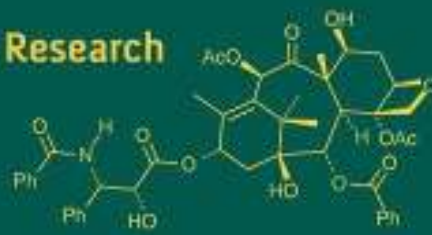
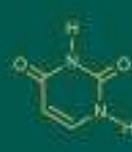
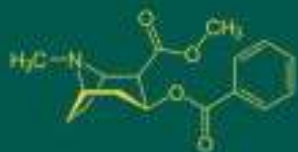


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Studies on the protein content in popular bivoltine double hybrid FC₁ x FC₂ of the silkworm *Bombyx mori* L. using fortified mulberry leaf

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Abstract

Sericulture is the large scale rearing of the silkworm for production of cocoons for extraction of silk fiber which is made up of two proteins. The protein is extracted from the mulberry leaf which gets reflected in the form of quality and quantity of silk in the cocoons. When the protein in the mulberry leaf is not as per desired standards, fortification is followed by adding various sources of protein externally. The present study was conducted by using spirulina and soya flour as fortification agents and their influence was evaluated on protein content in the silkworms of bivoltine double hybrid FC₁×FC₂ during the 5th instar larval stage across pre-monsoon, monsoon and post-monsoon seasons. Mulberry leaves were fortified with spirulina powder (200, 300 and 400 ppm) and soya flour powder (200, 300 and 400 ppm) and were fed to silkworm larvae, and protein content was estimated in the silk glands and haemolymph on the 3rd, 4th and 5th day of the 5th instar larvae. The results have revealed a progressive increase in protein content from day-3 to day-5 in both tissues, with the highest levels consistently recorded in SP-400 treatment, followed by SP-300 and SF-400, while the lowest values were observed in distilled water control and absolute control batches. Among the three seasons, monsoon exhibited significantly higher protein levels compared to post-monsoon and pre-monsoon. In FC₁×FC₂ hybrids, the maximum protein content in silk glands were seen followed by haemolymph under SP-400 treatment during the monsoon season. Statistical analysis using one-way ANOVA revealed highly significant differences ($p < 0.01$) among treatments, seasons and larval days. The present study clearly demonstrates that fortification using spirulina, particularly at 400 ppm significantly enhances protein metabolism in the double hybrid, thereby indicating its potential role in improving larval physiological efficiency and silk productivity.

Keywords: Spirulina, soya flour, silkworm, fortification

1. Introduction

The mulberry silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae), is a commercially important insect owing to its remarkable efficiency in converting mulberry leaf protein into silk fiber. Larval growth, silk gland development and overall silk productivity are directly influenced by the quality and quantity of nutrients available in the diet. Hence, nutritional improvement of mulberry leaves through fortification with proteins, carbohydrates, amino acids, vitamins, hormones and other bioactive compounds has been widely recognized as an effective method for enhancing larval growth and cocoon productivity (Laskar and Datta, 2000; Jeyapaul, *et al.*, 2003) [8, 5].

Protein is the most critical nutrient influencing the growth and metabolic efficiency of the silkworm larvae. Among various natural protein supplements, spirulina a cyanobacterial biomass has gained importance due to its exceptionally high protein content and rich profile of essential amino acids such as glycine, lysine, methionine, glutamine and phenylalanine, along with vital vitamins including tocopherol, riboflavin, biotin, niacin, folic acid and β -carotene. Spirulina fortification has been reported to enhance the larval physiology, improve digestion and increase the overall nutritional efficiency of *B. mori*. Similarly, soya flour also serves as an excellent plant-based protein source enriched with vitamins (A, B and D), minerals such as calcium and magnesium and essential amino acids. Dietary supplementation with soya proteins has been reported to improve larval growth, enhance metabolic activity and significantly elevate the economic traits of *Bombyx mori* by increasing cocoon weight, shell ratio and filament quality

(Kamaraj, *et al.*, 2017; Shruti, *et al.*, 2019) [6, 9]. Since nearly 70% of the silk filament protein is derived from mulberry leaf protein, efficient digestion and assimilation of dietary proteins are crucial for silk synthesis. The activity of midgut proteases responsible for converting mulberry proteins into silk proteins varies across pure breeds and hybrid combinations (Kumar and Kalpana, 2009) [7], indicating the importance of evaluating nutritional responses in specific genetic backgrounds.

Double hybrids as FC₁×FC₂ are widely used in commercial sericulture due to their hybrid vigour, but their physiological response to protein-enriched diets may differ due to genetic and metabolic variations. Therefore, understanding how spirulina and soya flour influence protein accumulation in these hybrids is vital for optimizing feeding strategies and improving silk productivity. Hence, the present study aims to evaluate the impact of spirulina and soya flour supplementation at different concentrations on the protein content of FC₁×FC₂ double hybrid of *Bombyx mori* L., with emphasis on larval physiology and nutritional efficiency to convert the mulberry protein to silk protein when fortified with external protein supplements.

