Original Research Article

Effect of Different Feeding Frequencies on the Commercial Characters of Silkworm (*Bombyx mori* L.)

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**Abstract**

Feeding is the most important factor in silkworm rearing, as it has a direct impact on growth and development of worms. Silkworm requires feed five times a day at an interval of five hours. However, the rearers of J&K state feed the worms two times only, resulting in lower cocoon yield. Feeding of quality mulberry leaves has a great impact on biology and physiology of silkworm *Bombyx mori* L. The study revealed 3 feedings per day was statistically at par with 4 feedings in terms of various parameters including larval survival percentage i.e. 93.50 ± 0.76 for 3 feedings and 95.00 ± 0.57 for 4 feedings, larval weight 40.00 ± 2.00 for 3 feedings and 40.66 ± 1.45 for 4 feedings, cocoon yield 14.10 ± 0.38 by wt. and 9183.30 ± 44.09 by no. for 3 feedings and 14.82±0.38 by wt. and 9366.70 ± 60.09, single cocoon wt. 1.54 ± 0.02 g for 3 feeds and 1.61 ± 0.0 for 4 feeds, single shell weight was recorded as 0.32± 0.00 and 0.33± 0.00 for 3 and 4 feeds respectively. Shell ratio percentage was recorded as 20.66 ± 0.26 and 20.95 ± 0.11 respectively for 3 and 4 feeds. However, total filament lengths 721.0 m and 765.3 m, non-breakable filament lengths 680.6 m and 765.3 m and filament size 3.0 d and 2.73 d for 3 and 4 feeds respectively. From this experiment it can safely be concluded that three feedings are obligatory for obtaining successful cocoon crop at field level.

**Keywords**

*Bombyx mori* L., Bivoltine, Breeds, Feeding, commercial traits, feeding frequency

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**Introduction**

The silkworm, *Bombyx mori*, is a monophagous lepidopteran insect which has been domesticated for more than five thousand years. The mulberry leaves mainly constitute proteins, carbohydrates, vitamins, sterols, phagostimulants and minerals. Such nutritional requirement in food consumption has direct impact on the all genetic characters like cocoon weight, quantity of silk produced, pupation, reproductive traits and quality (Ramesha et al., 2010). Mulberry leaves are suitable as food for silkworms, as they contain several chemical constituents such as water (80%), proteins (27%) carbohydrates (11%), mineral matters, vitamins and other extracts etc. As the leaves contain morin protein, they can easily eaten by the silkworms (Koul, 1989). Mulberry leaves are used as feed due to favourable physical features such as suitable tenderness, thickness and tightness.
In sericulture, feeding is a factor of paramount importance having a direct impact on growth, development, and silk yield on one side and the cost involvement of silkworm rearing on the other. Food intake and silk production in silkworms are very closely related to nutritional factors. Dietary efficiency of silkworms plays a major role in converting mulberry leaves consumed to silk. Ingestion of same amount of mulberry leaves under different environmental and feeding regimes shows significant differences in digestion, absorption, and conversion of the ingested amount of food to body matter. An optimum feeding frequency satisfies the appetite of the worms for their uniform and healthy growth besides ingestion and digestion which in turns get reflected by the commercial characters of the cocoon crop. The insufficient feeding in the fifth larval instar causes production of small sized cocoon with less number of eggs, owing to sequential disease in the yolk protein content of the oocyte (Kawaguchi et al., 1996). Legay (1958) has stated that silk production is dependent on the larval nutrition and nutritive value of mulberry leaves plays a very effective role in producing good quality cocoon. The present study was proposed to work out the frequency of feed required for obligatory and facultative phases to obtain quality cocoon production.

