

USE OF BEE POLLENS AS A NUTRITIONAL DIET TO IMPROVE THE QUALITY AND QUANTITY OF SILK PRODUCTION

Muhammad Ismail Kaleem¹, Hafiz Muhammad Tahir^{*1}, Aamir Ali¹,
Muhammad Ibrahim², Ayesha Muzamil², Hassan Mustafa²

^{1,1,1}Department of Zoology, Government College University, Lahore

^{2,2}Institute of Zoology, University of the Punjab, Lahore

²Department of Zoology, Government College University, Lahore

*dr.hafiztahir@gcu.edu.pk

ABSTRACT

To meet the increasing demand of silk, different research studies have been designed to give silkworms (*Bombyx mori* L.) (Lepidoptera: Bombycidae) the mulberry leaves fortified with natural and synthetic food additives to increase the production, quality and quantity of silk. The objective of this study was to investigate the effect of bee pollens coated mulberry leaves on the biological and economic parameters of silkworm. Different concentrations of bee pollens (0.5%, 1% and 2%) coated fresh mulberry leaves were given to 5th instar larvae while unfortified mulberry leaves were fed to control group. The results of the present study showed that there was significant ($P < 0.05$) increase in the larval weight of treatment group as compared to control group. Highest increase in larval weight ($1.047 \text{ g} \pm 0.028$) and % ratio of silk gland weight to body weight (30.306 ± 0.430) was observed in the group treated with 1% BP. Furthermore, the cocoon weight, cocoon length and cocoon width was also significantly higher in treatment groups as compared to control group. A comparable pattern was observed in the % shell ratio and % fibroin content. Moreover, the highest cocoon weight (0.654 ± 0.017), cocoon length (31.96 ± 0.217), cocoon width (17.55 ± 0.060), % shell ratio (52.968 ± 2.008) and % fibroin content (76.8 ± 0.335) was found in the group treated with 1% BP. The outcomes of the current experiment have illustrated that silkworm has good adaptability for artificial nutritional diet. By enhancing the protein content in the silkworm feed, the biological and economical parameters of silkworms can be improved. It is conferred from the current study that the use of bee pollens has positive impacts on the productivity of silk.

INTRODUCTION

Different arthropod like spiders, bees, mites produce silk but silkworm is the major contributor of silk production in the world (Shahzadi et al., 2022). Silkworm is known for its sleek and luxurious silk thread which is recognized as Queen of textile industry (Souyma et al., 2017; Gautam et al., 2022; Shahzadi et al., 2022). Sericulture is an agricultural based industry which involve rearing of silkworm (*Bombyx mori* L.) to get silk. Sericulture is a profitable business that is practiced in more than 60 countries in the world but China and India are major contributor of silk and provide a handsome source of income with low investment to poor people living in rural areas (Sharma and Kapoor, 2020; Jaiswal et al., 2021). China is producing 84% of total world silk with 15% production in India (Natarikar et al., 2023). The domestication of silkworm from its wild habitat started 5000 to 1000 years ago in China (Li et al., 2023; Sashina and Yakovleva, 2023).