2. Materials and Methods

The present study was conducted using bivoltine double hybrid namely FC₁×FC₂ known for their superior silk yield and hybrid vigour. The disease free layings (DFLs) of the hybrid was procured from the Silkworm Seed Production Centre (SSPC), NSSO, Mysuru, Karnataka. Upon receipt, the DFLs were incubated under controlled conditions (25±1 °C temperature and 80±5 relative humidity) until hatching. The young larvae were reared on S₃₆ mulberry variety during the first and second instars (chawki stage), and V₁ variety for the late age larvae (third, fourth and fifth instars). The rearing activities were carried out in the Department of Studies in Sericulture Science, University of Mysore, Manasa Gangothri, Mysuru following the standard protocols suggested by Dandin and Giridhar (2010) [3]. The various treatments were administered after the fourth moult during the fifth instar larval stage on day-2, day-3 and day-4 larvae were divided into subgroups and fed with mulberry leaves treated with the following formulations:

- Control batches: Distilled water control and absolute control (no treatment)
- Spirulina treatments: 200 ppm, 300 ppm and 400 ppm
- Soya flour treatments: 200 ppm, 300 ppm and 400 ppm

The powders of the above were dissolved using double distilled water and fresh mulberry leaves were smeared with the solutions prepared. The smeared leaves were air-dried for 15-30 minutes before feeding to the silkworm larvae. The larvae were fed with untreated leaves at 11:00 a.m. and the treated leaves at 4:00 p.m. during 2nd, 3rd and 4th day of the fifth instar as treatments followed by untreated mulberry leaf.

The samples such as the silk glands and haemolymph were dissected and collected from the silkworm larvae under sterile conditions. All samples were immediately processed or stored at appropriate temperatures (−20 °C for short-term storage) to preserve their biochemical integrity before analysis. The estimations were carried out following standard biochemical protocols for the silkworm (Chand Asaf and Mahavishnu, 2018) [2].

2.1 Procedure: In the Lowry's *et al.*, (1951) method for protein estimation, 1 ml of the sample, 1 ml of BSA standard and 1 ml of distilled water (blank) are taken in separate test tubes. To each tube, 5 ml of freshly prepared alkaline copper reagent is added, mixed thoroughly, and kept undisturbed for 10 minutes at room temperature. Following this, 0.5 ml of diluted (1:1) Folin-Ciocalteu reagent is added to each tube, mixed immediately and incubated for 30 minutes at room temperature for color development. After the incubation period, the optical density (OD) is measured at 660 nm using a spectrophotometer against the blank, and the protein concentration in the sample is determined by comparing the optical density (OD) with that of the standard values.

During the rearing of silkworm larvae, data collected pertaining to selected economic traits namely fecundity, hatching percentage, larval duration, matured larval weight, cocoon weight, shell weight, shell ratio, filament length, denier and renditta were collected to correlate the same with the protein estimation.

2.2 Statistical Analysis

The data collected were subjected to statistical analysis, including the calculation of mean and standard error. A ONE-WAY analysis of variance (ANOVA) was performed and the data were further analyzed using Factorial Analysis of Variance ($p \leq 0.05$, $p \leq 0.01$) (Sundarraj *et al.*, 1972) through SPSS for Windows, version 21.0 to assess the significance of treatments, seasons, and day-wise effects on the economic and biochemical parameters. Graphs were plotted using the MS-Excel application (version-2013).

3. Results

3.1 Protein content in silk glands

The protein content in the silk glands of FC₁×FC₂ double hybrid under various treatments (Fig. 1) was estimated across different developmental days (day-3, day-4 and day-5) in the 5th instar larval stage during pre-monsoon, monsoon and post-monsoon seasons. The results are presented in figure 1, the protein content is expressed in µg/mg and µg/ml depending on the tissue taken.

During the pre-monsoon season, protein content in silk glands increased progressively from day-3 to day-5 across spirulina and soya flour treatment batches. On day-3, the highest protein content among spirulina treatments was observed in SP-400 (117.6 µg/mg), while the lowest was in SP-200 (97.45 µg/mg). Among the soya flour treatments, the highest was recorded in SF-400 (116.2 µg/mg) and the lowest in SF-200 (95.51 µg/mg). On day-4, SP-400 treatment observed the highest protein level (142.3 µg/mg), whereas the lowest was in SP-200 (111.5 µg/mg); for soya flour treatments, the highest was SF-400 (140.7 µg/mg) and the lowest was SF-200 (110.8 µg/mg). On day-5, the highest protein content among spirulina treatments was noted in SP-400 (154.6 µg/mg), and the lowest in SP-200 (122.2 µg/mg); similarly, in soya flour treatments, SF-400 had the highest (151.2 µg/mg) and SF-200 the lowest (121.9 µg/mg).

In the monsoon season, a consistent rise in protein content was observed. On day-3, the highest protein level among spirulina treatments was found in SP-400 (120.7 µg/mg), while the lowest was in SP-200 (99.74 µg/mg); for soya

flour treatments, the highest was in SF-400 (119.2 µg/mg) and the lowest in SF-200 (98.71 µg/mg). On day-4, SP-400 recorded the highest protein content (147.3 µg/mg), and the lowest was in SP-200 (115.3 µg/mg); within soya flour treatments, the highest was observed in SF-400 (146.3 µg/mg), and the lowest in SF-200 (114.3 µg/mg). On day-5, the highest protein content among spirulina treatments was again found in SP-400 (158.7 µg/mg), while the lowest was in SP-200 (127.6 µg/mg); similarly, in soya flour batches, SF-400 had the highest value (156.3 µg/mg) and SF-200 the lowest (126.6 µg/mg).

