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EFFECT OF *SPIRULINA* SUPPLEMENTATION ON NUTRITIONAL INDICES PARAMETERS IN SILKWORM, *BOMBYX MORI* (L.)

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ABSTRACT

To find out the effect of *Spirulina* on nutritional indices in silkworm.. In the study of bioenergetics profile, it was found that the consumption, assimilation, metabolism, rates of consumption, assimilation and metabolism were found to decrease in *Spirulina* supplementation. The production, production rate, assimilation efficiencies (AD), the efficiencies for conversion ingested food (ECI) and conversion of digested food (ECD) were found to increase in *Spirulina* supplementation. In other words the larvae consumed less mulberry leaves were supplemented with *Spirulina*, a higher quantum of tissue production was noted. A possible explanation for this phenomenon is that larvae that fed on mulberry leaves supplemented with *Spirulina* allocated minimum energy for maintenance and channelized maximum energy towards tissue production. 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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INTRODUCTION

Geographically, Asia is the main producer of silk in the world and produces over 90% of the total global output. 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 (Kumari *et al*., 2011). The silk worm is a beneficial insect reared for the valuable commodity silk. The silk industry plays an important role in the Indian rural economy, so research on silkworm and mulberry crop enhancement is of high importance (Hiware, 2001).

In developing countries like India, agriculture and agro-based industries play a vital role in the improvement of rural economy. The limited availability of land, the limited cash returns, and agriculture being confined to one or two seasons in the year, have made villages to look for supporting rural industries, such as sericulture. Agriculture and sericulture are adopted simultaneously by the agriculturists in regions where the ecological conditions are favourable. In India, over three million people are employed in various fields of sericulture. It is a cottage industry and provides ample work for the womenfolk in the rural areas in rearing silk worms, while the male members work in the fields. Recently the enforcing of new ideas by research institutions both in mulberry cultivation and silk-worm-handling among sericulturists, the industry is now practiced as a main profession and as a important cash crop of the country (Srinivas and Ramadevi, 2001).

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 (Sannappa, 2002). Mulberry leaves have been supplemented with various nutrients for silkworm feeding to promote silk quality and quantity. The supplementation and fortification of mulberry leaves is a recent technique in sericulture research.

Nutritional study on silkworm is an essential prerequisite for its proper commercial exploitation. The physiological potential of an insect is influenced by its food intake, growth regulators and various biotic factors (Rath, 2005). In view of these observations, in the present study an attempt has been made to find out the efficiency *Spirulina* on nutritional indices parameters of silkworm. The selected *Spirulina* species was *Spirulina plantensis* (*Arthrospira platensis*) used as a supplement for this study.

MATERIALS AND METHODS

Experimental Animals

The egg cards of silkworm *B. mori* (cross breed; Local, a multivoltine x NB_4D_2, a bivoltine) were obtained 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.

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.

Treatements

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.

Nutritional indices:

Collection of food remains and faeces:

Unfed leaves and faecal pellets were removed everyday before offering the next consignment of food with least disturbance to the animals and the collected samples were dried separately at 80°C to a constant weight. Care was taken to separate fine unfed food particles from the faecal pellets.

Gravimetry:

All weighings were made in a monopan electronic balance with an accuracy of 0.0001mg. The accuracy of the balance was checked for every ten weighing. To findout the dry matter content of the test animal belonging to different experimental groups, a few test animals were sacrificed at the beginning as well as at the end of the fifth-instar and dried at 80 °C to a constant weight (Maynard and Loosli, 1962). Dried materials of sample animals, faeces, exuvia and food were powdered and stored in the desiccators for calorific analysis.