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Silkworm is monophagous insect that only eats mulberry leaves. *B. mori* has short reproductive cycle of 6-8 weeks with four different developmental stages that are eggs, larvae, pupa and adult moth. Within 10 days, eggs hatch to larva that undergoes complete metamorphosis (Soumya et al., 2017; Altman and Farrell, 2022). At 5th instar, larvae spin to produce silk cocoon and pupa goes inside and turn out with adult moth (Soumya et al., 2017; Ikeya et al., 2023). Ideal condition for better larval growth and development include 25-28°C temperature and 60-70% humidity. However, high temperature and humidity also effect the characteristics of cocoon i.e. length, color and rigidity (Offord et al., 2016). Silk is a fibrous protein which consist of two thread like filaments clinked together by sericin protein (Altman and Farrell, 2022). Fibroin protein is produced by posterior silk gland while sericin is synthesized by middle silk gland (Dong et al., 2019). Two type of silk is produced worldwide i.e. Mulberry Silk and Non-Mulberry silk. Mulberry silk is produced by Bombycidae family while non-mulberry silk is produced by family Saturniidae and family Lasiocampidae (Jasmine and Mandal, 2014). Many crops of silk are obtained in a year depending upon the type of silkworm specie i.e. univoltine, bivoltine, and multivoltine (Gautam et al., 2022). Every year, the demand of silk for both domestic and export purpose is increasing which is still not fulfilled (Holland et al., 2019). The estimated quantity of natural silk produced in the world is 83393 tons every year while the required demand is 92742 tons in each year, showing a massive deficit (Tenriawaru *et al.*, 2021). Sericulture industry is playing an importance role in sustaining the economy of many developing countries but in last few decades, silk industry is facing many problems like decrease in demand and supply proportion, economic crisis in farmers, poor economic strategies, lack of trained persons, conventional infrastructure for silkworm rearing and better jobs opportunities in other industries than sericulture (Popescu, 2013). Many other concerns and obstacles are arising in silkworm industry including management of mulberry, preservation of genetic resources, high equipment prices and lack of labor force (Gautam *et al.*, 2022; Habeanu *et al.*, 2023). Nutrition plays a crucial role in health, growth and development of larvae, quality, and quantity of silk of *B. mori*. Characteristics of silk fibers produced by silkworm depend on the intake of protein content along with mulberry leaves (Sharma *et al.*, 2022). A number of research activities demonstrated improvements in physiological and biochemical properties of silkworm when fortified mulberry leaves with different nutrients are given i.e. amino acids, vitamins, juvenile hormones, minerals, honey, royal jelly, and propolis (Helaly *et al.*, 2021; Mohanny *et al.*, 2022). Bee pollens are products of bee-hive rich in protein content, carbs, lipids, dietary fibers and many vitamins. Due to nutrient rich composition, bee pollens have been used in many research studies as food for insect (Ghouizi *et al.*, 2023). The current study has been planned to demonstrate the effect of bee pollens on the physiological characteristics of silkworm. Mulberry leaves are coated the with three different concentrations (0.5%, 1%, and 2%) of bee pollen and 5th instar larvae are fed with them (Moise *et al.*, 2020; Mohanny *et al.*, 2022).

MATERIAL AND METHODS

The experiment was performed in Applied Entomological and Toxicology Lab, Department of Zoology, Government College University Lahore and Sericulture Wing; Forestry, Wildlife and Fisheries Department, Ravi Road Lahore. Mulberry leaves coated with different concentrations (0.5%, 1% and 2%) of bee pollen were given to silkworm larva at 5th instar. The biological and economical parameters of silkworm were observed (Mohanny et al., 2022).

Source of Silkworm Eggs

Sericulture Wing; Forestry, Wildlife and Fisheries Department, Ravi Road Lahore provided us eggs of silkworm (*Bombyx mori* L.) of Chinese Race.

Eggs Hatching and Rearing Technique

Silkworm eggs were hatched in 8 days under optimal laboratory conditions having temperature (25-28°C), humidity (70-75%) (Helaly et al., 2021). Neonate larvae were kept in disinfectant rearing room. Disinfection was administered by using 3% formalin and equipment was sterilized by 0.05% spray of Asthra liquid

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possessing active chlorine (Aruna and Muruges, 2021). Fresh mulberry leaves were minced and fed to larva till 4th molting. The newly hatched larvae were kept on cotton trays for rearing with 20 larvae in each tray. At 5th instar, experiment was performed on randomly selected larva and fed with mulberry leaves augmented by bee pollens solution five times a day. The neonate beds were cleaned daily to remove dry leaves and larval feces.

Experimental Design

A batch of 60 larvae of silkworm was randomly divided into four groups, each group containing 15 larvae. One group was taken as control group which was treated with normal fresh mulberry leaves. Other three groups were designated as treatment groups that fed on mulberry leaves fortified with 0.5%, 1% and 2% of bee pollen.

Preparation of experimental solutions

Different concentrations of bee pollen (0.5%, 1% and 2%) were prepared by individually dissolving 0.5 gm, 1 gm and 2 gm of bee pollens in 100 ml distilled water. Furthermore, fresh mulberry leaves were soaked in prepared solutions for 20-30 seconds and fed experimental groups (Table 1).

Sr. No.	Groups	Treatment
1	Control Group	Larvae fed with non-coated mulberry leaves
2	Group 1	Larvae fed with 0.5% bee pollen concentration coated mulberry leaves
3	Group 2	Larvae fed with 1% bee pollen concentration coated mulberry leaves
4	Group 3	Larvae fed with 2% bee pollen concentration coated mulberry leaves

Table 1. Detail of control and experimental groups.