In the post-monsoon season, protein content followed the same increasing trend. On day-3, the highest protein content among spirulina treatments was recorded in SP-400 (119.5 µg/mg), and the lowest in SP-200 (99.52 µg/mg); for soya flour treatments, the highest was in SF-400 (118.7 µg/mg), and the lowest in SF-200 (97.05 µg/mg). On day-4, SP-400 exhibited the highest protein level (144.7 µg/mg), while the lowest was observed in SP-200 (113.7 µg/mg); in soya flour treatments, SF-400 had the highest (142.3 µg/mg), and SF-200 the lowest (112.7 µg/mg). On day-5, the highest protein content among spirulina treatments was found in SP-400 (156.7 µg/mg), with the lowest in SP-200 (125.5 µg/mg); in soya flour treatments, the highest was SF-400 (154.2 µg/mg) and the lowest was SF-200 (124.4 µg/mg). Among all three seasons, the highest protein content was recorded during the monsoon season, followed by post-monsoon and pre-monsoon seasons. The result revealed analysis of variance (one-way ANOVA) conducted on the protein (silk gland) estimation parameter revealed highly significant differences ($p < 0.01$) among seasons, day wise treatments and treatment batches.

3.2 Protein content in haemolymph

The protein content in the haemolymph of FC₁×FC₂ double hybrid under various treatments (Fig. 2) was estimated across different developmental days (day-3, day-4 and day-5) in the 5th instar larval stage during pre-monsoon, monsoon and post-monsoon seasons. The results are presented in figure 2.

During the pre-monsoon season, protein content in haemolymph increased progressively from day-3 to day-5 across spirulina and soya flour treatment batches. On day-3, the highest protein content among spirulina treatments was observed in SP-400 (110.2 µg/ml), while the lowest was in SP-200 (90.41 µg/ml). Among soya flour treatments, the highest was recorded in SF-400 (109.6 µg/ml) and the lowest in SF-200 (89.85 µg/ml). On day-4, SP-400 treatment observed the highest protein level (122.6 µg/ml), whereas the lowest was in SP-200 (102.3 µg/ml); for soya flour treatments, the highest was SF-400 (121.3 µg/ml) and the lowest was SF-200 (101.4 µg/ml). On day-5, the highest protein content among spirulina treatments was noted in SP-400 (129.7 µg/ml), and the lowest in SP-200 (110.1 µg/ml); similarly, in soya flour treatments, SF-400 had the highest (128.8 µg/ml) and SF-200 the lowest (109.4 µg/ml).

In the monsoon season, a consistent rise in protein content was observed. On day-3, the highest protein level among spirulina treatments was found in SP-400 (113.8 µg/ml), while the lowest was in SP-200 (93.21 µg/ml); for soya flour treatments, the highest was in SF-400 (113.2 µg/ml) and the lowest in SF-200 (93.06 µg/ml). On day-4, SP-400

recorded the highest protein content (126.7 µg/ml), and the lowest was in SP-200 (106.8 µg/ml); within soya flour treatments, the highest was observed in SF-400 (125.5 µg/ml), and the lowest in SF-200 (105.5 µg/ml). On day-5, the highest protein content among spirulina treatments was again found in SP-400 (134.8 µg/ml), while the lowest was in SP-200 (114.3 µg/ml); similarly, in soya flour batches, SF-400 had the highest value (132.3 µg/ml) and SF-200 the lowest (113.2 µg/ml).

In the post-monsoon season, protein content followed the similar increasing trend. On day-3, the highest protein content among spirulina treatments was recorded in SP-400 (112.8 µg/ml), and the lowest in SP-200 (92.25 µg/ml); for soya flour treatments, the highest was in SF-400 (111.5 µg/mg), and the lowest in SF-200 (91.32 µg/ml). On day-4, SP-400 exhibited the highest protein level (124.4 µg/ml), while the lowest was observed in SP-200 (104.3 µg/ml); in soya flour treatments, SF-400 had the highest (123.6 µg/ml), and SF-200 the lowest (103.3 µg/ml). On day-5, the highest protein content among spirulina treatments was found in SP-400 (131.7 µg/ml), with the lowest in SP-200 (112.2 µg/ml); in soya flour treatments, the highest was SF-400 (130.3 µg/ml) and the lowest was SF-200 (111.2 µg/ml). Among all three seasons, the highest protein content was recorded during the monsoon season, followed by post-monsoon and pre-monsoon seasons. The result revealed analysis of variance (one-way ANOVA) conducted on the protein (haemolymph) estimation parameter revealed highly significant differences ($p < 0.01$) among seasons, day wise treatments and treatment batches.

3.3 Economic Traits

The mean values of the selected economic traits of bivoltine double hybrid FC₁×FC₂ of the silkworm, *Bombyx mori* are presented in Table 1. Among the bivoltine hybrids selected for the study, SP400 on day-5, day-4, day-3 in fifth instar showed higher matured larval weight, cocoon weight and shell weight.