**Materials and Methods**

The present study was carried out at Division of Sericulture, Udheywalla, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu during spring-2017. The present experiment comprises of four treatments viz., single feeding (24 hrs gap), 2 feedings (12 hrs gap), 3 feedings (8 hrs gap) and 4 feedings (6 hrs gap). The experiment was laid out in Completely Randomized Block Design (CRD) with four replications. The seed of silkworm hybrid combination (CSR2 × CSR4) was procured from RSRS, Dehradun during spring-2017 and three disease free layings of hybrid were incubated for each treatment and reared as per the standard techniques of Dandin et al., (2003). The worms of the three disease free layings were brushed enmass and reared upto third moult. After third moult, the worms were reared in three replicates, taking a population size of 200 worms per replicate at random for each treatment. Ripe worms were picked for Seri-positioning and spinning was conducted on collapsible plastic mountages. The cocoons were harvested on sixth day after mounting. Cocoon samples were taken and stifled in hot air oven at 90°C-60°C for six hours for reeling purpose. Randomly selected cocoon samples were reeled for post cocoon parameters at Demonstration cum Technical Centre of Central Silk Board, Miran Sahib, Jammu. The observations were recorded for different characters at egg, larval, cocoon and post-cocoon stage.

**Egg stage**

Following observations were made for different characters of this stage:

**Fecundity**

It is the total number of eggs laid by a single mother moth and was calculated by counting the total number of eggs laid by the female moth. Average of three layings in each replicate was recorded for analysis purpose.

**Hatching percentage**

It is the number of larvae hatched out from total eggs laid by a mother moth and was recorded as an average of three layings in each replicate. It was calculated by the following formula:
Brushing percentage

It is the total number of larvae brushed out from each disease free laying for each replicate and was recorded in percentage.

It was calculated by the following formula:

\[
\text{Brushing percentage} = \frac{\text{Number of larvae brushed}}{\text{Total number of eggs per laying}} \times 100
\]

Larval stage

Following observations were made for different parameters at larval stage:

**IV age larval duration**

It is the duration of larvae from III moult out upto initiation of IV moult and was recorded in days and hours for each replicate.

**V age larval duration**

It is the duration of larvae from IV moult out upto pre-spinning and was recorded in days and hours for each replicate.

**Total larval life**

It was recorded as an average of total larval life in days and hours from brushing to pre-spinning stage including moultling duration in each instar of each replicate.

**Weight of 10 mature larvae (g)**

Ten mature larvae were picked randomly from each replicate from 4 to 6 day of fifth instar and weighed using digital balance.

The maximum larval weight was recorded in each replicate.

**Larval survival percentage**

The larval survival percentage represents the number of worms surviving during rearing up to pre spinning stage and was calculated by using the following formula:

\[
\text{Larval survival percentage} = \frac{\text{Number of larvae surviving at pre spinning stage}}{\text{Total number of larvae retained after III moult}} \times 100
\]

Cocoon stage

Following observations were made for different parameters at cocoon stage:

**Cocoon yield/10000 larvae**

This parameter was recorded as an average weight of cocoons harvested in kg and converted for 10,000 larvae and was worked out by using the by following formulae:

\[
\text{By weight (kg)} = \frac{\text{Cocoon yield in kg}}{\text{Total number of larvae retained after III moult}} \times 10,000
\]

**By number**

It was recorded as an average number of cocoons harvested and converted for 10,000 larvae and was worked out by using the by following formulae:

\[
\text{By number} = \frac{\text{Cocoon yield by number}}{\text{Total number of larvae retained after III moult}} \times 10,000
\]

**Pupation percentage**

This parameter represents the average number of live pupae obtained in each replicate and is represented in percentage. It was calculated by using the following formula:

\[
\text{Pupation percentage} = \frac{\text{Number of live pupae in harvested cocoon}}{\text{Total number of larvae retained after III moult}} \times 100
\]
Good cocoon percentage

Good cocoons were sorted out and counted in each replicate. Average number of reelable cocoons obtained was recorded. It was calculated by using the following formula:

\[
\frac{\text{Number of good cocoons harvested}}{\text{Total number of larvae retained after III moult}} \times 100
\]

Double cocoon percentage

This represented the average number of double cocoons obtained in each replicate and was determined by using the following formula:

\[
\frac{\text{Number of double cocoons harvested}}{\text{Total number of larvae retained after III moult}} \times 100
\]

Flimsy cocoon percentage

This parameter depicts the average number of flimsy cocoons counted in each replicate and was computed by using the following formula:

\[
\frac{\text{Number of flimsy cocoons harvested}}{\text{Total number of larvae retained after III moult}} \times 100
\]