Analysis of Biological parameters of silk cocoon

Larval weight

From day one to pupation, increase in larval weight of all molts were measured by using electric weighing balance and noted on daily basis under laboratory conditions.

Silk gland weight

Before the start of silk formation, 5 silkworms were taken from each group and dissected them and weigh their silk glands to check the effect of bee pollen coated mulberry leaves on them. Electric weighing balance was used for this purpose.

Analysis of Quantity of silk cocoon

Cocoon Weight

Silkworm larva at 5th instar started forming cocoon with speed 65 revolutions per minute. As cocoon formation completed, cocoons were kept in sun light to kill adult moth inside the cocoon. Cocoons produced by experiment and control group larvae were weight by using electric weighing balance. Cocoons length and width was also measured by using Vernier caliper.

Shell weight/Shell ratio

From each group, 5 cocoons were selected and cut them to remove adult dead moth inside cocoon. In order to know the production of silk by experimental and control groups, shell weight and shell ratio of both groups were found by using the given formula:

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Shell weight Formula

Shell weight = cocoon weight – pupa weight

Shell ratio Formula

$$\text{Shell ratio (\%)} = \frac{\text{Shell weight (gm)}}{\text{Cocoon weight (gm)}} \times 100$$

(Rahman, 2018; Fathy and Gad, 2022)

Analysis of Quality of silk cocoon

Sericin and Fibroin content in cocoon

Few cocoons were taken and cut them into small pieces and autoclaved them in particular concentrations for 2 hours at 121°C temperature. After autoclaving, the liquid was filtered by using filter paper and filtrate was dried at room temperature for 24 hours. Dried filtrate was weighed which gave fibroin content in the cocoons. Sericin content was determined by following formula:

$$\text{Sericin content (gm)} = \text{Initial dry weight of shell} - \text{dry weight of the filtrate after filtration}$$

$$\text{Fibroin content (gm)} = \text{Dry weight of the shell} - \text{Sericin content}$$

Statistical Analysis

Normality of the variables was assessed by employing Shapiro-wilk test. Biological aspects (% ratio of silk gland weight) and economical aspects including cocoon weight, cocoon length, cocoon width and % shell ratio were found by applying One-way ANOVA (Analysis of Variance) following Turkey's test and comparison was done between groups. The increase in larval weight was measured by applying repeated measure ANOVA. The significant value considered during Turkey's test was $P < 0.05$ (Shahzadi *et al.*, 2022).

RESULTS

Analysis of weight of Silkworm Larvae

The gradual increase in the weight of 5th instar larvae of silkworms was observed from day 1 to day 5 in both treatment and control groups (Table 2). The larvae fed with 1% bee pollen fortified mulberry leaves had shown significant increase in larval weight (Table 3) (Figure 6). At day 5, the average gain in weight of larvae was highest in treatment group with 1% bee pollen (1.047g ± 0.028) as compared to control group (0.884g ± 0.062).

Analysis of Percentage ratio of Silk gland weight

In all the treatment groups, the significant increase in % ratio of silk gland weight to larval body weight was observed as compared to control group ($F_{3, 16} = 4.669$; $P < 0.05$) (Table 4). Maximum increase in the % shell gland weight was observed in the group fed with 1% bee pollen coated mulberry leaves (30.306 ± 0.430) followed by 2% bee pollen coated mulberry leaves (28.606 ± 0.978) and 0.5% bee pollen coated mulberry leaves (27.674 ± 0.553) (Figure 7).

Cocoon weight, Width and Length

The significant difference between the cocoon weight of all treatment groups were observed as compare to control group ($F_{3, 16} = 3.630$; $P < 0.05$). There were increase in cocoon width of all treatment groups as compared to control group ($F_{3, 16} = 4.255$; $P < 0.05$). Similar trend was observed in the cocoon length ($F_{3, 16} = 11.047$; $P < 0.05$). The highest increase in cocoon weight, cocoon width and cocoon length was shown by the silkworms fed with 1% bee pollen fortified mulberry leaves followed by silkworms fed with 2% bee pollen fortified mulberry leaves and silkworms fed with 0.5% bee pollen fortified mulberry leaves (Table 5).

