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Influence of protein fortification on larval growth parameters of silkworm, *Bombyx mori* L.

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Abstract
Fortification of silkworm host plant leaves is a technique of recent application in sericulture research to increase its economic value. In the present investigation, the silkworm bivoltine double hybrid $FC_1 \times FC_2$ [$(CSR_6 \times CSR_{26}) \times (CSR_2 \times CSR_{27})$] was reared on mulberry leaves fortified with proteins namely drone brood, protinex and their combinations to study its effects on larval growth parameters and on economic growth parameter called Gland-Body Ratio. Protinex (10%) concentration caused the significant influence on larval body length, larval body weight and larval body perimeter of silkworm in terms of Overall Growth Rate by 46.56, 295 and 48.54 per cent with Compound Periodical Growth Rate of 6.32, 24.58 and 6.52 per cent, respectively. Moreover, in protinex (10%) and drone brood (6%) concentrations the impact on Gland-Body Ratio was found more pronounced when compared to other concentrations.

Keywords: Fortification, silkworm, protinex, drone brood

1. Introduction

Sericulture is an age old, agro-based, labour oriented, low investment cottage industry, the end product of which is silk. Silk is the most graceful textile in the world with unmatched splendour, natural shine and innate affinity for dyes, high absorbance, light weight, soft feel and high resilience and recognized as the “Queen of Textiles” over the world. Silk is a proteinaceous fibre produced by spinning of the larvae of silkworm to provide a protective casing to the delicate silkworm body during its pupal stage. Our country is the second-largest producer and the largest buyer of silk within the world. India has the unique distinction of being the sole country manufacturing all the five recognized profitable varieties of silk namely domesticated mulberry silk, semi-domesticated eri silk, wild tropical tasar silk, wild oak tasar silk and exclusive muga silk, the wild golden silk being exceptional and privilege of India. The entire production of raw silk in India was 34,903 MT in 2021-22 of which, mulberry raw silk production aggregated to 25,818 MT, tasar silk 1,466 T, eri silk 7,364 MT and muga silk 255 MT, respectively (Anonymous, 2022) [1]. Being the second-largest silk manufacturing country within the world next to China, India provides employment to over 8.8 million individuals within the whole silk production value chain from farm to fabric. Though, India ranks second next solely to China in raw silk production the manufacture of raw silk is not enough to fulfill the domestic demand owing to low output coupled with low-grade silk quality.

Fortification, a modern technique in advanced sericulture research and development industry is being used to improve the cocoon yield and silk content through supplement of different nutrients on mulberry leaf. Silkworm, *B. mori* requires sugars, amino acids, proteins and vitamins for its normal growth, survival and for the growth of silk gland (Sengupta *et al.*, 1972) [13]. Among these, one of the most vital is the amount of protein content in the mulberry leaves which influences the silk production. The formation of silk proteins throughout growth of the silkworm larvae was studied by Fukuda *et al.* in 1959 [4] wherein, they found that about 70 per cent of the silk protein produced by *B. mori* was taken directly from the mulberry leaves on which they were fed. It was also found that about 72-86 per cent of the amino acids and 60 per cent of the absorbed amino acids used for silk production are obtained by the silkworm larvae from the mulberry leaf (Lu and Jiang, 1988) [10]. It is predicted that the dietary amount of protein on mulberry leaves is about 30 per cent (Hamano and Okano, 1989) [5]. The nutritional composition of mulberry leaves does not always fulfil the nutritional protein requirement of the silkworm. The lower percentages of protein within the mulberry leaves may lead to lower quantity of cocoons leading to lower productivity (Takahashi *et al.*, 2001) [17]. Therefore, number of artificial foods such as royal jelly, honey, dietary proteins, amino acids, vitamins etc. can be supplied by the recent technique of fortification to silkworms as they have the capability to convert plant proteins to silk proteins, also helps in improving the qualities of silk fibre playing a vital role for yield enhancement in sericulture industry (Borgohain, 2015) [2].

