

Studies on the Protein Content in Popular Bivoltine Double Hybrid $FC_2 \times FC_1$ of the Silkworm *Bombyx Mori* L. Using Fortified Mulberry in Three Seasons

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Abstract—Sericulture relies on the efficient conversion of mulberry leaf protein into silk proteins by the silkworm, *Bombyx mori* L. In recent times nutritional enhancement of mulberry leaves through external fortification has been widely adopted to improve larval performance and silk yield. The present investigation was carried out to assess the influence of protein-rich fortifications such as spirulina and soya flour on the biochemical performance of the bivoltine double hybrid $FC_2 \times FC_1$ during the fifth instar larval stage under different seasonal conditions. Mulberry leaves were fortified with spirulina (200, 300 and 400 ppm) and soya flour (200, 300 and 400 ppm), were fed to the 5th instar silkworm larvae during the late instar. The protein content was estimated in the silk glands and haemolymph on the 3rd, 4th and 5th day of the fifth instar larvae after 24 h. of treatment. The results obtained have shown a gradual increase in protein levels with larval age, irrespective of treatments. Among the treatments, spirulina at 400 ppm recorded the highest protein accumulation followed by 400 ppm of soya flour, whereas control batches showed the lowest values. Seasonal influence was evident, with the monsoon season favouring higher protein synthesis compared to pre-monsoon and post-monsoon periods. The $FC_2 \times FC_1$ hybrid exhibited a strong response to nutritional fortifications, suggesting improved metabolic efficiency and nutrient utilization. The statistical analysis revealed significant differences among treatments, larval stages and seasons ($p < 0.01$). The study highlights the importance of fortification in enhancing biochemical attributes and indicates that spirulina fortification can be effectively used to improve silkworm performance and silk productivity.

Index Terms—Batches, Fortification, Spirulina, Soya flour, Season, Treatments.

I. INTRODUCTION

The success of sericulture largely depends on the efficient conversion of mulberry leaf protein into silk protein by the silkworm, *Bombyx mori* L. The nutritional composition of mulberry leaves play a decisive role in larval growth, silk accumulation in the silk glands, growth and development along with the cocoon quality (Krishnaswami, 1978; Koul and Tikoo, 1990). Since mulberry leaves are the sole food source for the silkworms, any imbalance in their nutrient content directly affects physiological performance and silk productivity (Sengupta and Singh, 1974; Rahmathulla, 2012). Therefore, improving the nutritional quality of mulberry leaves through external fortification has become an important strategy in modern sericulture practices (Ranjitha and Umakanth, 2025a, 2025b and 2026).

Among various nutrients, proteins are considered fundamental for silkworm metabolism, as they are directly involved in tissue building, enzyme synthesis and silk protein formation (Ito, 1967; Horie and Watanabe, 1983). A major proportion of silk fibre proteins is derived from ingested mulberry leaf proteins, highlighting the importance of efficient digestion and assimilation mechanisms (Bhattacharyya *et al.*, 2016). However, natural variations in mulberry leaf quality across seasons often necessitate the use of dietary supplements to maintain optimal larval performance (Lamberti, *et al.*, 2019).

In recent years, the use of nutritionally enriched additives such as spirulina and soya-based products has gained considerable attention. Spirulina is a unicellular blue-green alga known for its high protein

content, balanced amino acid composition and presence of vitamins and antioxidants, which collectively enhance metabolic efficiency and growth in silkworm larvae (Venkataramana, *et al.*, 2003; Ranjitha and Umakanth, 2025a, 2025b and 2026). On the other hand, soya flour serves as an economical plant protein source that improves nutrient availability and supports better cocoon and filament characteristics (Kamaraj, *et al.*, 2017; Shruti, *et al.*, 2019). Apart from nutritional factors, the genetic constitution of silkworm hybrids plays a crucial role in determining their response to dietary inputs. Reciprocal hybrids often exhibit differential performance due to maternal effects and cytoplasmic inheritance, which influence physiological and biochemical processes. Such variations necessitate independent evaluation of each hybrid combination rather than assuming similar responses across crosses. Furthermore, the environmental factors particularly seasonal variations, significantly influence silkworm metabolism by affecting feeding behaviour, digestion and enzymatic activity. Hence, studying the interaction between nutrition, genotype and season becomes essential for optimizing silkworm productivity under field conditions. In this context, the present study was designed to investigate the effect of spirulina and soya flour fortification on protein content in the bivoltine reciprocal hybrid FC₂ × FC₁ of the silkworm *Bombyx mori* L. during the fifth instar larval stage under different seasonal conditions, with an emphasis on understanding nutrient utilization and biochemical efficiency.

II. MATERIALS AND METHODS

The present study was conducted using popular bivoltine double hybrid namely FC₂×FC₁ 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. The DFLs were incubated under standard 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). 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 additives were dissolved using double distilled water and fresh mulberry leaves were smeared with the solutions of various concentrations 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 (Lowry's *et al.*, 1951; Chand Asaf and Mahavishnu, 2018).