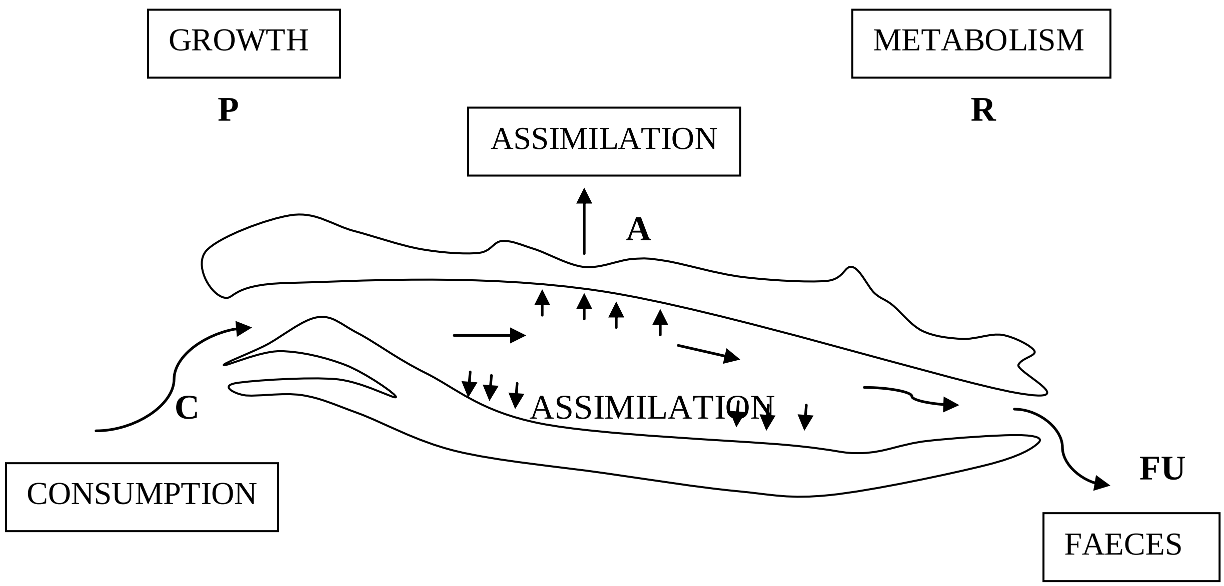
Calorimetry:

Calorific content of the sample was determined using Semi Micro Calorimeter following the stand procedure described in the instruction manual for calorimetry. After every 10 to 12 estimations, the bomb was standardized using a standard benzoic acid pellet. Whenever the material for bombing was inadequate, a known amount of standard benzoic acid was added and bombed, and necessary corrections were made for the amount of benzoic acid added.

Nutritional indices:

The scheme of energy balance followed in the present study (Fig- 1) is based on the IBP formula (Petrusewice and Mac Fadyed, 1970) usually represented as,

Figure 1 Bioenergetic scheme in silkworm.



$$C = P + R + FU$$
$$A = C - FU = P + R$$
$$C = P + R + FU \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where C = Food energy consumed

P = Growth

R = Energy released as heat due to metabolism.

F + U = Loss of energy through faeces and nitrogenous excretory products.

In majority of the insects the quantity of uric acid (U) excreted in the faeces, is negligible and amounts to 0.2 to 0.5 percent of the total quality of faeces egested (Lawton,1969). The total food consumption of the fifth-instar larvae was also calculated similarly. The above quantitative estimations of C were made in terms of joules (J). Food energy converted into body substance (P = Growth) was estimated as the difference between the energy content of the test animals at the beginning and the end of the fifth-instat. Food energy assimilated (A) was calculated by subtracting the mean faecal energy from that of food consumed.

$$A = C - (F + U) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

The rates of consumption (C_r), assimilation (A_r) and production (P_r) were calculated by dividing the respective amounts of energy by the product of mid body weight (g) of the animal and the duration (day) required for the completion of the fifth-instars; the rates are expressed in terms of J/g live insect/day. The efficiencies of conversion of ingested matter to body substances (ECI) and digested matter to body substances (ECD) were calculated relating P to C and A separately and expressed as percentages.