Dead cocoon percentage

This represented the average number of cocoons with dead pupae obtained in each replicate and was calculated by using the following formula:

\[
\frac{\text{Number of dead cocoons harvested}}{\text{Total number of larvae retained after III moult}} \times 100
\]

Single cocoon weight (g)

Twenty five male and twenty five female cocoons were randomly selected and weighed on digital balance to determine the average cocoon weight by using the following formula:

\[
\frac{\text{Weight of 25 male (g) + 25 female cocoon (g)}}{50}
\]

Single shell weight (g)

Same twenty five male and twenty five female cocoon shells from each replicate were weighed on digital balance to determine average single shell weight. The formula applied was;

\[
\frac{\text{Weight of 25 male (g) + 25 female cocoon shells (g)}}{50}
\]

Shell ratio percentage

It is the average ratio of twenty five male and twenty five female cocoon shell to that of average cocoon weight of same cocoons per replicate and was calculated by using the following formula:

\[
\frac{\text{Average weight (g) of 25 cocoon shells of each sex}}{\text{Average weight of same cocoons of each sex}} \times 100
\]

Post cocoon stage

The reeling was conducted at Demonstration Cum Technical Service Centre of Central Silk Board, Miran Sahib, Jammu and data recorded for different post cocoon parameters.

Total filament length (m)

Filament length indicates the total reelable length of silk filament obtained from a single cocoon in meters. It is the average length of the silk reeled from a single cocoon.

\[
\text{TFL} = \frac{\text{Length of raw silk reeled (m) \times Number of cocoons maintained per end}}{\text{Number of reelable cocoons}}
\]

*Number of reelable cocoons = Number of cocoons taken for testing – Number of new unreelable cocoons/Number of converted carry over cocoons
Non-breakable filament length (m)

It is a length at which cocoon filament breaks and is replaced by another cocoon. It was recorded as per the following formula:

\[ \text{NBFL} = \frac{\text{Length of silk filament reeled} \times \text{No. of cocoons maintained per end}}{\text{No. of reeling ends} \times \text{Weight (g) of raw silk reeled}} \times 9000 \text{ (m)} \]

*Indicates number of castings + Number of carry over cocoons – Number of converted carry over cocoon.

Filament size (d)

It was determined by using filament reeled from ten cocoons from each replicate and was calculated by using the following formula:

\[ \frac{\text{Weight (g) of raw silk reeled}}{\text{Length (m) of silk reeled} \times \text{No. of cocoons maintained per end}} \times 9000 \text{ (m)} \]

Statistical analysis

The data presented for various parameters was tabulated and subjected to Analysis of Variance techniques by using statistical package (SPSS 16.0). Effects of treatments on the physiological and metric parameters were analyzed by using one way ANOVA. Differences between means were tested by using Tukey’s HSD (P <0.05).

Results and Discussion

Feeding is the most important factor in silkworm rearing as it has a direct impact on growth and development of worms. The present experiment comprising of four treatments viz., single feeding (24 hrs gap), 2 feedings (12 hrs gap), 3 feedings (8 hrs gap) and 4 feedings (6 hrs gap) was carried out in order to find out the quantum/frequency of feed required for obligatory and facultative phases on fourteen most important commercial characters. The results obtained during the investigation are presented as under:

Egg stage

The experimental trial was started after IIIrd moult. However, upto IIIrd age mass rearing was conducted after brushing randomly 4 dfIs (disease free layings) for each treatment and average fecundity, hatching percentage and brushing percentage of four treatments was calculated.

The data presented in the Table 1 revealed that different feeding treatments didn’t exhibit any significant effect on fecundity. Numerically, higher value of (594.66 ± 3.52) for fecundity was recorded in 4th treatment i.e. 4 feeding frequency followed by (560.00 ± 2.64). In case of 1st treatment i.e. single feeding frequency (24 hrs gap), 3rd treatment with 3 feeding frequency recorded a value of (555.00 ± 17.67) in comparison to 2 feeding frequency having 12 hrs gap (551.66 ± 24.05). Different feeding frequencies did not record any significant variations in terms of hatching as it depicted 98.09 ± 0.52 value for treatment 1st, 97.50 ± 0.57 for treatment 2nd, 97.08 ± 0.59 for treatment 3rd and 97.02 ± 0.19 for treatment 4th. In case of brushing percentage character 96.78 ± 1.08 for 1st, 96.44 ± 1.10 for 2nd, 95.56 ± 1.16 for 3rd and 95.85 ± 0.42 for 4th treatment was recorded.