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) were 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 obtained.

During the rearing of silkworm larvae, the 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 used to correlate the same with the protein content by quantitative analysis.

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).

III. RESULTS

3.1. Protein content in silk glands:

The protein content in the silk glands of $FC_2 \times FC_1$ double hybrid under various treatments 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 $\mu\text{g}/\text{mg}$ and $\mu\text{g}/\text{ml}$ depending on the tissue taken for estimation.

During the pre-monsoon season, the 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 (115.1 $\mu\text{g}/\text{mg}$), while the lowest was in SP-200 (95.65 $\mu\text{g}/\text{mg}$). Among soya flour treatments, the highest content was recorded in SF-400 (114.2 $\mu\text{g}/\text{mg}$) and the lowest in SF-200 (93.45 $\mu\text{g}/\text{mg}$). On day-4, SP-400 treatment observed the highest protein level (140.2 $\mu\text{g}/\text{mg}$), whereas the lowest was in SP-200 (109.5 $\mu\text{g}/\text{mg}$); for soya flour treatments, the highest was SF-400 (138.9 $\mu\text{g}/\text{mg}$) and the lowest was SF-200 (108.1 $\mu\text{g}/\text{mg}$). On day-5, the highest protein content among spirulina treatments was noted in SP-400 (152.9 $\mu\text{g}/\text{mg}$), and the lowest in SP-200 (120.2 $\mu\text{g}/\text{mg}$); similarly, in soya flour treatments, SF-400

had the highest (149.7 $\mu\text{g}/\text{mg}$) and SF-200 the lowest (119.8 $\mu\text{g}/\text{mg}$).

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 (119.9 $\mu\text{g}/\text{mg}$), while the lowest was in SP-200 (98.55 $\mu\text{g}/\text{mg}$); for soya flour treatments, the highest was in SF-400 (118.9 $\mu\text{g}/\text{mg}$) and the lowest in SF-200 (97.65 $\mu\text{g}/\text{mg}$). On day-4, SP-400 recorded the highest protein content (144.6 $\mu\text{g}/\text{mg}$), and the lowest was in SP-200 (113.2 $\mu\text{g}/\text{mg}$); within soya flour treatments, the highest was observed in SF-400 (142.9 $\mu\text{g}/\text{mg}$), and the lowest in SF-200 (112.4 $\mu\text{g}/\text{mg}$). On day-5, the highest protein content among spirulina treatments was again found in SP-400 (156.8 $\mu\text{g}/\text{mg}$), while the lowest was in SP-200 (125.5 $\mu\text{g}/\text{mg}$); similarly, in soya flour batches, SF-400 had the highest value (154.7 $\mu\text{g}/\text{mg}$) and SF-200 the lowest (124.9 $\mu\text{g}/\text{mg}$).

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 (117.9 $\mu\text{g}/\text{mg}$), and the lowest in SP-200 (97.55 $\mu\text{g}/\text{mg}$); for soya flour treatments, the highest was in SF-400 (116.4 $\mu\text{g}/\text{mg}$), and the lowest in SF-200 (95.82 $\mu\text{g}/\text{mg}$). On day-4, SP-400 exhibited the highest protein level (142.6 $\mu\text{g}/\text{mg}$), while the lowest was observed in SP-200 (111.2 $\mu\text{g}/\text{mg}$); in soya flour treatments, SF-400 had the highest (141.2 $\mu\text{g}/\text{mg}$), and SF-200 the lowest (110.2 $\mu\text{g}/\text{mg}$). On day-5, the highest protein content among Spirulina treatments was found in SP-400 (154.3 $\mu\text{g}/\text{mg}$), with the lowest in SP-200 (123.3 $\mu\text{g}/\text{mg}$); in Soya flour treatments, the highest was SF-400 (153.1 $\mu\text{g}/\text{mg}$) and the lowest was SF-200 (122.2 $\mu\text{g}/\text{mg}$). 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 glands) 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 $FC_2 \times FC_1$ hybrid under various treatments 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 16.

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 (108.1 µg/ml), while the lowest was in SP-200 (88.75 µg/ml). Among soya flour treatments, the highest was recorded in SF-400 (107.1 µg/ml) and the lowest in SF-200 (87.75 µg/ml). On day-4, SP-400 treatment observed the highest protein level (121.2 µg/ml), whereas the lowest was in SP-200 (100.2 µg/ml); for soya flour treatments, the highest was SF-400 (119.7 µg/ml) and the lowest was SF-200 (99.13 µg/ml). On day-5, the highest protein content among spirulina treatments was noted in SP-400 (127.6 µg/ml), and the lowest in SP-200 (108.8 µg/ml); similarly, in Soya flour treatments, SF-400 had the highest (126.5 µg/ml) and SF-200 the lowest (107.4 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 (112.6 µg/ml), while the lowest was in SP-200 (92.05 µg/ml); for soya flour treatments, the highest was in SF-400 (111.2 µg/ml) and the lowest in SF-200 (91.25 µg/ml). On day-4, SP-400 recorded the highest protein content (124.3 µg/ml), and the lowest was in SP-200 (104.5 µg/ml); within soya flour treatments, the highest was observed in SF-400 (123.5 µg/ml), and the lowest in SF-200 (103.5 µg/ml). On day-5, the highest protein content among spirulina treatments was again found in SP-400 (132.2 µg/ml), while the lowest was in SP-200 (112.3 µg/ml); similarly, in soya flour batches, SF-400 had the highest value (131.3 µg/ml) and SF-200 the lowest (111.2 µg/ml).