Formulae used to calculate the parameters of nutritional indices.

$$\text{Consumption rate } (C_r) = \frac{\text{C (J/individual)}}{\text{Mid body weight (g) x duration (day)}} \quad \ldots (3)$$

$$\text{Assimilation rate } (A_r) = \frac{\text{J/individual)}}{\text{Mid body weight (g) x duration (day)}} \quad \ldots (4)$$

$$\text{Production rate } (P_r) = \frac{\text{P(J/individual)}}{\text{Mid body weight (g) x duration (day)}} \quad \ldots (5)$$

$$\text{Metabolic rate } (M_r) = \frac{\text{A (J/individual)}}{\text{Mid body weight (g) x duration (day)}} \quad \ldots (6)$$

Assimilation efficiency (AD) = A/C x 100 …………. (7)

Efficiency of conversion of ingested matter to body substance (ECI) = P/C x 100 …………(8)

Efficiency of conversion of digested matter to body substance (ECD) = P/A x 100 …………(9)

Statistical Analysis:

The values were expressed as mean ± SD for fifty animals in each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey's test for multiple comparisons. The results were statistically analyzed by Graphpad Instat Software (Graphpad Software, San Diego, CA, USA) version 3 was used and $p < 0.05$ was considered to be significant.

RESULTS

Mulberry (Morus species) leaf is the solo food and source of nutrition for the silkworm, *Bombyx mori* L. due to the presence of morin (Tribhuwan *et al*., 1989). The supplementation and fortification of mulberry leaves is a recent technique in sericulture research. The growth and development of larvae, and subsequent cocoon production are greatly influenced by nutritional quality of mulberry leaves. In recent years attempts have been made in sericulture with nutrient supplements such as proteins, carbohydrates, amino acids, vitamins, sterols, hormones, antibiotics etc. for better performance and get higher yield, quantity and quality of cocoons (Sannapa, 2002).

The present investigation of the economic, biochemical and reproductive characters of silkworm supplemented with *Spirulina* were worked out. Growth parameters were also measured on dry weight basis. The indices such as relative consumption rate (RCR), relative growth rate (RGR), Growth Index (GI), approximate digestibility were worked out. The silk sericin (SS)-capped AgNPs were successfully synthesized in 1, 3 and 5% concentrations of *Spirulina* supplemented silkworm cocoon and evaluated the antibacterial activity of SS-capped AgNPs.

EFFECTS OF *SPIRULINA* ON NUTRITIONAL EFFICIENCY PARAMETERS

Studies on the nutritional indices were performed in *B. mori* by Hiratsuka (1972). Thereafter quantitative and qualitative aspects of insect nutrition have been studied with great importance. It is known that paramount importance to be given to the studies of nutritional indices which in turn helps in obtaining good cocoon quality and quantitiy.

The control female and male fifth – instar larvae consumed 54.26 and 52.12 KJ/individual respectively but it declined gradually with increased concentration of *Spirulina* (Table 1). For instance at 1,3 and 5 per cent *Spirulina* supplementation to female larval consumption decreased to 53.14, 49.62 and 47.21 KJ/individual respectively and male larval consumption decreased to 51.02, 49.46 and 48.46 KJ/individual respectively. The results show that a significant negative linear relationship in female and male exists between the *Spirulina* concentration and the consumption.

The control female and male of fifth – instar larval production was 9.21 and 8.94 KJ/individual but it increased gradually with increased concentration of *Spirulina* (Table 1). For instance at 1,3 and 5 per cent *Spirulina* supplementation to female larval production increased to 9.26, 9.12and 8.12 KJ/individual respectively and male larval production increased to 8.96, 8.99 and 9.20 KJ/individual respectively. The results showed that a significant positive linear relationship in female and male exists between the *Spirulina*.

The control female and male fifth – instar larval assimilation 20.00 and 19.00 KJ/individual but it declined gradually with increased concentration of *Spirulina* (Table 1). For instance at 1, 3 and 5 per cent *Spirulina* supplementation to female larval assimilation reduced to 19.95, 18.64 and 17.37 KJ/individual respectively and male larval assimilation reduced to 19.98, 19.82 and 19.32 KJ/individual respectively. The results showed that a significant negative linear relationship in female and male exists between the *Spirulina* concentration and the assimilation.