Larval stage

At larval stage the observations were recorded for IVth and Vth larval age and weight 10 mature larvae. The larval developmental period (D:HR) varied significantly in terms of IVth instar duration as (5.08 ± 0.00 for 1st, 4.73 ± 0.28 for 2nd, 4.07± 0.00 for 3rd and 4.05± 0.00 for 4th treatment (F=12.83; df= 3; P= 0.002). Fifth instar larval duration with (8.04± 0.01), (7.09± 0.00), (6.09 ± 0.00) and (6.07 ± 0.00) for single, double, three and four times
feeding frequencies (F 1.32; df=3; P = 0.000). However, maximum larval duration in IVth instar was found in case of treatment 1st i.e. single feeding frequency (5.08 ± 0.00) and in Vth instar (8.04± 0.01) for single feed (Fig. 1).

The data presented in (Table 2 and Fig. 2) revealed that different feeding treatments exhibited significant results in respect of weight of 10 mature larvae (F = 29.87; df = 3; P = 0.000) where it was maximum in 4th treatment i.e. 4 feedings (40.66 ± 1.45) followed by 3rd treatment i.e. 3 feedings (40.00 ± 2.00), 38.33 ± 1.20 for 2nd treatment i.e. 2 feedings and least in case of 1st treatment i.e. single feed (40.66 ± 1.45) followed by 3 feedings (38.33 ± 1.20), 2 feedings (36.66 ± 1.00) least in case of single feed (24.33 ± 0.66) and larval survival percentage (F = 78.73; df = 3; P = 0.000) was observed maximum in case of 4 feedings (95.00 ± 0.57) followed by 3 feedings(93.50 ± 0.76), 2 feedings (97.50 ± 0.76) least in case of single feed (77.83 ± 1.58).

**Cocoon stage**

The following observations on cocoon parameters were recorded for different feeding frequency treatments on experimental material of silkworm hybrid CSR2 x CSR4.

The analyzed data on cocoon parameters is presented in (Table 3) and it reveals that the cocoon yield by weight in Kg (F = 183.88; df = 3; P = 0.000) was maximum with four feedings a day (14.82 ± 0.38) followed by 3 feedings (14.10 ± 0.14) significantly superior over two feedings (12.95 ± 0.07 ) and single feed a day (7.68 ± 0.23) by number (F =6.603; df = 3; P = 0.015), the maximum numbers of cocoons per 1000 larvae was with four feedings i.e., (9366.7 ±600.92 ) and significantly superior over single feed (6100.50 ± 199.0) where as good cocoon percentage (F =263.34; df = 3; P = 0.00), pupation percentage (F =267.14; df = 3; P = 0.000) was also higher in treatments with four feeding i.e., (90.50 ±1.04) and (93.66 ±0.60) respectively. The maximum double cocoon (F =8.045; df = 3; P = 0.008) and flimsy cocoon (F =167.85; df = 3; P = 0.000) were found in the treatment one feed a day i.e. (3.16 ± 0.10) and (16.83 ±0.88) respectively. However, single cocoon weight (F=94.85; df=3; P=0.00), single shell weight (F = 415.284; df = 3; P = 0.000) and shell ratio (F = 0.202; df = 3; P = 0.892) significantly different when compared among different feeding frequency treatments and was found maximum with four feedings i.e., (1.61 ± 0.00), (0.33± 0.00) and (20.95 ± 0.11) respectively followed by three feedings, two feedings and single feed a day. However, no significant difference was observed in shell ratio per cent among various feeding treatments (Fig. 3 and 4).

**Post cocoon stage**

Following observation were recorded for different parameters of post cocoon characters.