2. Methodology: The present investigation was conducted at Sericulture Research Laboratory of Division of Sericulture, Sher-e-Kashmir University of Agricultural Science and Technology of Jammu, Main Campus, Chatha during spring season of 2021-22. For the experiment, the test insect, bivoltine double hybrid $FC_1 \times FC_2$ $[(CSR_6 \times CSR_{26}) \times (CSR_2 \times CSR_{27})]$ silkworm, *B. mori* was used. The silkworm eggs were procured from the Sericulture Development Department of Jammu and Kashmir Union Territory and then incubated at ambient temperature of 25 ± 1˚ C and relative humidity of 80 ± 5 per cent until hatching. The newly hatched worms were then transferred to rearing beds and reared as per the rearing method suggested by Krishnaswami (1978) [9].

Drone brood and protinex powder were used for the present investigation as the feed supplementation proteins. Drone brood was collected from the Apiary maintained by Division of Entomology of SKUAST-Jammu. However, protinex powder was procured from the nearby market. Mass rearing of silkworm larvae was done till 3rd moult. After 3rd moult, the silkworm larvae were divided into nine treatments including control. Each treatment replicated thrice and per replication 60 worms were reared. The larvae were fed with mulberry leaves three times a day *viz.*, 8 AM, 2 PM and 8 PM. From the stock solutions of drone brood and protinex proteins, required concentrations were prepared by using distilled water. The fresh mulberry leaves were soaked in each concentration for 15 minutes and then shade dried for about 15 minutes. The mulberry leaves fortified with different concentrations of different proteins were then fed to 5th instar silkworm larvae, once a day from the beginning of the 5th instar till spinning stage. Silkworm larvae in control batch were fed on fresh mulberry leaves dipped in distilled water.

2.1 Larval growth parameters: Data was collected on three larval growth parameters namely larval body length, body weight and body perimeter during 5th instar larval developmental period and on economic growth parameter called Gland-Body Ratio (GBR). The instar-wise and day-wise growth trends were analysed respectively by Overall Growth Rate (OGR) and Compound Periodical Growth Rate (CPGR).

2.1.1 Larval body weight (g)
The mean body weight was determined by measuring the weight of 20 randomly selected larvae in an electronic balance (AG135 METTLER TOLEDO) during first and last day as initial and final weight of 5th larval instars. The body weight was expressed in grams.

2.1.2 Body length (cm)
The mean body length was determined by measuring the length of 20 randomly selected silkworm larvae by using Vernier Caliper, during first and last day as initial and final body length during 5th larval instars. The body length was expressed in centimeters.

2.1.3 Body perimeter (cm)
The mean body perimeter was determined by measuring the perimeter (the whole outer boundary) of 20 randomly selected silkworm larvae by using Vernier Caliper, during first and last day as initial and final body perimeter during 5th larval instars. The body perimeter was expressed in centimeters.

2.1.4 Gland-Body Ratio (%)
The growth of the silk gland in relation to the body growth was determined by an economic growth parameter called Gland-Body Ratio and the same was computed by dividing the silk gland weight by larval body weight. During fifth day of 5th instar from each replication of each treatment including control, 10 silkworm larvae were randomly selected, weighted and dissected to measure the weight of the silk glands isolated from the same larvae. The mean weight of silk gland and larval body was expressed in grams.

2.1.5 Overall Growth Rate (%)
The Overall Growth Rate (OGR) represents the instar-wise growth trend in silkworm larvae. It was computed by using the following formula:

$$\text{OGR} = \frac{\text{Final value} - \text{Inital value}}{\text{Initial value}} \times 100$$

2.1.6 Compound Periodical Growth Rate (%)
The Compound Periodical Growth Rate (CPGR) represents the day-wise growth trend in silkworm larvae and was calculated by using the following formula:

$$\text{CPGR} = (\text{End value}/\text{Start value})^{1/n-1} - 1$$

The end value refers to growth after specified period, the start value to growth at the beginning of the experiment and 'n' to the time component which is split into number of days. The value so obtained is then expressed in percentage form by multiplying it with 100 (Sivaprasad, 2012) [14].