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 (110.5 µg/ml), and the lowest in SP-200 (90.92 µg/ml); for soya flour treatments, the highest was in SF-400 (109.7 µg/mg), and the lowest in SF-200 (89.45 µg/ml). On day-4, SP-400 exhibited the highest protein level (122.9 µg/ml), while the lowest was observed in SP-200 (102.3 µg/ml); in soya flour treatments, SF-400 had the highest (121.6 µg/ml), and SF-200 the lowest (101.4 µg/ml). On day-5, the highest protein content among spirulina

treatments was found in SP-400 (130.3 µg/ml), with the lowest in SP-200 (110.1 µg/ml); in soya flour treatments, the highest was SF-400 (128.7 µg/ml) and the lowest was SF-200 (109.7 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 $FC_2 \times FC_1$ of the silkworm, *Bombyx mori* are presented in Table 1 to Table 3. 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 (47.29 g) was recorded in SP400 treatment in day-5 compared to day-4 and day-3, whereas the lowest value (41.08 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 (41.18 g) in SP400 in day-5 compared to day-4 and day-3; minimum of (33.41 g) in SP200 treatment in day-3 compared with day-4 and day-5 of 5th instar. The cocoon weight was highest (20.81 g) treated with SP400 on day-5 and lowest (16.06 g) in SP200 on day-3, while shell weight showed a maximum (4.233 g) for SP400 treatment in day-5 and a minimum (2.893 g) in SP200 treatment in day-3 compared to day-4 of 5th instar larvae. The filament length ranged from (1409 m) in SP400 in day-5 and (1171 m) SP200 of day-3; while in soya flour the highest matured larval weight (46.71 g) was recorded in SF400 treatment in day-5 compared to day-4 and day-3, whereas the lowest (41.72 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 (39.80 g) in SF400 in day-5 compared to day-4 and day-3, and a minimum of (32.33 g) in SF200 treatment in day-3 compared with day-4 and day-5 of 5th instar. The cocoon weight was highest (20.25 g) in SF400 on day-5 and lowest (15.92 g) in SP200 on day-3; the

shell weight was found to be maximum (4.140 g) in SF400 on day-5 and a minimum (2.960 g) in SF200 treatment in day-3 compared to day-4 and day-5. The filament length ranged from (1277 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 (50.23 g) was recorded in SP400 treatment in day-5 compared to day-4 and day-3, whereas the lowest value (43.65 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 (41.95 g) in SP400 in day-5 compared to day-4 and day-3; minimum of (35.36 g) in SP200 treatment in day-3 compared with day-4 and day-5 of 5th instar. The cocoon weight was highest (21.54 g) treated with SP400 on day-5 and lowest (16.86 g) in SP200 on day-3, while shell weight showed a maximum (4.640 g) for SP400 treatment in day-5 and a minimum (3.220 g) in SP200 treatment in day-3 compared to day-4 of 5th instar larvae. The filament length ranged from (1435 m) in SP400 in day-5 and (1182 m) SP200 of day-3; while in soya flour the highest matured larval weight (48.60 g) was recorded in SF400 treatment in day-5 compared to day-4 and day-3, whereas the lowest (43.54 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 (41.04 g) in SF400 in day-5 compared to day-4 and day-3, and a minimum of (33.52 g) in SF200 treatment in day-3 compared with day-4 and day-5 of 5th instar. The cocoon weight was highest (21.38 g) in SF400 on day-5 and lowest (16.87 g) in SP200 on day-3; the shell weight was found to be maximum (4.620 g) in SF400 on day-5 and a minimum (3.107 g) in SF200 treatment in day-3 compared to day-4 and day-5. The filament length ranged from (1365 m) in SF400 in day-5 and (1202 m) SP200 of day-3 of 5th instar.