For total filament length (F =24.60; df = 3; P = 0.000) and filament size depicted (F = 8.24; df = 3; P = 0.008) which varied significantly among treatments. Numerically, highest value for total filament length recorded was (765.33 ± 16.01) for 4 feeding frequency followed by721.00 ± 4.16 and 621.33 ± 10.03 for 3 and 2 feeding system. Least value of 498.66 ± 43.64 was scored by single feed frequency. Similarly, non-breakable filament length recorded a value of 765.33 ± 16.01 followed by 721.00 ± 4.16 and 621.33 ± 10.03 for 3 and 2 feeding system. Least value of 498.66 ± 43.64 was recorded in single feed system. However, filament size2.47 ± 0.14 was recorded for single feed regime followed by 2.73± 0.11 for 4 feeding frequency 3.00 ± 0.03 for 3 feeding frequency and 3.10 ± 0.03 for 2 feeding frequency (Table 4 and Fig. 5).

The quality and quantity of food plant can play an important role in growth and
development of silkworm, particularly during larval stage, which in turn influences the expression of cocoon productivity traits. It also leads to the increase in body size and dry weight of cellular mass which are dependent on environmental conditions in each instar in the rearing bed (Ahmed et al., 2015a). Feeding frequency and overcrowding in rearing bed affects the economics of cocoon crop significantly, as over feeding leads to leaf wastage and higher leaf cocoon ratios (Ahmed et al., 2015b). Overcrowding of silkworm in rearing bed leads to insufficient consumption of feeds, poor growth and higher incidence of disease resulting in low cocoon yield and inferior cocoon quality (Krishnaswami et al., 1977).

Table.1 Mean Performance of bivoltine silkworm hybrid for egg traits (CSR2 X CSR4)

| Frequency of Feeds/day | Fecundity | Hatching % | Brushing % |
|------------------------|-----------|------------|------------|
| 1 feed                 | 560.00 ± 2.64 | 98.09 ± 0.52 | 96.78 ± 1.08 |
| 2 feed                 | 551.66 ± 24.05 | 97.50 ± 0.57 | 96.44 ± 1.10 |
| 3 feed                 | 555.00 ± 17.67 | 97.08 ± 0.59 | 95.56 ± 1.16 |
| 4 feed                 | 594.66 ± 3.52 | 97.02 ± 0.19 | 95.85 ± 0.42 |

Values are Means ± SE
Means within a column followed by different letters are significantly different P<0.05

Table.2 Mean Performance of CSR2 X CSR4 bivoltine silkworm hybrid for IVth and Vth age larval duration

| Frequency of Feeds/day | IVth Instar(D:Hrs.) | Vth Instar(D:Hrs.) |
|------------------------|---------------------|--------------------|
| 1                      | 5.08 ± 0.00 a       | 8.04± 0.01 a       |
| 2                      | 4.73 ± 0.28 a       | 7.09± 0.00 b       |
| 3                      | 4.07± 0.00 b        | 6.09 ± 0.00 c      |
| 4                      | 4.05± 0.00 b        | 6.07 ± 0.00 c      |

Values are Means ± SE
Means within a column followed by different letters are significantly different P<0.05

Table.3 Mean Performance of CSR2 X CSR4 bivoltine silkworm hybrid for cocoon traits

| Frequency of Feeds / day | Cocoon yield/10,000 larvae | Good cocoon percentage | Pupation Percentage | Double cocoon percentage | Flimsy cocoon percentage | Single cocoon weight (g) | Single shell weight (g) | Shell ratio Percentage |
|--------------------------|----------------------------|------------------------|---------------------|--------------------------|-------------------------|--------------------------|-------------------------|------------------------|
| By wt.                   | By No.                     |                        |                     |                          |                         |                          |                          |                        |
| 1                        | 7.68 ± 0.23 c              | 6100.50 ± 199.0 b      | 41.00 ± 2.51 c      | 58.16 ± 1.85 c           | 0.16 ± 0.10 b           | 16.83 ± 0.88 a          | 1.26 ± 0.01 c           | 20.79 ± 0.37           |
| 2                        | 12.95 ± 0.07 b             | 8866.70 ± 333 a        | 81.00 ± 0.28 b      | 88.66 ± 0.33 b           | 2.66 ± 0.60 a           | 2.33 ± 0.60 b           | 1.47 ± 0.01 b           | 20.79 ± 0.23           |
| 3                        | 14.10 ± 0.14 a             | 9183.30±4.40 a         | 85.00 ± 0.50 ab     | 91.50 ± 0.50 ab          | 2.16 ± 0.44 a           | 2.16 ± 0.16 b           | 1.54 ± 0.02 a           | 20.66 ± 0.26           |
| 4                        | 14.82 ± 0.38 a             | 9366.7 ±600.92 a       | 90.50 ± 1.04 a      | 93.66 ± 0.60 a           | 1.16 ± 0.16 ab          | 0.83 ± 0.44 b           | 1.61 ± 0.00 a           | 20.95 ± 0.11           |