3. Results
3.1 Effect of mulberry leaves fortified with different proteins on silkworm larval growth parameters
3.1.1 Larval body length:
The Overall Growth Rate (OGR) of larval body length ranged from 42.18 to 46.56 per cent. Notably, in protinex (10%) concentration a significant elevation in OGR by 46.56 per cent was recorded, representing an additional elevation of 4.38 per cent over control with CPGR of 6.32 per cent. However, the drone brood (6%) and drone brood (5%) + protinex (5%) concentrations recorded OGR for larval body length to the tune of 45.93 and 45.45 with CPGR of 6.21 and 6.16 per cent, respectively. However, drone brood (2%), protinex (6%), protinex (2%), drone brood (3%) + protinex (3%), drone brood (1%) + protinex (1%) and control were found statistically at par with each other with respect to larval body length (Table 1).

3.1.2 Larval body weight:
The Overall Growth Rate (OGR) of larval body weight ranged from 265 to 295 per cent. The mulberry leaves fortified with protinex (10%) and drone brood (5%) + protinex (5%) concentration exhibited significant influence on body weight of silkworm larvae having OGR of 295 and 294 per cent with CPGR of 24.58 and 24.54 per cent, respectively. However, drone brood (6%), protinex (6%), drone brood (2%), drone brood (3%) + protinex (3%), drone brood (1%) +

protinex (1%) and protinex (2%) and concentrations also showed significant increase in larval body weight in terms of OGR and CPGR by 288, 286, 286, 282, 282 and 280; 24.26, 24.09, 24.03, 23.93, 23.80 and 23.81 per cent, respectively. Whereas the larval body weight of the control batch showed an OGR of 265 per cent with CPGR of 23.04 per cent (Table 1).

3.1.3 Larval body perimeter
The Overall Growth Rate (OGR) for larval body perimeter was recorded maximum (48.54%) at protinex 10 per cent concentration with CPGR of 6.52 per cent. However, in drone brood (6%), drone brood (5%) + protinex (5%), protinex (6%), drone brood (2%), protinex (2%) and drone brood (3%) + protinex (3%) concentrations the OGR for larval body perimeter were recorded to the tune of 47.43, 47.08, 46.07, 45.67, 45.66 and 44.60 per cent with CPGR of 6.41, 6.36, 6.25, 6.20, 6.20 and 6.06 per cent, respectively. Whereas the body perimeter of silkworm larvae fed on mulberry leaves fortified with drone brood (1%) + protinex (1%) and control batch were found statistically at par with each other having OGR of 42.38 and 41.07 per cent, respectively (Table 1)

Table 1: Effect of mulberry leaves fortified with different concentrations of proteins on larval growth parameters of silkworm, *B. mori*