The control female and male the energy spent for metabolism was 11.79 and 12.06 KJ/individual but it declined gradually with increased concentration of *Spirulina* (Table 1). At 1,3 and 5 per cent *Spirulina* supplementation the female larval metabolism reduced to 14.17, 10.52 and 10.25 KJ/individual respectively and male larval metabolism reduced to 12.02, 11.83 and 12.12 KJ/individual respectively. The results show that a significant negative linear relationship in female and male exists between the *Spirulina* concentration and the metabolism.

The consumption rate of control male (5171.32 J/g) and female (5402.16 J/g) live insect/day, group decreased to 5013.16, 4642.12 and 4541.14 J/g live insect/day in 1, 3 and 3 percent *Spirulina* respectively for female and male consumption rate reduced to 5285.73, 4717.84 and 4642.71 J/g live insect/day in 1, 3 and 5 percent Spirulina respectively (Table 1). The results show that a significant negative linear relationship in female and male exists between the Spirulina t concentration and consumption rate.

The assimilation rate of male and female 2124.11 and 2036.74 J/g live insect/day in control group decreased to 2064.52, 1912.35 and 1885.65 J/g live insect/day in 1, 3 and 5 percent Spirulina respectively for female and male assimilation rate reduced to 1937.37, 1781.58 and 1775.88 J/g live insect/day in 1, 3 and 5 percent Spirulina respectively (Table 1). The results showed that a significant negative linear relationship in female and male exists between the Spirulina concentration and assimilation rate.

The control male and female production rate was 772.65 and 792.42 KJ/individual but it increased gradually with increased concentration of Spirulina. For instance at 1,3 and 5 per cent Spirulina supplementation to female larval production increased to 794.16, 803.14 and 815.64 KJ/individual respectively and male larval production increased to 779.78, 791.21 and 792.12 KJ/individual respectively (Table 1). The results show that production rate was significant influenced by the Spirulina concentration.

The metabolic rate of male and female 1352.46 and 1245.42 J/g live insect/day in control group decreased to 1144.21, 979.44 and 961.24 J/g live insect/day in 1, 3 and 5 percent Spirulina respectively for female and in male metabolic rate reduced to 1285.74, 1122.14 and 1094.53 J/g live insect/day (Table 1). The results show that a significant negative linear relationship in female and male exists between the Spirulina concentration and metabolic rate.

Assimilation efficiency (AD), efficiency of conversion of ingested matter to body substance (ECI) and efficiency of conversion of digested matter to body substance (ECD) recorded a higher value in the larvae fed with mulberry leaves supplemented with different concentrations of Spirulina when compared to that of the control group (Table 2). The assimilation efficiency such as (AE) of 37.16and 35.67 percent in control of male and female increased to 37.94, 38.83 and 40.70 percent at 1, 3 and 5 percent of Spirulina supplementation. Similarly, the AE of female 41.10, 41.28 and 41.32 percent at 1, 3 and 5 percent Spirulina supplementation respectively (Table 2).

The ECI such as 15.53 and 15.41 percent in control male and female and it is increased to 15.91, 16.48 and 17.27 percent at 1, 3 and 5 percent Spirulina supplementation. Similarly, the ECI of female namely 15.84, 16.70 and 16.79 percent at 1, 3 and 5 percent Spirulina supplementation respectively (Table 2). The ECD such as 41.78 and 43.21 percent in control male and female and it is increased to 41.93, 42.45 and 42.54 percent at 1, 3 and 5 percent Spirulina supplementation. Similarly, the ECD of female are 38.54, 46.03 and 46.49 percent at 1, 3 and 5 percent Spirulina supplementation respectively (Table 2).

The results revealed that the Assimilation efficiency (AD), efficiency of conversion of ingested matter to body substance (ECI) and efficiency of conversion of digested matter increased steadily with the increasing concentrations of Spirulina

DISCUSSION

Silkworms are economically important for silk production. Diet supplementation of mulberry leaves fed to silkworms, offer a new source of nutrients, which are reported to have great potential of protein supplementation, one such source is Spirulina. 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 economic characters, nutritional indices, reproductive potential, silk production and biochemical profile of haemolymph of silk Bombyx mori L. The observations made on different groups of experimental and control groups were compared.

