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RESEARCH PAPER

Growth and economic traits of silkworm, *Bombyx mori* under the influence of flour supplementation

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Abstract
Nutrition is an important factor affecting the growth and development of silkworm that directly influences the cocoon and post cocoon parameters. A successful production of cocoon by mulberry silkworm mainly depends upon the nutritional quality of mulberry leaves they feed on. India is the second largest producer of silk in the world. The demand of Indian silk is more as compared to production and hence ways to increase the quality and quantity of silk produced is a major area of concern. Supplementing mulberry leaves with nutritive additives and feeding to silkworm is one of the most useful techniques used to increase economic value of cocoons. The present study was thus undertaken to study the impact of various flour supplements viz. corn, soy and multigrain on the growth and various cocoon parameters of *Bombyx mori.* From the present study it can be concluded that oral administration of mulberry leaves supplemented with corn and soy flour increases various growth and economic traits of *Bombyx mori*. Addition of corn or soy flour with mulberry leaves can be an easy and cheap way that can be adopted by the rearers specifically the local farmers that will help to increase silk productivity without much investment.

Keywords Silkworm · Mulberry · Fortification · Flour

Introduction

Sericulture involves rearing of silkworm and production of raw silk. It is a labour oriented, small scale industry with low investment that suits both marginal and small land holders for its high returns within a short gestation period (Kori et al. 2022). It creates ample employment opportunities to both men and women. Silkworms are also known as serigenous insects that belongs to order Lepidoptera, family Bombycidae/ Saturnidae and class Insecta. One important feature of silkworm is their ability to convert plant protein into silk (Ude et al. 2014). Silk, also known as queen of textile have wide range of applications in various fields (Khyade and Yamanaka 2018; Ageitos et al. 2019). Several silkworm species are used commercially for producing silk viz. mulberry, eri, muga and tassar. Asia is the largest producer of silk in the world and produces more than 95% of the total output. India is the second largest producer of silk after China and contributes nearly 18% to the total world raw silk production (Kori et al. 2022). Mulberry silk alone contributes more than 90% of India's total silk production.

Bombyx mori (*Lepidoptera*), also called mulberry silkworm feeds on mulberry leaves. Nutrition is a vital factor governing the growth and development of silkworm that directly affects the cocoon and post cocoon traits (Smitha and Rao 2010; Mala and Vijila, 2021). Silkworm reared in low nutrient feed are more susceptible to diseases that increases mortality. This also reduces the effective rate of rearing (Borah and Boro 2020). The demand of Indian silk is more as compared to production. Hence ways to increase the quality and quantity of silk produced is a major area of concern. Supplementing mulberry leaves with nutritive additives and feeding to silkworm is one of the most useful techniques used to increase the economic value of cocoons (Masthan et al. 2017). In recent years various research has been carried out on fortified mulberry leaves fed to silkworms to study their impact on their growth and development. Shifa 2016 studied about the impact of *Zea mays* flour on various parameters of mulberry silkworm.Various other supplements used by different researchers includes addition of ascorbic acid (Nirwani and Kaliwal, 1996), amino acid

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(Saranya et al. 2019) multi-vitamins, folic acid (Etebari and Fazilati 2003; Etebari et al. 2004), thiamin (Saha and Khan 1996), natural dyes (Selvisabhanayakam et al. 2014) etc. The present study was thus undertaken to study the impact of various flour supplements viz. corn, soy and multigrain on the growth and various cocoon parameters of *Bombyx mori* which has not been studied well. This comparative study will also enable us to propose the best supplements that can be adopted by the farmers to enhance the quality and quantity of silk produced.

Materials and methods

Rearing of silk worm

Five hundred disease free layings (DFLs) of silkworm were procured from local rearers and disinfected by spraying 2% solution of formalin. The rearing experiments were carried out in the Department of Zoology, DHSK College, Dibrugarh, Assam. The rearing tools and instruments were cleaned and disinfected properly before rearing (Dandin et al. 2003). The DFLs were kept in trays by spreading paraffin papers and were incubated under standard conditions of light (12 h light: 12 h dark), temperature (25 ± 1 °C) and relative humidity ($75 \pm 5\%$) for 8 days (Zhen et al. 2021). On the 9th day of laying, the eggs were subjected to black boxing for 24 h and on the next day they were exposed to light in order to achieve synchronized and uniform hatching (Gobina and Bhaskar, 2015). After hatching, the larvae were fed on chopped mulberry leaves up to first moult.