During the post-monsoon season, the highest matured larval weight (48.06 g) was recorded in SP400 treatment in day-5 compared to day-4 and day-3, whereas the lowest value (42.53 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 (41.65 g) in SP400 in day-5 compared to day-4 and day-3; minimum of (35.33 g) in SP200 treatment in day-3 compared with day-4 and day-5 of 5th instar. The cocoon weight was highest (21.17 g) treated with SP400 on day-5 and

lowest (16.78 g) in SP200 on day-3, while shell weight showed a maximum (4.393 g) for SP400 treatment in day-5 and a minimum (3.100 g) in SP200 treatment in day-3 compared to day-4 of 5th instar larvae. The filament length ranged from (1425 m) in SP400 in day-5 and (1175 m) SP200 of day-3; while in soya flour the highest matured larval weight (47.43 g) was recorded in SF400 treatment in day-5 compared to day-4 and day-3, whereas the lowest (42.30 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 (40.82 g) in SF400 in day-5 compared to day-4 and day-3, and a minimum of (33.00 g) in SF200 treatment in day-3 compared with day-4 and day-5 of 5th instar. The cocoon weight was highest (20.94 g) in SF400 on day-5 and lowest (16.61 g) in SP200 on day-3; the shell weight was found to be maximum (4.330 g) in SF400 on day-5 and a minimum (3.020 g) in SF200 treatment in day-3 compared to day-4 and day-5. The filament length ranged from (1332 m) in SF400 in day-5 and (1183 m) SP200 of day-3 of 5th instar.

IV. DISCUSSION

The present study clearly demonstrated that fortification of mulberry leaves with protein-rich additives significantly enhances protein accumulation in both silk glands and haemolymph of the double hybrid $FC_2 \times FC_1$ of *Bombyx mori* L. The gradual increase in protein content from day-3 to day-5 of the fifth instar indicates intensified metabolic activity during the late larval stage, which is primarily associated with silk protein synthesis and glandular development.

Higher protein levels observed in the silk glands compared to haemolymph confirm that silk glands serve as the principal site for protein deposition and utilization. This is mainly due to the active synthesis of fibroin and sericin proteins during the later stages of larval growth, as supported by studies on dietary protein utilization and silk gland physiology in silkworms (Horie and Watanabe, 1983; Akai, 1984; Krishnaswami, 1978). Among the different treatments, spirulina supplementation, particularly at higher concentrations (400 ppm), resulted in significantly increased protein levels. This may be attributed to its superior nutritional profile, including essential amino acids, vitamins and growth-

promoting bioactive compounds that enhance digestion and metabolic efficiency. Similar improvements in larval growth and biochemical parameters under nutrient supplementation have been reported in the silkworms *Bombyx mori* L. (Venkataramana *et al.*, 2003; Krishnaswami, 1978, Ranjitha and Umakanth, 2025a, 2025b, 2026).

Soya flour treatments also showed a positive effect on protein accumulation, although the magnitude was comparatively lower than spirulina. As a plant-derived protein source, soya flour contributes to improved nutrient availability and supports larval growth and physiological functions. The beneficial effects of plant-based dietary supplements on silkworm performance have also been documented by Etebari and Matindoost (2005). The seasonal variation significantly influenced protein metabolism, with the monsoon season showing relatively higher protein content in both the silk glands and haemolymph. This could be due to favourable environmental factors such as optimal temperature and humidity, which enhance feeding activity and enzymatic processes. Similar observations were made by Benchamin and Jolly (1986), who reported that environmental conditions greatly affect silkworm growth and productivity.

The differential response observed in the $FC_2 \times FC_1$ double hybrid further indicates the importance of genetic constitution in nutrient utilization. Reciprocal crosses are known to exhibit variations due to maternal and cytoplasmic inheritance, which can influence metabolic pathways and physiological performance. The improved response of this hybrid to nutritional supplementation suggests its efficiency in converting dietary nutrients into silk proteins under enhanced feeding conditions. Overall, the results of the present investigation highlight the significant role of dietary fortification in improving protein metabolism and larval efficiency in silkworms. Enhanced protein accumulation in silk glands ultimately contributes to better silk production, and the findings emphasize the importance of evaluating double hybrid $FC_2 \times FC_1$ independently for optimizing sericultural practices.

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VI. CONCLUSION

The present study clearly demonstrates that dietary enrichment of mulberry leaves with protein-rich fortifications significantly enhances protein metabolism in the double hybrid $FC_2 \times FC_1$ of *Bombyx mori* L. A consistent increase in protein content was observed from day-3 to day-5 of the fifth instar larvae in both silk glands and haemolymph, but compared to haemolymph, silk glands exhibited higher protein content, indicating intensified metabolic activity by way of protein accumulation during the later stages of larval development. Among the treatments, spirulina fortification, particularly at higher concentration (SP-400), proved to be the most effective in improving protein accumulation, followed by soya flour treatments in all three concentrations. The superior performance of spirulina may be attributed to its rich nutritional composition and better assimilation efficiency.

The seasonal influence was also evident, with the monsoon season providing favourable conditions for enhanced protein synthesis compared to pre-monsoon and post-monsoon periods. The study further highlights that the double hybrid $FC_2 \times FC_1$ of mulberry silkworm responds effectively to nutritional fortification, suggesting improved nutrient utilization efficiency. These findings emphasize the importance of fortifications and hybrid evaluation in optimizing silkworm performance and silk productivity under varying environmental conditions. Overall, the results provide valuable insights for developing improved feeding strategies in sericulture, thereby contributing to enhanced cocoon quality and economic returns. If applied in large scale the silk content enhancement also helps in increased income to the farmers who practice sericulture for a better life quality.