The growth and development of larva, and subsequent cocoon production are greatly influenced by nutritional quality of mulberry leaves. Supplement in silkworm nutrition like fortified with Spirulina are needed for nutritional requirement among several insects (Subburathinam and sulochana Chetty,1991). Larval nutrition plays a vital role in producing good quality cocoons. Improving the economic characters is the main criteria of supplementing the silkworm with various nutrients (Birendro Nath Saha and Atuar Rahman Khan, 1997). Fortification of mulberry leaves by supplementary Spirulina and using them for feeding the silkworms is another useful modern technique to increase their economic and commercial value (Nirmala,1998). The present investigation was worked out in economic and commercial parameters of silkworm supplemented with Spirulina.

Consumption of nutrition is an important growth-regulating factor in silkworm, and with this background, the study investigated the growth of silkworm larvae fed on mulberry leaves enhanced with Spirulina. The present investigation was worked out in growth parameters of silkworm supplemented with Spirulina.

Nutrition involves chemical and physiological activities, which transform food elements into body elements. Insect nutrition concerned primarily with the chemical substances in foodstuff to set in motion and maintain a long series of production in silkworm are very closely related to nutritional factors (Dash *et al*., 1992). Dietary efficiency of silkworm plays a major role in converting the mulberry leaves consumed by them to silk (Etebari *et al*., 2004).

The results revealed that the rates of consumption, assimilation and metabolism showed a declining trend, whereas the production rate increased steadily with the increasing concentrations of Spirulina.

Effect of *Spirulina* on nutritional efficiency

The results of the present study showed a maximum growth of the larvae fed on mulberry leaves supplemented with 5% concentration of Spirulina. The consumption and assimilation was decreased with the increase of the concentration of Spirulina. In general, the larvae feeding on a diet could compensate and acquire protein by elevating their consumption and assimilation (Hamilton *et al*., 1990; Cooper and Schal, 1992).

Table 1 Effects of *Spirulina* on growth and Nutritional efficiency parameters

PARAMETERS	CONTROL		GROUP I (1%)		GROUP II (3%)		GROUP III (5%)	
	Male	Female	Male	Female	Male	Female	Male	Female
Consumption (KJ/Individual)	52.12±3.64	54.26±3.29	51.02±3.57	53.14±3.71	49.46±3.46*	49.62±3.47*	48.46±3.39*	47.21±3.30*
Faeces (KJ/Individual)	33.12±2.31	35.26±2.46	32.04±2.24	31.71±2.21	30.64±2.14*	31.98±2.23*	29.14±2.03*	30.84±2.15*
Production (KJ/Individual)	8.94±0.62	9.21±0.64	8.96±0.62	9.26±0.64	8.99±0.62*	9.12±0.63*	9.20±0.64*	8.12±0.56*
Assimilation (KJ/Individual)	19.00±1.33	20.00±1.40	19.98±1.39	19.95±1.37	19.82±1.38*	18.64±1.30*	20.32±1.42*	17.37±1.21*
Metabolism (KJ/Individual)	12.06±0.84	11.79±0.82	12.02±0.84	14.17±0.99	11.83±0.82*	10.52±0.73*	12.12±0.84*	10.25±0.71*
Consumption rate (J/g live insect/day)	5171.32±361.99	5402.16±378.15	5013.16±350.92*	5285.73±370.00*	4642.12±324.94*	4717.84±330.24*	4542.40±317.96*	4642.71±324.98*
Assimilation rate(J/g live insect/day)	2124.11±148.68	2036.74±142.57	2064.52±144.51*	1937.37±135.61*	1912.35±133.86*	1781.58±1211*	1885.65±131.99*	1775.88±124.31*
Production rate (J/g live insect/day)	772.65±54.08	792.42±55.46	779.78±54.58*	794.16±55.59*	791.21±55.38*	803.14±56.21*	792.12±55.44*	815.64±57.09*
Metabolic rate(J/g live insect/day)	1352.46±94.67	1245.42±87.17	1285.74±90.00*	1144.21±80.09*	1122.14±78.54*	979.44±68.56*	1094.53±76.61*	961.24±67.28*