During the pre-monsoon season, the highest matured larval weight (48.00 g) was recorded in SP400 treatment in day-5 compared to day-4 and day-3, whereas the lowest value (42.79 g) was observed in SP200 treatment in day-3 compared with day-4 and day-5 of 5th instar larvae. The larval weight was found to be higher (42.21 g) in SP400 in day-5 compared to day-4 and day-3; minimum of (35.30 g) in SP200 treatment in day-3 compared with day-4 and day-5 of 5th instar. The cocoon weight was highest (20.60 g) treated with SP400 on day-5 and lowest (16.74 g) in SP200 on day-3, while shell weight showed a maximum (4.200 g) for SP400 treatment in day-5 and a minimum (3.300 g.) in SP200 treatment in day-3 compared to day-4 of 5th instar larvae. The filament length ranged from (1405 m) in SP400 in day-5 and (1188 m) SP200 of day-3; while in soya flour the highest matured larval weight (47.12 g) was recorded in SF400 treatment in day-5 compared to day-4 and day-3, whereas the lowest (42.02 g) was observed in SF200 treatment in day-3 compared with day-4 and day-5 of 5th instar. The larval weight was found to be maximum (41.05 g) in SF400 in day-5 compared to day-4 and day-3, and a minimum of (34.17 g) in SF200 treatment in day-3 compared with day-4 and day-5 of 5th instar. The cocoon weight was highest (20.10 g) in SF400 on day-5 and lowest

(16.69 g) in SP200 on day-3; the shell weight was found to be maximum (4.247 g) in SF400 on day-5 and a minimum (3.060 g) in SF200 treatment in day-3 compared to day-4 and day-5. The filament length ranged from (1283 m) in SF400 in day-5 and (1176 m) SP200 of day-3 of 5th instar.

During the monsoon season, the highest matured larval weight (51.12 g) was recorded in SP 400 treatment in day-5 compared to day-4 and day-3, whereas the lowest (44.35 g) was observed in SP200 treatment in day-3 compared with day-4 and day-5 of 5th instar. The larval weight was found maximum (42.44 g) in SP400 in day-5 compared to day-4 and day-3 of 5th instar; minimum of (35.85 g) in SP200 treatment in day-3 compared with day-4 and day-5 of 5th instar. The cocoon weight was highest (21.82 g) in SP400 on day-5 and lowest (17.17 g) in SP200 on day-3, while shell weight showed a maximum (5.100 g) for SP400 treatment in day-5 and a minimum (3.693 g) in SP200 treatment in day-3 compared to day-4 of 5th instar. The filament length ranged from (1473 m) in SP400 in day-5 and (1197 m) SP200 of day-3; while in Soya flour the highest matured larval weight (49.67 g) was recorded in SF 400 treatment in day-5 compared to day-4 and day-3, whereas the lowest (44.67 g) was observed in SF200 treatment in day-3 compared with day-4 and day-5 of 5th instar. The larval weight was found to be maximum of (41.53 g) in SF400 in day-5 compared to day-4 and day-3, and a minimum of (34.98 g) in SF200 treatment in day-3 compared with day-4 and day-5 of 5th instar. The cocoon weight was highest (21.58 g) in SF400 on day-5 and lowest (17.10 g) in SP200 on day-3; shell weight was found to be maximum (4.727 g) in SF400 on day-5 and a minimum (3.353 g) in SF200 treatment in day-3 compared to day-4 and day-5. The filament length ranged from (1411 m) in SF400 in day-5 and (1212 m) SP200 of day-3 of 5th instar.

During the post-monsoon season, the highest matured larval weight (49.03 g) was recorded in SP 400 treatment in day-5 compared to day-4 and day-3, whereas the lowest value (44.76 g) was observed in SP200 treatment in day-3 compared with day-4 and day-5 of 5th instar. The larval weight was found to be higher (42.12 g) in SP400 in day-5 compared to day-4 and day-3; minimum of (35.78 g) in SP200 treatment in day-3 compared with day-4 and day-5 of 5th instar larvae. The cocoon weight was highest (21.20 g) in SP400 on day-5 and lowest (16.99 g) in SP200 on day-3, while shell weight showed a maximum value (4.96 g) for SP400 on day-5 and a minimum (3.56 g) in SP200 treatment in day-3 compared to day-4 of 5th instar larvae. The filament length ranged from (1453 m) in SP400 on day-5 and (1189 m) SP200 of day-3; while in soya flour treatment the highest matured larval weight (48.96 g) was recorded in SF 400 in day-5 compared to day-4 and day-3, whereas the lowest (43.65 g) was observed in SF200 treatment in day-3

compared with day-4 and day-5 of 5th instar. The larval weight was found to be maximum of (41.29 g) in SF400 in day-5 compared to day-4 and day-3 and was minimum (34.40 g) in SF200 treatment in day-3 compared with day-4 and day-5 of 5th instar. The cocoon weight was highest (21.10 g) in SF400 on day-5 and lowest (16.72 g) in SP200 on day-3, the shell weight was found to be maximum (4.42 g) in SF400, day-5 and a minimum (3.19 g) in SF200 treatment in day-3 compared to day-4 and day-5. The filament length ranged from (1383 m) in SF400 in day-5 and (1194 m) SP200 of day-3 of 5th instar larvae.

4. Discussion

The silkworm (*Bombyx mori*) growth and silk productivity are largely determined by nutritional status, enzymatic efficiency, and environmental conditions. Biochemical components such as proteins, carbohydrates, and enzymes like amylase and phosphatases play crucial roles in larval metabolism and silk synthesis. Dietary supplementation is widely recognized as an effective approach to enhance larval physiological performance and cocoon quality, and its impact may vary with tissue type, developmental stage, and seasonal conditions.