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Cocoon Shell Ratio

The cocoon shell ratio was increased in all the treatment groups as compared to control group. Moreover, the significant difference in cocoon shell ratio was observed in the treatment group fed with 1% bee pollen fortified mulberry leaves as compared to control group ($F_{3, 16} = 4.693$; $P < 0.05$). All other groups were showing non-significant difference in cocoon shell ratio as compared to control group. Furthermore, the best result was shown by the silkworms fed with 1% bee pollen fortified mulberry leaves (52.968 ± 2.008) followed by treatment group fed with 0.5% bee pollen fortified mulberry leaves (51.198 ± 1.052) and treatment group fed with 2% bee pollen fortified mulberry leaves (49.664 ± 1.236) (Table 5).

Analysis of % Fibroin and % Sericin Content

There was significant difference in percentage fibroin content of all treatment groups as compared to control group ($F_{3, 16} = 25.893$; $P < 0.05$). However, the maximum % fibroin content was shown by the silkworms fed with 1% bee pollen coated mulberry leaves (76.8 ± 0.335), followed by treatment group fed with 0.5% bee pollen coated mulberry leaves (74.8 ± 0.335) and treatment group fed with 2% bee pollen coated mulberry leaves (74.6 ± 0.219) (Table 5).

In case of percentage sericin content, the control group gave the maximum percentage of sericin content ($F_{3, 16} = 25.893$; $P < 0.05$). The highest % sericin content was present in control group (27.6 ± 0.3580) while the treatment group fed with 1% bee pollen coated mulberry leaves gave least % sericin content (23.2 ± 0.335) (Table 5).

Table 2. Results of AVOVA followed by Tukey's Test showing multiple comparison of increase in larvae weight.

No. of days	ANOVA	Tukey's Test
Day1	($F_{3, 56} = 6.678$; $P < 0.05$)	The increase in larval weight from day 1 to day 5 varied significantly as compared to control group.
Day2	($F_{3, 56} = 5.078$; $P < 0.05$)	
Day3	($F_{3, 56} = 3.678$; $P < 0.05$)	
Day4	($F_{3, 56} = 4.139$; $P < 0.05$)	
Day5	($F_{3, 56} = 3.252$; $P < 0.05$)	

Table 3. Increase in Larval weight (g) in control and experimental groups.

Treatments	Day 1	Day 2	Day 3	Day 4	Day 5
Control	0.145 ^a ± 0.009	0.329 ^a ± 0.015	0.550 ^a ± 0.025	0.712 ^a ± 0.017	0.884 ^a ± 0.062
0.5% BP	0.203 ^b ± 0.016	0.391 ^{ab} ± 0.016	0.603 ^{ab} ± 0.026	0.791 ^{ab} ± 0.046	0.943 ^{ab} ± 0.019
1% BP	0.236 ^b ± 0.012	0.431 ^b ± 0.017	0.660 ^b ± 0.023	0.923 ^b ± 0.064	1.047 ^b ± 0.028
2% BP	0.209 ^b ± 0.019	0.402 ^b ± 0.025	0.613 ^{ab} ± 0.017	0.892 ^b ± 0.044	0.986 ^{ab} ± 0.020

Note: Values in column with same superscripts showed non-significant difference while values with different superscripts showed significant difference. The values after ± are representing Standard Error.

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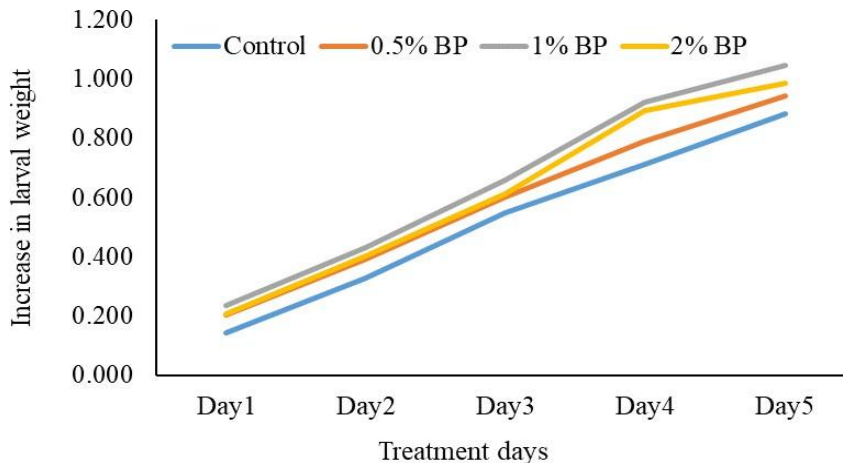


Figure 6. Mean plot of larvae weight (g) of treatment and control groups in 5th instar larva.