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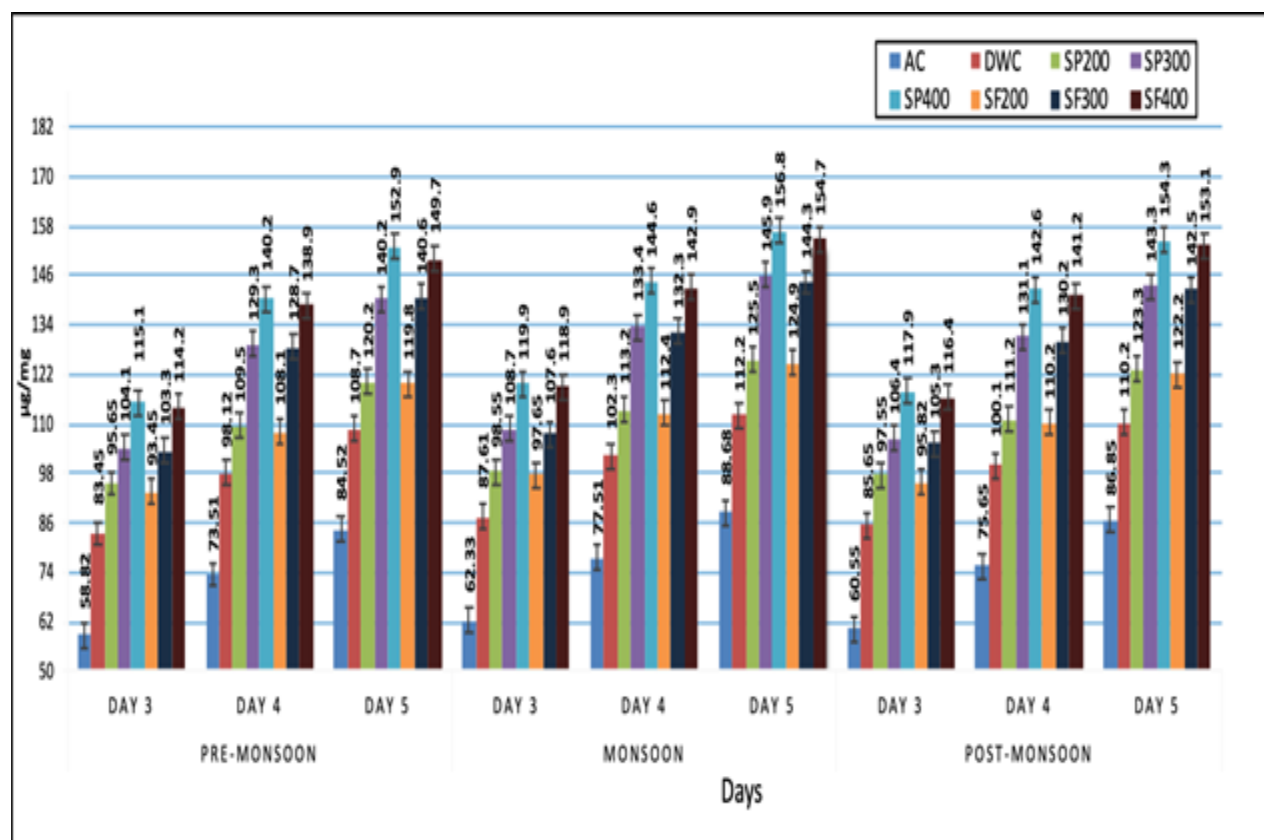


Figure 1: Effect of fortification at varied concentrations on protein content in the silk glands in double hybrid FC₂xFC₁ of the silkworm *Bombyx mori*.