The protein content in $FC_1 \times FC_2$ is a vital indicator of metabolic and synthetic potential in silkworm larvae. In the present study, the protein content of $FC_1 \times FC_2$ were significantly influenced by both the type of dietary supplement and tissue type studied. In the silk glands, spirulina fortification at SP400 produced the highest protein content, followed by SP300 and SP200, indicating that enriched protein sources directly enhance silk protein synthesis. In contrast, soya flour treatments (SF400, SF300, and SF200) showed moderate increases, while control batches (DWC and AC) exhibited the lowest protein levels. Similar trends were observed in the haemolymph, reflecting systemic metabolic enhancement, where protein accumulation suggests improved storage and mobilization for biosynthetic purposes. Seasonal variation revealed that the monsoon season supported higher protein levels across tissues, likely due to optimal temperature and humidity for metabolic activity.

Among the seasons, larvae reared during the monsoon season exhibited higher protein accumulation than the larvae reared during pre-monsoon and post-monsoon seasons, possibly due to optimal temperature and humidity favoring digestion and metabolism. Across all treatment batches, a steady increase in protein content was noted from day-3 to day-5 of 5th instar larva.

Among all three seasons, the highest protein content is found in silk glands when compared with haemolymph was recorded during the monsoon season, followed by post-monsoon and pre-monsoon seasons.

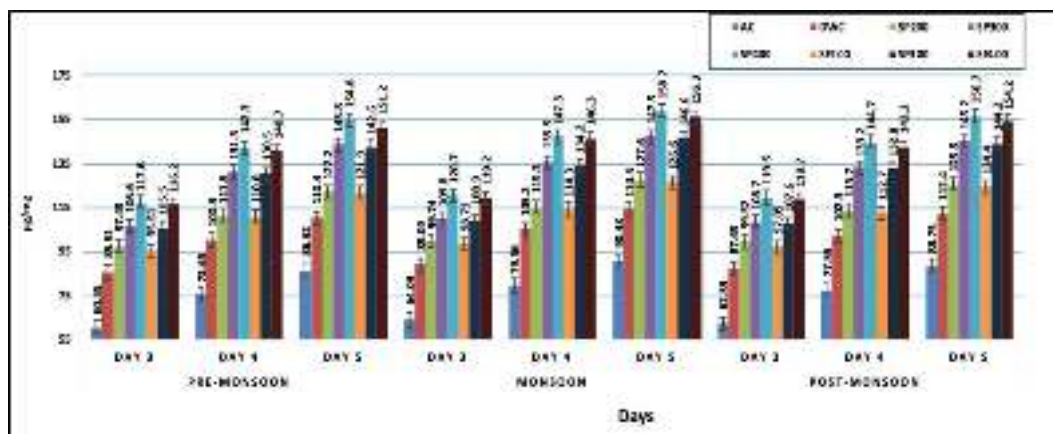


Fig 1: Effect of fortification at varied concentrations on protein content in the silk glands in double hybrid FC₁xFC₂ of the silkworm *Bombyx mori*.

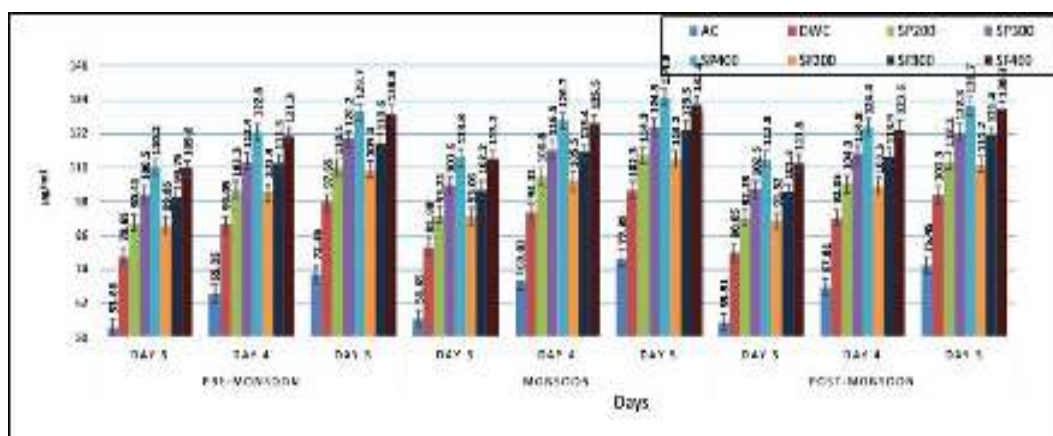


Fig 2: Effect of fortification at varied concentrations on protein content in the haemolymph in double hybrid FC₁xFC₂ of the silkworm *Bombyx mori*.

