.27	3.75 ± 0.26	4.11 ± 0.28*	4.17 ± 0.29*
Note: *Significantly different from control P<0.05								

Figure 9. Effects of *Spirulina* on antioxidant defence.



It is known that diapause and embryo development of the silkworm are controlled by the Diapause Hormone (DH). The weights of testes and ovaries had increased following the increase in weights of the larvae after administration of *Spirulina*. The increased Gono-Somatic Indices (GSI) of both the testes and ovaries suggest that this was due to its treatment with *Spirulina* and was greater for gonadal growth. The increased weight of the larval body and silk gland, testis and ovarian tissues during the course of larval maturation was caused by the active accumulation of proteins, carbohydrates and lipids [12].

Total carbohydrates, glucose, glycogen, lactic acid, pyruvic acid, aldolase and LDH activity were increased in the testes and ovaries of *Spirulina* treated silkworm larvae. The enhanced carbohydrate content in gonads indicates a higher extent of utilization of these energy sources in testicular and ovarian function. The increase in carbohydrate indicates that the increased metabolic activity [13,14].

Aldolase and LDH are important enzymes in carbohydrate metabolism and related to energy production in the live cell. The enhanced activity level of aldolase and LDH in gonads indicates active energy metabolism in the gonads. When the larvae are treated with *Spirulina*, the energy sources are stored in the gonads for further metabolic activity resulting in a maximum accumulation of carbohydrate resources [15]. The food consumption has a direct relevance on the weight of larvae, cocoon, pupae and shell, the independent parameters of consumption and productivity vary depending upon the type of nutrition and silkworm breeds. The feeding efficacy of different mulberry feed varieties such as *Spirulina* treated mulberry leaves on larval growth and development was also reported. Chakrabarty and Kaliwal, suggested that the positive effect of mineral nutrition on the silkworm economic characters such as cocoon weight, shell weight, silk filament length and weight, denier, cocoon-shell ratio has been increased by the nutritional role of some mineral salts such as potassium iodide and cobalt chloride. Kavitha et al., confirmed the filament length and its weight have been elevated by zinc salts. The high level of accumulated energy resources suggests that a direct relationship exists between the presence of *Spirulina* and energy resources of the gonads. Since carbohydrates are an essential energy source, their accumulation in the gonads under the influence of *Spirulina* is also suggestive of an increased energy metabolism of the silkworm larvae.

CONCLUSION

The main constituents of the testes and ovaries are carbohydrates which are used as energy resources for growth and development of the sperm and ovum. In the present experiment, the content of glucose, aldolase and LDH activity significant by increased in the testes and ovaries of *Spirulina* supplemented to silkworm. A possible explanation for this phenomenon is that *Spirulina* induces an increased energy metabolism which supports gonadal growth and embryo development of the silkworm. It is hoped that the results of this study on supplementation of 5% *Spirulina* to silkworm to be beneficial to sericulture industry in India by ultimately increasing the quantum of quality silk production economically.

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