Food supplements

Corn flour, soy flour and multigrain flour were used as food supplements. 10% aqueous solutions of different supplements were prepared in distilled water. Fresh mulberry leaves were chopped and sprayed separately with 5 ml of 10% solution of corn flour (T1), 10% solution of soy flour (T2) and 10% solution of multigrain flour (T3).

Experimental design

The second instar larvae were used for the feeding experiments. Healthy and active second instar larvae were randomly divided into four groups, each group containing 120 larvae. Group 1 was the control group and the larvae were fed with fresh mulberry leaves, while groups 2–4 were experimental groups that were fed with T1,T2 and T3 respectively. The larvae were fed thrice a day at 0600, 1400, and 2200 h till full maturity was reached. The mature larvae were transferred to bamboo mountage for spinning of cocoons. Proper cleaning was done at regular intervals (Zhen et al. 2021).

Data collection

The following growth and economic traits of *Bombyx mori* were recorded as given below (Zhen et al. 2021; Gobina and Bhaskar, 2015).

Moulting duration (h)

The moulting duration for the last two larval moult (3rd and 4th) were recorded.

Larval duration (days)

Total larval duration for all the instar were recorded.

Larval weight (g)

Ten larvae were picked randomly from each treatment and their weights were recorded at the end of 3rd, 4th and 5th instar.

Effective rate of rearing (ERR) %

ERR was calculated by adopting the following formula:

$$\mathrm{ERR} = (\mathrm{Z/W}) \times 100,$$

where, Z is number of harvested cocoons and W is the initial number of population of larvae brushed.

Cocoon, pupal and shell weight (g)

Ten cocoons were selected randomly from each group and weighed to get the cocoon weight. The pupae were removed by cutting the cocoon and weighed. Similarly the shell weight was also obtained for each group and recorded separately.

Cocoon shell ratio (CSR) %

CSR is calculated by using the following formula:

$$\mathrm{CSR} = (\mathrm{SW/CW}) \times 100,$$

where, SW is the shell weight and CW is cocoon weight.

Table 1 Affect of flour supplements on moulting and larval duration

Treatments	Moulting duration (h)		Larval duration (days)				
	III Moult	IV Moult	I instar	II Instar	III Instar	IV Instar	V Instar
T1	11.6 ± 0.7	13 ± 0.7	3 ± 0.7	2 ± 0	4 ± 0	6 ± 0.8	6 ± 0.2
T2	11.9 ± 0.8	13.5 ± 0.5	3 ± 0.7	4 ± 1.3	4 ± 1.3	5 ± 0	7 ± 2
T3	14 ± 1.0	16 ± 1.1	3 ± 0.5	4 ± 1.3	6 ± 0.8	6 ± 1.0	9 ± 1.3
Control	12 ± 1	15 ± 2	3 ± 0.5	3 ± 0.7	4 ± 1.3	6 ± 1.0	6 ± 0

Physical cocoon parameters

Some physical parameters of the cocoons like colour, shape, size and compactness were recorded for each group.

Silk filament length (m)

Three cocoons were randomly taken from each group and individually reeled on a cocoon reeler (epprouvette). Silk filament lengths (L) were calculated by adopting the following formula:

$$L = R \times 1.125,$$

where, R is number of revolutions recorded by epprouvette whose circumference is 1.125.

Silk filament weight (g)

The reeled silk (three cocoons) were dried in an oven at 85 °C and weighed on an electronic balance.

Denier

Denier or the thickness of the filaments were calculated by the following formula:

$$\text{Denier} = (\text{SFW}/\text{SFL}) \times 9000,$$

where, SFW is the weight of silk filament (g) and SFL is the length of silk filament (m).

Silk productivity (SP)

SP is expressed in cg per day and was calculated by the formula given below:

$$SP = SW/FILD,$$

where, SW is shell weight and FILD is fifth instar larval duration.