Table 1: Showing mean values of economic traits during the pre-monsoon season for the bivoltine double hybrid FC₁ X FC₂

PRE-Monsoon Season										
Treatments	Days of fifth instar	Larval duration (h)	Matured larval weight (g) (10 larva)	Larval weight (g)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)	Filament length (m)	Denier (d)	Renditta (kgs.)
AC	3	543.0±1.732	40.08±0.319	30.36±0.380	12.79±0.282	1.967±0.018	15.38±0.201	1083±5.250	2.538±0.007	8.683±0.363
	4		41.12±0.347	31.62±0.847	13.80±0.115	2.127±0.064	15.40±0.349	1086±12.29	2.506±0.006	8.977±0.631
	5		42.00±0.404	32.00±0.577	14.93±0.406	2.467±0.240	16.62±2.023	1092±3.961	2.413±0.007	6.023±0.054
DWC	3		41.56±0.349	32.35±0.867	14.99±0.052	2.367±0.041	15.78±0.311	1142±4.433	2.535±0.008	5.460±0.087
	4		42.87±0.353	32.66±0.670	16.00±0.115	2.553±0.029	15.96±0.253	1161±7.862	2.474±0.007	5.773±0.094
	5		43.65±0.351	33.00±0.577	16.76±0.151	3.067±0.067	18.30±0.407	1102±8.933	2.407±0.006	6.480±0.264
SP200	3		42.79±0.248	35.30±0.351	16.74±0.181	3.300±0.050	19.71±0.216	1188±1.984	2.224±0.007	5.580±0.059
	4		43.89±0.347	35.69±0.400	16.93±0.176	3.373±0.013	19.92±0.158	1194±5.902	2.246±0.008	5.533±0.118
	5		44.88±0.392	36.08±0.087	19.12±0.14	3.707±0.058	19.39±0.332	1157±4.555	2.235±0.008	6.807±0.177
SP300	3		43.56±0.295	36.66±0.667	17.16±0.349	3.480±0.031	20.30±0.582	1197±6.538	2.281±0.006	5.620±0.065
	4		44.93±0.382	38.00±0.577	17.40±0.231	3.580±0.042	20.66±0.435	1206±13.33	2.216±0.006	5.360±0.025
	5		46.01±0.378	39.38±0.061	19.79±0.064	3.733±0.067	18.86±0.286	1267±14.75	2.142±0.006	6.120±0.174
SP400	3		45.91±0.384	40.66±0.667	20.09±0.093	3.930±0.141	20.60±0.397	1397±9.093	2.126±0.006	5.233±0.056
	4		46.89±0.371	41.00±0.577	20.29±0.357	4.147±0.074	20.76±0.674	1441±49.14	2.057±0.009	4.927±0.166
	5		48.00±0.462	42.21±0.234	20.60±0.305	4.200±0.306	20.36±1.250	1405±15.40	2.043±0.007	5.470±0.052
SF200	3		42.02±0.214	34.17±0.436	16.69±0.093	3.060±0.087	18.33±0.481	1176±8.143	2.422±0.006	5.743±0.042
	4		43.23±0.271	34.58±0.290	16.70±0.270	3.307±0.087	19.79±0.384	1199±1.666	2.312±0.006	5.660±0.065
	5		44.21±0.323	34.98±0.255	17.90±0.058	3.427±0.207	17.95±0.093	1171±6.599	2.302±0.006	6.427±0.114
SF300	3		43.41±0.211	34.29±0.293	16.74±0.06	3.240±0.023	19.35±0.085	1194±4.918	2.305±0.008	5.643±0.049
	4		44.09±0.361	35.00±0.577	16.77±0.093	3.340±0.023	19.91±0.143	1200±6.202	2.271±0.006	5.543±0.241
	5		45.31±0.323	36.21±0.330	19.54±0.136	3.667±0.067	18.77±0.470	1210±5.534	2.187±0.006	6.480±0.216
SF400	3		45.07±0.394	38.10±1.054	19.47±0.268	3.900±0.031	20.03±0.240	1362±1.175	2.184±0.007	5.133±0.080
	4		46.03±0.318	39.72±0.364	19.97±0.229	4.027±0.027	20.17±0.114	1370±38.60	2.148±0.007	5.180±0.031
	5		47.12±0.313	41.05±0.508	20.10±0.097	4.247±0.352	19.26±0.181	1283±5.662	2.118±0.007	5.850±0.077

Table 2: Showing mean values of economic traits during the monsoon season for the bivoltine double hybrid FC₁ X FC₂.