Significantly different from control * $P<0.05$

Table 2 Effects of *Spirulina* on growth and Nutritional efficiency parameters

PARAMETERS	CONTROL		GROUP I (1%)		GROUP II (3%)		GROUP III (5%)	
	Male	Female	Male	Female	Male	Female	Male	Female
Assimilation Efficiency (AE)	37.16±0.17	35.67±0.91	37.94±0.62*	41.10±0.94*	38.83±0.90*	41.28±0.62*	40.70±0.96*	41.32±0.91*
Efficiency of conversion of ingested material (ECI)	15.53±0.46	15.41±0.41	15.91±0.49*	15.84±0.44*	16.48±0.59*	16.70±0.71*	17.27±0.62*	16.79*
Efficiency of conversion of digested (ECD)	41.78±4.01	43.21±3.11	41.93±12	38.54±4.16	42.45±3.10	46.03±2.84	42.54±3.81*	46.49±2.91*

Significantly different from control * $P<0.05$

In the present study, this was the reason for the increased consumption and assimilation in control group than that of supplemented groups.

The data obtained in the present study showed a reduction in tha assimilation and consumption rate with an increase in the concentration of *Spirulina* supplementation. This corroborates with the findings of Venkataramana *et al*., (2003); Kumar *et al*.,(2009) and Masthan *et al*., (2011).

Increase in ingesta, digesta and excreta per larva with advancement in age of silkworm in temperate breeds (Hori and Inokuchi, 1978), as well as in tropical breeds and its variation among breeds and seasons are well known on artificial diet and mulberry leaves (Yamamota and Fujimaki, 1982). An overall analysis of different *Spirulina* treatment, showed significant change in relative growth rate on all the treated groups when compared to that of control group. Approximate digestibility observed for all the *Spirulina* treatment was significantly superior to that of the control. Higher approximate digestibility was observed at 5% *Spirulina*.

The higher value of approximate digestibility indicates the greater suitability of the food plants. The ingesta and digesta required to produce gram dry weight were low in instars and had increased gradually. It is evident in fifth instars; the approximate digestibility and rate of assimilation of food were high when compared to late instars. This is due to superior quality and high water content in the food, which has a direct regulation on the phagostimulation, digestion and efficiency of conversions (Paul et a., 1992). It is established that the water content of the feed has direct regulation over the ingestion, AE, ECI and ECD (Waldbauer, 1968; Paul *et al*., 1992). Significant differences were observed in efficiency of conversion of ingested food among the different *Spirulina* tested. However, 5% *Spirulina* had higher efficiency of conversion ingested food than the control group tested. The efficiency of conversion of ingested food is an overall measure of the ability of larvae to utilize the ingested food.

The bioenergetic profile of silkworm *B.mori* on supplementation showed that the feeding activity of this larva was highly influenced by *Spirulina*. The results of the feeding parameters indicate that 5% *Spirulina* are the suitable concentrations to improve cocoon yield in sericulture industry because it shortened the larval duration and facilitated higher growth and ECD with lesser consumption.

In the study of bioenergetics profile, it was found that the consumption, assimilation, metabolism, rates of consumption, assimilation and metabolism were found to decrease in *Spirulina* supplementation. On the other hand, the production, production rate, assimilation efficiencies (AD), the efficiencies for conversion ingested food (ECI) and conversion of digested food (ECD) were found to increase in *Spirulina* supplementation. In other words the larvae consumed less mulberry leaves were supplemented with *Spirulina*, a higher quantum of tissue production was noted. A possible explanation for this phenomenon is that larvae that fed on mulberry leaves supplemented with *Spirulina* allocated minimum energy for maintenance and channelized maximum energy towards tissue production. 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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