Fortification of mulberry leaves with	Growth parameters											
	Larval length (cm)		OGR (%)	CPGR (%)	Larval weight (g)		OGR (%)	CPGR (%)	Larval perimeter (cm)		OGR (%)	CPGR (%)
	Initial	Final			Initial	Final			Initial	Final		
Drone brood (6%)	4.92±0.01	7.18±0.01^{bcd}	45.93	6.21	1.19±0.00	4.62±0.01^{ef}	288	24.26	9.93±0.01	14.64±0.03^{de}	47.43	6.41
Drone brood (2%)	4.91±0.01	7.04±0.02^{abc}	43.38	5.92	1.17±0.01	4.52±0.00^{cd}	286	24.03	9.92±0.02	14.45±0.02^{bcd}	45.67	6.20
Protinex (10%)	4.94±0.01	7.24±0.04^{d}	46.56	6.32	1.19±0.02	4.70±0.01^{f}	295	24.58	9.93±0.00	14.75±0.02^{e}	48.54	6.52
Protinex (6%)	4.92±0.00	7.07±0.03^{abcd}	43.70	5.97	1.18±0.01	4.56±0.01^{de}	286	24.09	9.92±0.00	14.49±0.02^{bcd}	46.07	6.25
Protinex (2%)	4.91±0.00	7.02±0.01^{abc}	42.97	5.90	1.17±0.01	4.45±0.03^{bc}	280	23.81	9.90±0.01	14.42±0.03^{bc}	45.66	6.20
Drone brood (5%) + Protinex (5%)	4.95±0.02	7.20±0.07^{cd}	45.45	6.16	1.19±0.01	4.69±0.00^{f}	294	24.54	9.94±0.01	14.62±0.01^{cde}	47.08	6.36
Drone brood (3%) + Protinex (3%)	4.92±0.01	7.07±0.01^{abcd}	43.70	5.98	1.18±0.01	4.51±0.02^{bcd}	282	23.93	9.91±0.02	14.33±0.03^{b}	44.60	6.06
Drone brood (1%) + Protinex (1%)	4.91±0.03	7.01±0.00^{ab}	42.77	5.87	1.16±0.00	4.43±0.01^{b}	282	23.80	9.91±0.02	14.11±0.08^{a}	42.38	5.81
Control	4.86±0.01	6.91±0.04^{a}	42.18	5.80	1.15±0.02	4.20±0.01^{a}	265	23.04	9.86±0.02	13.91±0.04^{a}	41.07	5.66

Each value is mean ± standard error of three replications

Figures followed by same letter in column are non-significant by Tukey's HSD test

3.2 Effect of mulberry leaves fortified with different concentrations of proteins on Gland-Body Ratio (%) of silkworm
The Gland-Body Ratio of silkworm recorded positive growth trends under the impact of mulberry leaves fortified with different concentrations of proteins. In protinex (10%) and drone brood (6%) concentrations the impact on Gland-Body Ratio was found more pronounced when compared to other concentrations. However, the highest Gland-Body Ratio (32.91%) was recorded for protinex at 10 per cent concentration which was found statistically at par with drone brood (6%) concentration with 32.84 per cent of Gland-Body Ratio. While for drone brood (5%) + protinex (5%), drone brood (2%), protinex (2%), protinex (6%), drone brood (3%) + protinex (3%) and drone brood (1%) + protinex (1%) it was 32.05, 31.83, 30.63, 30.58, 29.65 and 28.65 per cent, respectively. However, the least Gland-Body Ratio of 22.07 per cent was observed in control batch (Fig. 1).

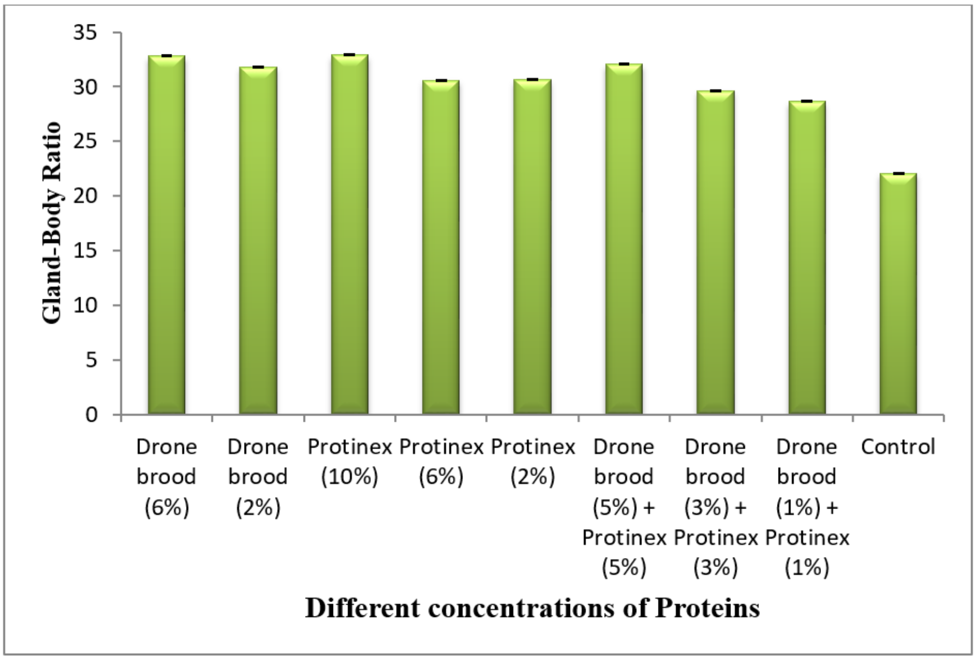

Fig 1: Effect of protein fortification on Gland-Body Ratio of silkworm, *Bombyx mori*