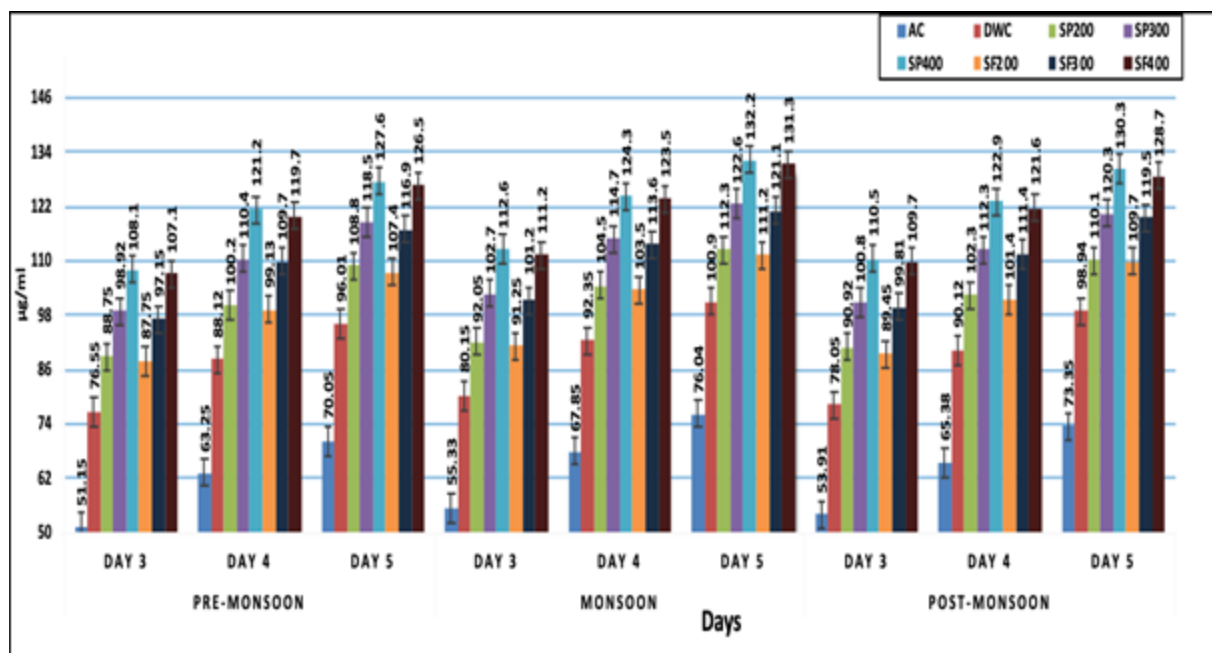


Figure 2: Effect of fortification at varied concentrations on protein content in the haemolymph in double hybrid FC₂xFC₁ of the silkworm *Bombyx mori*.

PRE-MONSOON SEASON										
Treatments	Days of fifth instar	Larval duration (h)	Matured larval weight (g) (10 larvae)	Larval weight (g)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)	Filament length (m)	Denier (d)	Renditta (kgs.)
AC	3	543.0±1.732	39.34±0.223	30.01±0.508	12.38±0.194	1.827±0.047	14.76±0.617	1047±3.000	2.524±0.008	8.683±0.363
	4		40.12±0.160	31.00±0.577	13.33±0.406	2.013±0.013	15.12±0.458	1077±22.13	2.486±0.008	8.977±0.631
	5		41.53±0.233	31.10±0.810	14.33±0.240	2.200±0.115	15.33±0.563	1072±28.97	2.442±0.006	5.927±0.139
DWC	3		40.52±0.263	31.35±0.324	14.59±0.068	2.273±0.037	15.57±0.191	1131±1.647	2.492±0.006	5.460±0.087
	4		41.63±0.217	31.72±0.162	15.53±0.291	2.453±0.053	15.81±0.636	1134±3.340	2.465±0.008	5.780±0.150
	5		42.47±0.241	31.72±0.175	15.93±0.176	2.733±0.067	17.16±0.494	1142±17.88	2.376±0.008	6.227±0.158
SP200	3		41.08±0.191	33.41±0.301	16.06±0.176	2.893±0.134	18.00±0.927	1171±36.00	2.235±0.008	5.557±0.129
	4		42.35±0.229	34.35±0.353	16.60±0.200	3.287±0.059	19.79±0.244	1188±7.130	2.298±0.007	5.727±0.134
	5		43.27±0.233	36.33±0.333	18.40±0.503	3.467±0.240	18.82±1.094	1147±4.176	2.131±0.006	6.747±0.511
SP300	3		42.56±0.230	35.00±0.577	16.96±0.057	3.387±0.013	19.96±0.080	1195±8.775	2.163±0.007	5.603±0.059
	4		43.26±0.236	35.30±0.351	17.00±0.115	3.433±0.033	20.19±0.104	1197±7.477	2.148±0.007	5.380±0.101
	5		45.36±0.	37.98±0.641	19.47±0.	3.613±0.	18.55±0.	1219±13	1.964±0.	6.427±0.