Monsoon Season										
Treatments	Days of fifth instar	Larval duration (h)	Matured larval weight (g) (10 larva)	Larval weight (g)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)	Filament length (m)	Denier (d)	Renditta (kgs.)
AC	3	519.00±4.619	42.65±0.333	31.89±0.087	14.16±0.018	2.113±0.029	14.93±0.220	1111±6.513	2.673±0.006	8.553±0.318
	4		43.78±0.377	32.28±0.307	14.26±0.145	2.273±0.057	15.93±0.271	1153±11.09	2.692±0.006	8.250±0.312
	5		44.967±0.376	32.95±0.103	15.20±0.382	2.980±0.064	19.64±0.904	1158±1.500	2.729±0.007	7.680±0.190
DWC	3		43.76±0.352	33.88±0.076	15.44±0.101	2.720±0.061	17.60±0.282	1153±11.71	2.576±0.006	5.207±0.052
	4		44.67±0.354	34.04±0.124	16.32±0.083	2.833±0.044	17.36±0.180	1175±8.793	2.648±0.007	5.500±0.026
	5		45.61±0.323	34.98±0.255	17.07±0.165	3.273±0.041	19.18±0.351	1186±6.852	2.569±0.008	5.183±0.044
SP200	3		44.35±0.353	35.85±0.127	17.17±0.048	3.693±0.052	21.50±0.245	1197±30.27	2.554±0.008	5.213±0.058
	4		45.67±0.376	36.83±0.314	17.48±0.131	3.707±0.035	21.19±0.193	1212±8.123	2.499±0.008	5.187±0.032
	5		46.79±0.378	37.38±0.130	19.76±0.243	4.013±0.081	20.30±0.062	1257±8.230	2.416±0.006	5.597±0.079
SP300	3		45.67±0.342	39.12±0.127	17.52±0.068	3.713±0.024	21.19±0.208	1224±6.514	2.496±0.006	5.057±0.068
	4		46.81±0.364	39.69±0.287	17.82±0.068	3.887±0.18	20.60±0.282	1220±10.85	2.324±0.008	4.960±0.046
	5		48.03±0.348	39.88±0.100	20.15±0.282	4.187±0.064	20.78±0.349	1281±18.72	2.322±0.006	5.383±0.086
SP400	3		47.81±0.404	40.82±0.271	21.46±0.069	4.607±0.024	21.46±0.144	1419±10.21	2.188±0.007	5.300±0.080
	4		48.78±0.377	41.80±0.180	21.67±0.077	4.673±0.018	21.56±0.147	1469±8.448	2.118±0.007	5.003±0.070
	5		51.12±0.405	42.44±0.318	21.82±0.134	5.100±0.064	23.62±0.412	1473±37.13	2.092±0.006	4.953±0.058
SF200	3		44.67±0.354	34.98±0.174	17.10±0.122	3.353±0.199	19.62±1.285	1212±6.510	2.651±0.006	5.470±0.076
	4		45.90±0.379	35.43±0.229	17.23±0.078	3.520±0.061	20.42±0.271	1228±3.591	2.571±0.006	5.323±0.124
	5		47.12±0.347	35.80±0.087	18.41±0.225	3.664±0.085	19.65±0.160	1260±14.62	2.548±0.007	5.557±0.055
SF300	3		45.92±0.325	35.55±0.098	17.40±0.066	3.540±0.031	20.41±0.233	1227±4.129	2.505±0.007	5.267±0.043
	4		46.71±0.363	35.98±0.115	18.01±0.240	3.663±0.106	20.44±0.189	1236±6.852	2.478±0.007	5.083±0.090
	5		48.09±0.377	36.42±0.212	19.82±0.092	3.907±0.035	19.71±0.168	1240±2.644	2.452±0.006	5.567±0.087
SF400	3		46.89±0.347	39.91±0.067	20.81±0.068	4.393±0.070	21.11±0.402	1381±5.533	2.374±0.007	5.270±0.056
	4		48.34±0.406	40.63±0.295	20.95±0.064	4.513±0.018	21.45±0.307	1367±32.08	2.316±0.007	5.130±0.100
	5		49.67±0.354	41.53±0.139	21.58±0.125	4.727±0.207	20.97±0.326	1411±6.511	2.271±0.006	5.047±0.099

Table 3: Showing mean values of economic traits during the post-monsoon season for the bivoltine double hybrid FC₁ X FC₂.

Post-Monsoon Season										
Treatments	Days of fifth instar	Larval duration (h)	Matured larval weight (g) (10 larva)	Larval weight (g)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)	Filament length (m)	Denier (d)	Renditta (kgs.)
AC	3	591.0±1.155	41.23±0.376	31.23±0.318	13.96±0.033	2.073±0.052	14.84±0.404	1092±2.598	2.601±0.006	8.960±0.182
	4		42.45±0.375	31.80±0.102	14.15±0.169	2.213±0.057	15.63±0.258	1118±8.383	2.588±0.007	8.707±0.472
	5		43.14±0.375	32.63±0.318	15.12±0.391	2.807±0.066	18.60±0.883	1120±1.386	2.536±0.008	7.800±0.235
DWC	3		42.35±0.407	33.00±0.577	15.34±0.095	2.540±0.020	16.56±0.227	1148±10.57	2.581±0.006	5.327±0.023
	4		43.75±0.375	33.70±0.69	16.13±0.119	2.673±0.048	16.57±0.328	1168±8.871	2.588±0.007	5.503±0.054
	5		44.67±0.376	34.87±0.355	16.90±0.141	3.193±0.024	18.88±0.161	1171±4.562	2.514±0.008	5.253±0.077
SP200	3		43.86±0.352	35.78±0.385	16.99±0.077	3.507±0.048	20.63±0.266	1189±29.69	2.516±0.006	5.393±0.095
	4		44.56±0.375	36.26±0.633	17.30±0.127	3.620±0.081	20.00±0.244	1205±5.531	2.376±0.009	5.397±0.057
	5		45.87±0.376	37.05±0.195	19.29±0.119	3.927±0.024	20.35±0.063	1243±1.664	2.364±0.008	5.793±0.041
SP300	3		44.76±0.352	38.34±0.329	17.26±0.197	3.580±0.031	20.75±0.405	1206±6.951	2.473±0.007	5.277±0.094
	4		45.78±0.377	39.12±0.502	17.50±0.142	3.707±0.037	21.18±0.345	1213±11.66	2.325±0.008	5.160±0.023
	5		47.02±0.347	39.51±0.127	19.96±0.008	4.140±0.070	20.73±0.347	1274±14.71	3.322±0.006	5.737±0.033
SP400	3		46.71±0.435	40.65±0.157	20.55±0.254	4.387±0.024	20.81±0.103	1403±10.32	2.241±0.006	5.380±0.015
	4		47.80±0.404	41.45±0.275	21.12±0.083	4.473±0.052	20.80±0.147	1452±49.56	2.155±0.008	5.070±0.044
	5		49.03±0.376	42.21±0.234	21.20±0.105	4.967±0.064	23.42±0.374	1453±3.382	2.136±0.008	5.050±0.087
SF200	3		43.65±0.354	34.40±0.180	16.72±0.068	3.193±0.134	19.09±0.802	1194±10.91	2.519±0.008	5.447±0.092
	4		44.87±0.376	34.97±0.578	16.82±0.285	3.373±0.094	20.04±0.428	1207±2.100	2.457±0.007	5.397±0.057
	5		45.71±0.383	35.46±0.137	18.01±0.035	3.487±0.037	19.35±0.173	1215±15.10	2.448±0.007	5.690±0.038
SF300	3		44.12±0.347	35.00±0.577	16.83±0.208	3.433±0.029	20.15±0.262	1197±1.984	2.412±0.006	5.393±0.064
	4		44.96±0.376	35.92±0.399	17.10±0.412	3.523±0.038	20.64±0.373	1204±6.599	2.388±0.007	5.303±0.171
	5		46.43±0.347	36.15±0.229	19.75±0.017	3.827±0.027	19.37±0.124	1227±3.811	2.394±0.008	5.757±0.088
SF400	3		46.03±0.375	39.66±0.275	20.60±0.080	4.193±0.037	20.35±0.238	1369±1.048	2.345±0.008	5.347±0.054
	4		47.34±0.376	40.10±0.237	20.84±0.251	4.300±0.050	20.84±0.190	1362±28.52	2.276±0.008	5.287±0.122
	5		48.96±0.376	41.29±0.247	21.10±0.128	4.420±0.220	20.93±0.921	1383±9.472	2.262±0.006	5.367±0.019