Table 4. Comparison of % ratio of silk gland weight to body weight in treatment and control group.

Groups	% ratio of silk gland weight
Control	26.196 ^a ± 0.765
0.5% BP	27.674 ^{ab} ± 0.553
1% BP	30.306 ^b ± 0.430
2% BP	28.606 ^{ab} ± 0.978

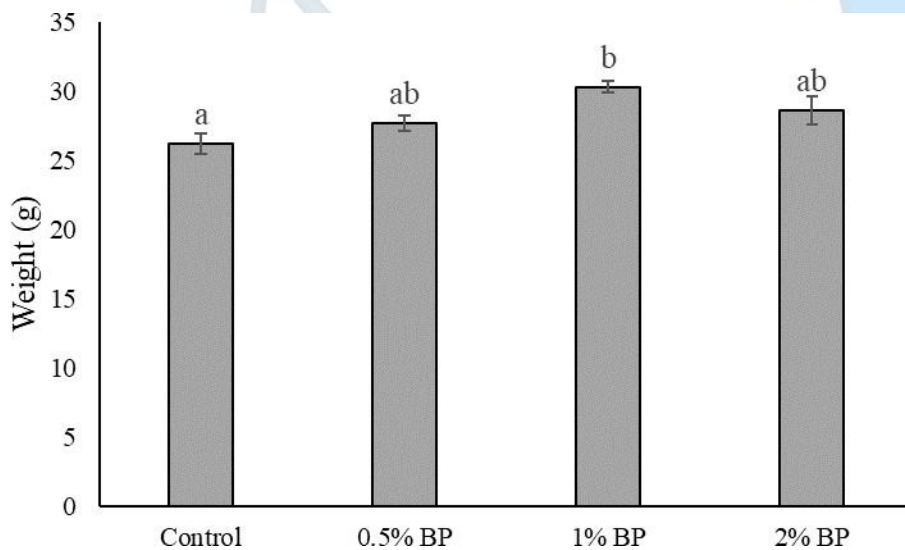


Figure 7. Percentage ratio of silk gland weight to the total body weight of larvae in control and treatment groups.

Table 5. Comparison of economic traits in experimental groups and control group.

Groups	Cocoon weight (g)	Cocoon length (mm)	Cocoon width (mm)	% Shell ratio	% Fibroin content	% Sericin content
Control	0.552 ^a	30.07 ^a	16.45 ^a	45.09 ^a	72.4 ^a	27.6 ^c

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0.5% BP	± 0.007 0.622 ^{ab}	± 0.185 31.742 ^{bc}	± 0.220 17.194 ^{ab}	± 1.049 51.198 ^{ab}	± 0.358 74.8 ^b	± 0.358 25.2 ^b
1% BP	± 0.034 0.654^b	± 0.347 31.96^c	± 0.271 17.55^b	± 1.052 52.968^b	± 0.335 76.8^c	± 0.335 23.2^a
2% BP	± 0.017 0.644 ^{ab}	± 0.217 30.7 ^{ab}	± 0.060 17.16 ^{ab}	± 2.008 49.664 ^{ab}	± 0.335 74.6 ^b	± 0.335 25.4 ^b
	± 0.020	± 0.166	± 0.184	± 1.236	± 0.219	± 0.219

Note: Values in column with same superscripts showed non-significant difference while values with different superscripts showed significant difference. The values after \pm are representing Standard Error.

DISCUSSION

Sericulture is profitable agro-farming that is providing a source of income to rural and poor farmers. From the last few decades, the demand of silk is increasing globally. To meet the global demand of silk, researchers are improving the feed of silkworm in order to increase its production. Different nutritional elements such as probiotics, protein, honey, multi-vitamins, royal jelly, pollen, cow milk, egg albumin and glycine are used as a supplement in the feed of silkworm to enhance the production of silk (Etebari and Matindoost, 2005; Saad et al., 2019; Helaly et al., 2021). The nutritional value of mulberry leaves is crucial in promoting the larval growth and development of silkworms. Beside this, nutrient rich diet also influences the economic aspects of silkworm cocoon (Muhammed and Ayoub, 2023). Sharma et al. (2023) reported that the growth parameters of silkworm larvae were significantly increased when they were given with enriched mulberry leaves containing additional proteins such as protinex powder, drone brood. The present study was conducted to increase the productivity, quality and quantity of silk by giving bee pollen fortified leaves to silkworm.