Values are Means ± SE
Means within a column followed by different letters are significantly different P<0.05
Table.4 Mean Performance of CSR2 X CSR4 bivoltine silkworm hybrid for post cocoon traits

| Frequency of Feeds/day | Total filament length (m) | Non-breakable filament length (m) | Filament size Denier |
|------------------------|---------------------------|-----------------------------------|----------------------|
| 1                      | 498.66± 43.64c            | 498.66±43.64 c                    | 2.47 ± 0.14 b        |
| 2                      | 621.33± 10.03 b           | 553.33±43.85 bc                   | 3.10 ± 0.03 a        |
| 3                      | 721.00 ± 4.16 ab          | 680.66±37.44 ab                   | 3.00 ± 0.03 a        |
| 4                      | 765.33 ± 16.01a           | 765.33±16.0 1a                    | 2.73± 0.11 ab        |

Values are Means ± SE
Means within a column followed by different letters are significantly different P<0.05

Fig.1 Performance of CSR2 X CSR4 bivoltine silkworm hybrid for IVth and Vth age larval duration

Fig.2 Performance of CSR2 X CSR4 bivoltine silkworm hybrid for IVth and Vth age larval traits
Fig. 3 Effect of different feeding schedules on cocoon traits of CSR2 X CSR4

Fig. 4 Effect of different feeding schedules on cocoon traits of CSR2 X CSR4

Fig. 5 Effect of different feeding schedules on post cocoon traits of CSR2 X CSR4
Superior quality of silkworm feeds should be fed to young and late age larvae. Quantitative differences in feed influence both the larval growth and cocoon characters of mulberry silkworms. Therefore, it is crucial to determine quantity of feed required per day for each instar and feeding frequencies according to environmental conditions. It has been clarified unequivocally that *Bombyx mori* L. grows very fast and needs adequate nutrition during the last two larval instars because Vth instar feeding stage consuming about 80-85 per cent of the total feed (Krishnaswami, 1988). After the last ecdysis, quick development of larvae takes place and exponential growth of the silk gland is accompanied by a parallel increase in the cellular content of DNA, RNA and proteins (Prudhomme and Couble, 1979).

The utmost important aspect of commercial silkworm rearing, aiming at production of good cocoon crop, is the uniformity in larval growth and development along with, minimum intake of food and labour (Babu, 2014 and Srinath, 2014). In the present study, an attempt has been made to rationalize the food quantity for commercial exploitation. The results show high degree of variability by hybrid (CSR2 x CSR4) with regard to various economically important characters for different feeding frequencies. Variation in manifestation of different characters can be ascribed to the fact that feeding frequency during larval instars is one of the crucial issues for successful commercial cocoon crop and if the feeding frequency of silkworm is minimized, the larvae show retarded growth and development, yielding both lower quality and quantity of cocoon production, due to weakness of larvae. However, if the feeding frequency of silkworm is increased, it leads to robust growth and development and ultimately better production of quality and quantity of cocoons (Roy Choudhury et al., 1993).

In conclusion, in the present investigation, the fourteen commercial quantitative and qualitative traits revealed broad variability between different feeding frequencies. This may be attributed to the adaptability of silkworm larvae to different feeding frequencies during the rearing of the worms. The present investigation indicates that rearing of silkworm, with feeding frequency of four and three times, is suitable for commercial rearing of bivoltine hybrid CSR2 x CSR4 at field level.

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