			318		068	013	077	.57	008	114
SP400	3		44.83±0.334	38.99±0.179	19.71±0.669	4.020±0.130	20.39±0.069	1350±29.80	1.943±0.007	5.350±0.295
	4		45.61±0.258	41.08±0.696	19.86±0.481	4.067±0.067	20.47±0.188	1374±18.42	2.122±0.006	5.023±0.050
	5		47.29±0.346	41.18±0.261	20.81±0.477	4.233±0.289	19.79±0.203	1409±46.14	2.032±0.006	5.343±0.143
SF200	3		41.72±0.211	32.33±0.882	15.92±0.073	2.960±0.060	18.58±0.339	1176±7.191	2.279±0.008	5.633±0.003
	4		42.26±0.182	33.68±0.942	16.51±0.162	3.080±0.023	18.65±0.129	1186±5.560	2.374±0.008	5.833±0.078
	5		43.66±0.296	35.00±0.577	17.75±0.101	3.127±0.463	17.63±2.672	1238±38.44	2.142±0.006	6.603±0.215
SF300	3		42.63±0.328	33.66±0.333	16.06±0.521	3.060±0.087	18.92±1.736	1178±1.662	2.231±0.006	5.497±0.235
	4		43.27±0.235	35.44±0.260	16.56±0.186	3.260±0.031	19.68±0.359	1178±12.16	2.196±0.007	5.620±0.638
	5		44.68±0.289	36.08±0.087	18.21±0.014	3.367±0.203	18.48±1.104	1210±8.177	2.152±0.006	6.360±0.140
SF400	3		44.41±0.258	37.66±0.333	19.04±0.579	3.767±0.064	19.83±0.906	1267±7.533	2.127±0.006	5.440±0.193
	4		45.23±0.217	39.53±0.233	19.82±0.072	3.960±0.023	19.98±0.081	1320±4.793	2.136±0.007	5.427±0.046
	5		46.71±0.377	39.80±0.157	20.25±0.392	4.140±0.301	19.21±0.148	1277±1.984	2.056±0.006	5.883±0.185

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

MONSOON-SEASON										
Treatments	Days of fifth-instar	Larval duration (h)	Matured larval weight (g) (10-larvae)	Larval weight (g)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)	Filament length (m)	Denier (d)	Renditta (kgs.)
AC	3	519.00±4.619	41.34±0.234	31.27±0.151	14.08±0.107	2.027±0.048	14.39±0.375	1102±10.80	2.551±0.007	8.657±0.354
	4		42.58±0.262	31.72±0.175	14.20±0.187	2.193±0.024	15.44±0.289	1147±9.863	2.632±0.006	8.173±0.472
	5		43.61±0.344	32.32±0.122	14.90±0.042	2.347±0.081	15.74±0.534	1154±2.110	2.665±0.008	7.570±0.030
DWC	3		42.78±0.244	33.43±0.229	15.20±0.023	2.487±0.044	16.36±0.306	1140±3.437	2.484±0.007	5.417±0.054
	4		43.56±0.260	33.61±0.256	15.82±0.266	2.727±0.029	17.24±0.393	1144±4.792	2.615±0.008	5.373±0.064
	5		44.70±0.252	34.44±0.249	16.34±0.076	2.960±0.061	18.11±0.357	1155±4.230	2.662±0.007	5.360±0.040
SP200	3		43.65±0.237	35.36±0.188	16.86±0.093	3.220±0.121	19.08±0.621	1182±34.59	2.426±0.006	5.373±0.052
	4		44.71±0.320	35.52±0.238	17.08±0.115	3.593±0.041	21.04±0.219	1200±6.508	2.473±0.007	5.167±0.013
	5		45.70±0.361	36.64±0.117	18.71±0.424	3.820±0.072	20.41±0.112	1212±12.57	2.421±0.006	5.680±0.170
SP300	3		44.36±0.260	37.84±0.195	17.27±0.048	3.593±0.024	20.80±0.148	1215±10.80	2.296±0.006	5.080±0.031
	4		45.13±0.318	38.34±0.416	17.68±0.141	3.600±0.012	20.36±0.219	1215±7.697	2.292±0.007	5.050±0.050
	5		47.43±0.348	38.84±0.395	20.08±0.200	4.120±0.012	20.52±0.150	1261±14.64	2.289±0.008	5.530±0.050
SP400	3		46.61±0.320	40.69±0.369	21.08±0.104	4.413±0.058	20.93±0.230	1379±22.09	2.031±0.006	5.537±0.027
	4		47.42±0.347	41.10±0.248	21.31±0.070	4.487±0.018	21.05±0.057	1433±9.649	2.042±0.006	5.117±0.101
	5		50.23±0.470	41.95±0.277	21.54±0.110	4.640±0.136	20.55±0.036	1435±7.149	2.161±0.006	5.070±0.020
SF200	3		43.54±0.352	33.52±0.116	16.87±0.116	3.107±0.048	18.41±0.405	1202±10.91	2.518±0.007	5.507±0.101
	4		44.63±0.291	35.27±0.440	16.94±0.207	3.327±0.070	19.64±0.603	1201±5.219	2.591±0.006	5.553±0.203
	5		46.70±0.321	36.12±0.502	18.01±0.044	3.520±0.080	19.54±0.427	1242±2.646	2.492±0.006	5.700±0.080
SF300	3		44.57±0.261	34.18±0.140	17.32±0.082	3.513±0.047	20.20±0.378	1206±8.073	2.396±0.006	5.347±0.073
	4		45.42±0.289	36.20±0.334	17.58±0.171	3.540±0.020	20.46±0.283	1216±14.87	2.481±0.006	5.187±0.032
	5		47.50±0.346	36.33±0.354	18.38±0.046	3.747±0.188	19.40±0.177	1239±1.890	2.445±0.008	5.510±0.040
SF400	3		45.76±0.295	39.25±0.154	20.66±0.110	4.227±0.114	20.45±0.541	1303±17.38	2.214±0.007	5.597±0.067
	4		47.73±0.410	40.75±0.311	20.79±0.210	4.460±0.053	21.45±0.307	1366±9.922	2.316±0.006	5.207±0.054
	5		48.60±0.351	41.04±0.468	21.38±0.193	4.620±0.180	20.62±0.318	1365±24.08	2.372±0.006	5.290±0.010