Results and discussion

Food is one of the most important factor that influences the growth and development of silkworm and also the quality and quantity of cocoons produced. Feeding *Bombyx mori* with fortified mulberry leaves decreased the moulting duration in both corn supplemented and soy supplemented mulberry leaves as compared to control. The shorter moulting duration (11.6 and 13 h) was seen when the larvae were treated with corn supplementation for the last two moults (Table 1). Decreased moulting duration by feeding silkworm with leaves supplemented with plant extracts has also been recorded by Gobena and Bhaskar 2015. No significant change in the larval duration was seen in any of the treated groups (except for T3 in which the larval duration showed slight increase from the control) as compared to the control (Table 1). Our results are in accordance with the findings of Rahmathulla et al. 2007 in which significant difference in the larval duration was seen when larvae were fed with folic acid supplements.

Increased larval weight was recorded both at the beginning of third and fourth instar, and at the end of fifth instar in T1 and T2 as compared to the control group. Maximum increase was found in T1 followed by T2 as shown in Table 2. Such increase in weight is due to the growth promoting properties of the additives and over consumption by the worms due to phagostimulation (Gobena and Bhaskar 2015). Various workers have also reported increased larval weight when they were fed on leaves added with supplements like amino acids, folic acid, zea mays flour, medicinal plant extracts, probiotics etc. (Saranya et al. 2019; Gobena and Bhaskar 2015; Shifa 2016; Muzamil et al. 2023; Zhen et al. 2021; Rahmathulla et al. 2007). T3 however showed

Table 2 Affect of flour supplements on larval weight and ERR

Treatments	Larval weight (g/10)			ERR (%)
	III (Initial)	IV (Initial)	V (Mature)	
T1	1.2 ± 0.2	5.67 ± 0.3	36 ± 1.5	59.1
T2	1.4 ± 0.2	4.8 ± 0.7	33 ± 3	51.6
T3	0.96 ± 0.01	4.2 ± 0.9	30 ± 1.5	38.3
Control	0.98 ± 0.02	4.7 ± 0.6	32 ± 2	47.5

Table 3 Affect of flour supplements on cocoon traits

Treatments	Cocoon weight (g/10)	Pupal weight (g/10)	Shell weight (g/10)	CSR (%)
T1	12.6 ± 1.4	10.2 ± 1.6	2.4 ± 0.7	19.04
T2	11.4 ± 1.2	9.3 ± 1.4	2.1 ± 0.7	18.42
T3	9.7 ± 1.3	9.2 ± 1.4	0.5 ± 0.2	5.15
Control	11 ± 1.7	9.8 ± 1.8	1.2 ± 0.3	10.9

Table 4 Physical cocoon parameters

Treatments	Colour	Shape	Size	Compactness (++++)
T1	Off White	Oval	Uniform	++++
T2	Creamy	Spindle	Less Uniform	++++
T3	Yellowish	Mostly spindle	Less Uniform	++
Control	White	Mostly Oval	Mostly Uniform	+++

decreased larval weight as compared to the control. Multigrain flour were less preferred by the worms which may be the reason for their decreased weight. Supplementation of mulberry leaves by corn and soy flour also increased the ERR as compared to the control. Highest ERR was recorded in T1 (59.1%) followed by T2 (51.6%) and T3(38.3%) as shown in Table 2 .

Highest cocoon weight (12.6 g/10), pupal weight (10.2 g/10), shell weight (2.4 g/10) and cocoon shell ratio (19.04%) were registered in silkworms fed with corn fortified mulberry leaves (T1) as shown in Table 3. Cocoon weight and shell weight are important parameters that determines the amount of silk that can be obtained. Our findings are in line with the findings of several other workers like Saranya et al. 2019; Gobena and Bhaskar 2015; Shifa 2016; Muzamil et al. 2023; Zhen et al. 2021 and Rahmathulla et al. 2007. Minimum cocoon traits were seen in T3 which were even lower than the control. The physical cocoon parameters such as colour, shape, size and compactness are shown in Table 4. Larval group fed with corn supplement produced off white, oval shaped and uniform cocoons with better degree of compactness. Soy supplemented larvae produced cream colour, spindle shaped cocoons with less uniformity but with better compactness as compared to control group. Multigrain fed larvae produced cocoons with less uniformity and compactness. Based on various features, the cocoons were classified as good or defective as shown in Table 5. It was observed that T1 produced highest number of good cocoons followed by T2. Cocoons produced by T3 were mostly of defective type. Quality of cocoon is very important as it plays an important role in fixing their price. Defective cocoons are economically not suitable as it increases silk waste and decreases reelablility percentage.