5. Conclusion

The present investigation clearly establishes that fortification of mulberry leaves with spirulina and soya flour markedly influences protein metabolism in FC₁×FC₂ double hybrid of *Bombyx mori*. A consistent and significant

increase in protein content was observed in silk glands and haemolymph from day-3 to day-5 of the 5th instar larvae, indicating enhanced metabolic activity and improved assimilation efficiency for conversion of leaf protein to silk protein.

Among the treatments, spirulina at 400 ppm (SP-400) has proved to be the most effective nutritional supplement, followed by SP-300 and SF-400, while the absolute control exhibited the lowest protein values. Seasonally, the monsoon period recorded the highest protein accumulation, followed by post-monsoon and pre-monsoon seasons, highlighting the favourable environmental influence on protein synthesis and utilization. The hybrids, FC₁×FC₂ responded positively to dietary enrichment, though slight variations were observed in their protein accumulation patterns, reflecting genetic differences in nutrient assimilation capacity. The statistically significant differences ($p < 0.01$) among treatments, seasons and larval days confirm the strong impact of nutritional supplementation on biochemical performance. Overall, fortification of mulberry leaves with spirulina at 400 ppm emerges as an effective strategy to enhance protein content in silkworm larvae, thereby contributing to improved physiological vigour, silk gland development and potential enhancement of cocoon and silk quality. These findings provide a valuable nutritional approach for optimizing productivity in commercial sericulture.

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References

1. Ananda Kumar MD, Michael AS. Changes in the activities of proteins and carbohydrates in haemolymph of silkworm, *Bombyx mori* L. treated with green leaf. International Journal of Environmental Sciences. 2012;3(1):119-125.
2. Chand A, Mahavishnu. Morphometric studies of silkworm, *Bombyx mori* L. fed with Spirulina treated MR2 mulberry leaves. Plant Archives. 2018;18:316-319.
3. Dandin SB, Jayaswal J, Giridhar K. Handbook of sericulture technologies. Bangalore: Central Silk Board; 2003. p. 259.
4. Hassan EM. Comparative study on effects of some high protein content nutritional additives on some hybrids of mulberry silkworm *Bombyx mori* L. Journal of Plant Protection and Pathology. 2020;11(8):403-410.
5. Jeyapaul C, Padmalatha C, Ranjit Singh JA, Murugesan AG, Dhasarathan P. Effect of plant extracts on nutritional efficiency in mulberry silkworm, *Bombyx mori*. Indian Journal of Sericulture. 2003;42(3):128-131.
6. Kamaraj S, Pandiaraj T, Prabhu IG, Kumari S, Sinha AK. Effect of soya protein enriched fortified feed of tasar silkworm (*Antheraea mylitta* Drury) on rearing performance and economical cocoon characters. International Journal of Advanced Biological Research. 2017;7(1):61-64.
7. Kumar V, Kalpana GV. Variation in protease enzyme activity among different silkworm breeds of *Bombyx mori* L. Journal of Insect Physiology. 2009;55(3):245-250.
8. Laskar N, Datta RK. Effects of nutritional quality of mulberry leaves on silkworm growth and cocoon characters. Sericologia. 2000;40(4):525-533.
9. Shruti KS, Nagaraju N, Kumar P. Economics of probiotic feed supplementation to mulberry silkworm (*Bombyx mori* L.). Journal of Entomology and Zoology Studies. 2019;7(2):382-385.
10. Umakanth RS, Roopashree S. Quantitative estimation of protein content in the silkworm, *Bombyx mori* L. using two feeding schedules. Mysore: University of Mysore; 2014.