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

POST-MONSOON SEASON										
Treatments	Days of fifth-instar	Larval duration (h)	Matured larval weight (g) (10 ³ larvae)	Larval weight (g)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)	Filament length (m)	Denier (d)	Renditta (kgs.)
AC	3	591.0±1.155	40.26±0.328	30.33±0.333	13.83±0.191	1.993±0.047	14.41±0.369	1059±5.532	2.538±0.007	9.103±0.432
	4		41.40±0.322	31.40±0.476	14.08±0.298	2.113±0.024	15.02±0.407	1102±14.98	2.557±0.006	8.803±0.503
	5		42.70±0.321	32.02±0.041	14.71±0.047	2.287±0.075	15.53±0.479	1119±9.705	2.571±0.006	7.837±0.203
DWC	3		41.40±0.265	32.66±0.667	15.02±0.026	2.433±0.037	16.19±0.274	1134±1.299	2.467±0.006	5.463±0.063
	4		42.83±0.406	33.21±0.435	15.76±0.260	2.600±0.023	16.51±0.419	1140±4.332	2.552±0.006	5.603±0.100
	5		43.53±0.328	33.94±0.107	16.20±0.046	2.873±0.105	17.73±0.619	1141±2.643	2.539±0.008	5.407±0.117
SP200	3		42.53±0.318	35.33±0.333	16.78±0.090	3.100±0.076	18.47±0.364	1175±35.55	2.362±0.006	5.657±0.050
	4		43.76±0.353	35.45±0.277	16.88±0.116	3.413±0.077	20.21±0.494	1194±6.311	2.411±0.007	5.530±0.038
	5		44.90±0.346	35.67±0.393	18.66±0.430	3.773±0.044	20.23±0.231	1204±16.50	2.376±0.006	5.697±0.223
SP300	3		43.83±0.406	36.66±0.333	17.00±0.040	3.507±0.027	20.62±0.187	1200±9.750	2.274±0.008	5.243±0.030
	4		46.30±0.379	37.66±0.333	17.24±0.105	3.540±0.031	20.52±0.300	1204±6.250	2.208±0.007	5.177±0.090
	5		44.53±0.348	38.67±0.485	19.91±0.024	4.053±0.035	20.35±0.063	1222±29.62	2.241±0.006	5.847±0.038
SP400	3		44.40±0.322	40.34±0.329	20.89±0.127	4.313±0.029	20.64±0.043	1353±30.85	1.966±0.009	5.620±0.085
	4		46.83±0.406	40.66±0.667	20.96±0.070	4.347±0.029	20.73±0.206	1421±9.472	2.054±0.008	5.193±0.092
	5		48.06±0.348	41.65±0.379	21.17±0.234	4.393±0.024	20.75±0.311	1425±7.153	2.168±0.007	5.153±0.108
SF200	3		42.30±0.265	33.00±0.577	16.61±0.127	3.020±0.050	18.18±0.418	1183±5.053	2.423±0.007	5.567±0.098
	4		43.53±0.328	35.32±0.166	16.69±0.128	3.193±0.024	19.13±0.166	1192±5.685	2.491±0.006	5.710±0.126
	5		44.73±0.328	35.65±0.460	17.89±0.079	3.433±0.052	19.19±0.289	1213±6.180	2.512±0.006	5.92±0.079
SF300	3		43.70±0.361	33.60±0.346	16.72±0.068	3.373±0.055	20.02±0.326	1188±2.250	2.278±0.007	5.403±0.079
	4		44.10±0.404	34.38±0.202	16.85±0.058	3.400±0.050	21.18±0.345	1176±7.387	2.302±0.006	5.423±0.482
	5		45.86±0.376	35.74±0.224	18.29±0.035	3.663±0.058	19.06±0.167	1222±7.474	2.452±0.006	5.673±0.105
SF400	3		45.40±0.265	38.00±0.529	20.21±0.213	4.080±0.101	20.19±0.615	1274±7.902	2.178±0.007	5.643±0.063
	4		46.46±0.410	39.40±0.299	20.42±0.031	4.193±0.098	20.53±0.487	1323±6.538	2.221±0.006	5.440±0.062
	5		47.43±0.348	40.82±0.605	20.94±0.140	4.330±0.171	20.02±0.489	1332±3.815	2.366±0.006	5.573±0.058

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