The influence of additives on various post cocoon traits like silk filament length, silk filament weight, denier and silk productivity are shown in Table 6. The silk filament length and silk filament weight were found to be higher in T1 (891.37 m and 0.223 g) and T2 (997.50 m and 0.291 g) as compared to T3 and control. The highest silk productivity (4 cg/day) was recorded in T1, when mulberry leaves were fortified with corn flour followed by T2, soy fortified leaves (3 cg/ day). Increase in silk productivity can also be directly related to the bio constituents present in the food

Table 5 Influence of food supplementation on cocoon type

Cocoon Type	Traits	T1	T2	T3	Control
Good	Uniformity in shape, size and colour	√	√	×	√
	Good in hardness	√	√	√	√
	Presence of fully matured larva inside	√	√	×	√
	Tight ends	√	×	×	×
	Filament length	√	√	×	×
	Denier	√	√	×	×
Defective	Doubled	×	×	×	×
	Inside stained	×	×	√	×
	Outside stained	×	×	√	√
	Pierced	×	×	×	×
	Thin end	×	×	×	×
	Printed	×	×	×	×
	Thin shelled	×	×	×	×
	Malformed	×	×	√	×

Table 6 Affect of flour supplements on post cocoon traits

Treatments	Silk filament length (m)	Silk filament weight (g)	Denier	Silk Productivity (cg/day)
T1	891.37 ± 2.83	0.223 ± 0.005	2.25	4
T2	997.50 ± 5.66	0.291 ± 0.006	2.63	3
T3	578.62 ± 5.77	0.195 ± 0.008	3.03	0.55
Control	664.87 ± 4.77	0.198 ± 0.007	2.68	2

supplements. In our study it was found that the flour supplements considerably affect the denier. Considerable decrease in denier was recorded in T1 followed by T2 (2.25 and 2.63 respectively) as presented in Table 6. Low denier indicates improved quality of silk filament. Denier was highest in T3. Raja and Kumar 2016 had studied the impact of algal extract on economic traits of mulberry silkworm and reported significant increase in economic traits which is in accordance with our study.

In our investigation it was found that the larval, cocoon and post cocoon parameters of *Bombyx mori* increased considerably in treated groups 1 and 2 as compared to control. Corn flour is a good source of carbohydrates (around 75%) and fiber. It is rich in various vitamins (A, B, E, and K), minerals (magnesium, calcium, potassium, sodium and phosphorus), phenol, flavonoids, phytosterols, lignins etc. (Siyuan et al. 2018). The presence of various nutrients makes them more palatable by the silkworm and hence promote their growth and development. The bioactive compounds like phenols and flavanoids have disease preventive properties that can reduce mortality of silkworm larva and hence increase their performance. Soy flour is also rich in protein, essential amino acids minerals like, calcium, magnesium and iron (Bressani 1975).

Increase silk productivity is a major aim of any rearer in order to gain profit. Thus ways to increase silk productivity is a major aspect looked upon by various researchers. The impact of various food supplements have been studied by many workers and they have also reported beneficial impact on various larval, cocoon and post cocoon traits when silkworm is fed with mulberry leaves supplemented with algal extract, proteins, minerals, medicinal plant extracts, nanoparticles and growth hormones (Singh 1997; Sunder et al. 2000; Kumar et al. 2009; Prabu et al. 2011; Bai and Bai 2012). Addition of corn or soy flour with mulberry leaves can be an easy and cheap way that can be adopted by the rearers specifically the local farmers that will help to increase silk productivity without much investment. However convincing the farmers may be a challenging task.

Conclusion

Nutrition is a vital factor that influences the growth and development of silkworm. From the present study it can be concluded that oral administration of mulberry leaves supplemented with corn and soy flour increases various growth and economic traits of the silkworm, *Bombyx mori*. Thus, corn and soy flour supplementation can be of great benefit to the farmers to increase the quality and quantity of silk.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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