4. Discussion

The larval growth parameters of silkworm larvae were found greatly influenced when fortified mulberry leaves with proteins were fed to silkworm. Our findings are in line with Thulasi and Sivaprasad (2015) [16], who reported that the 5 per cent of alfalfa fortified mulberry diet the OGR of 5^{th} instar silkworm larvae were elevated by 309 with CPGR of 25.53, showing an overall increase of 6 per cent over control. Similarly, Madhavi *et al*. (2018) [11] also reported that honey at 2 per cent concentration elicited maximal response in all larval parameters. The OGR of body weight, length and perimeter grew in addition by 8.3, 5.0 and 9.0 per cent in 5^{th} instar at honey (2%) when compared to control. Likewise, CPGR of body weight, length and perimeter grew additionally by 0.46, 0.55 and 0.69 per cent in 5^{th} instar when compared to control batch. Likewise, Sivaprasad and Thulasi (2014) [15] also reported that honey at 2 per cent leads to optimum response in body growth of silkworm. Whereas, Sivaprasad and Thulasi (2015) [16] found that at 1 per cent of nutrilite the 5^{th} instar larval body weight recorded an OGR of 266 per cent with a CPGR of 24.14 per cent whereas in control batch the OGR was 206 per cent with CPGR of 20.52 per cent, respectively.

The results demonstrated that the Gland-Body Ratio (GBR) of silkworm recorded positive growth trends under the impact of mulberry leaves fortified with different concentrations of proteins. Silk glands attain maximum growth towards the end of the fifth instar owing to fibroin synthesis and it is obvious that the silk gland weight is one of the most important parameters for assessing the silk production potential of the larvae. In silkworms, silk fibroin is derived mainly from four amino acids *i.e.,* alanine, serine, glycine and tyrosine which come from their dietary source of protein and amino acids (Kaplan and McGrath, 1997) [7]. The formation of silk proteins throughout growth of the silkworm larvae was studied by Fukuda *et al.* in 1959 [4] and they found that 70 per cent of the silk protein produced by *B. mori* was directly taken from mulberry leaves. About 72-86 per cent of the amino acids and 60 per cent of the absorbed amino acids used for silk production were obtained by the silkworm larvae from the mulberry leaf (Lu and Jiang, 1988) [10]. The possible reason for the increased GBR of silkworms in the present investigation may be due to availability of optimum proteins in fortified mulberry leaves that were fed to silkworm larvae during 5^{th} instar development period. Results of the present study are also in confirmation with the findings of Madhavi *et al.* (2018) [11], who reported that the impact of honey at 2 per cent was more pronounced when compared to other concentrations including control. The 2 per cent honey fortified mulberry leaves yielded higher returns in Gland-Body ratio.

5. Conclusion

Protein is one of the most important constituents of the leaves of the host plant of silkworm. Especially, silk substance consists of proteins. Consequently, protein must be given to silkworms paying a special attention to the ratio of protein in the host plants. Food additives produced from different sources of protein, raise the protein content of the larvae of silkworm which affect the natural silk stored in the silk glands, which in turn leads to the higher production of silk thread and reduces breeder effort for providing larvae with mulberry leaves. The effect of mulberry leaves enriched with different proteins on the larval growth parameters of *Bombyx mori* L, has been studied in this investigation. At protinex 10 per cent concentration silkworm larvae recorded significant effect on silkworm larval growth parameters and on Gland-Body Ratio.

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