In the present study, the highest increase in larval weight was observed in the treatment group fed with 1% BP fortified mulberry leaves ($1.047g \pm 0.028$) as compared to control group ($0.884g \pm 0.062$). Similar results were also reported by Saad et al. (2019), who fed silkworm larvae with 1% glycine dipped mulberry leaves and observed significant increase in the larva weight of silkworm. The use of Multi-vitamins in silkworm feed resulted 10.2% weight increase in treatment group as compared to control group (Etebari and Matindoost, 2005).

The results of our study showed that the significant increase in % ratio of silk gland weight to larval body weight were observed when larvae consumed mulberry leaves fortified with 1% BP. Comparable findings were reported by Esaivani et al. (2014) with increase in % shell ratio and % ratio of silk gland weight to body weight of silkworm when larvae were given with probiotics (*Saccharomyces cerevisiae*). Similar increase in % shell ratio was also reported by Prihatin et al. (2023) when Eri silkworms were fed with cassava leaves soaked in 1.5% glycine. Mulberry leaves treated with 1% cinnamon showed similar increase in % shell ratio of cocoon (Fathy and Gad, 2022). The major constituents of the silk gland are proteins, predominantly fibroin, which constitutes the main element of silk fibers (Cui et al., 2018). A protein-rich diet supplies the essential components for silk protein synthesis, resulting in increase in % ratio of silk gland weight to body weight (Lattala et al., 2014). Furthermore, the increase in nutrients such minerals, vitamins, availability also fosters the development of silk gland, resulting in its weight gain (Muhammed and Ayoub, 2023).

The results of our study showed that the significant increase was observed in cocoon length, cocoon width and cocoon weight in the experimental group fed with 1% BP coated mulberry leaves as compared to control group. Hossain et al. (2022) reported that treatment group fed with 0.75% sericin coated mulberry leaves showed maximum increase in the cocoon length, width of cocoon and cocoon weight. Similar increase in the cocoon length, width of cocoon and cocoon weight were reported by Muhammed and Ayoub, (2023) when

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larvae were fed on mulberry leaves enriched with vitamin C, probiotic and honey. The productivity of silk is associated with the nutritional value of mulberry leaves. The major portion of mulberry leaves is composed of essential amino acids i.e. glycine, alanine, serine (Borah and Boro, 2020). Bee pollen contains a diverse range of nutrients, comprising proteins, carbohydrates, enzymes, antioxidants, beneficial fats and essential vitamins like B-complex and vitamin C (Sanyal et al., 2023). This nutritional diversity makes BP a valuable supplement for silkworm larvae and result significant difference among biological and economical traits of silkworm.

The food supplements are source of vital vitamins, minerals and proteins that might be deficient in the mulberry leaves. By enhancing their nutritional supplements, silkworms become healthier and stronger, leading to an increase in silk production (Borah and Boro, 2020; Mahanta et al., 2023). Furthermore, their immune system gets stronger which enable them to show resistance against illnesses and microbes (Kunz et al., 2016; Shahzadi et al., 2022). As a results, the rate of mortality of silkworm larvae decreases and increases the silk production and brings better economic conditions for sericulture farming and silkworm farmers (Muhammed et al., 2023). The fortification of mulberry leaves with bee pollen increases the essential amino acids in silkworm feed which resulted improvements in quality and quantity of silk. But, these improvements are observed up to a particular limit of bee pollen in mulberry leaves (Moise et al., 2020). Fortification of mulberry leaves with 5% BP gave significant improvements in economic characteristics of silkworm as compared to 2% and 10% concentration of BP (Mohanny et al., 2022). Moreover, a higher concentration of BP would disturb silkworm growth by creating toxicity, digestive issues, stress and allergic reactions (Vegh et al., 2021). As the concentration of BP increases, different minerals and vitamins in BP accelerate the metabolism of nutrients which in turn increase more energy generation within body and effect negatively in further feed absorption (Attia et al., 2011).

Conclusion

The results of the present study represented that silkworm larvae showed significant improvement in larval weight, cocoon weight, cocoon length, cocoon width, % shell ratio, % ratio of silk gland weight to body weight and fibroin content of cocoon of silkworm (*B. mori* L.) when they were given with 1% BP coated mulberry leaves as compared to control. It is concluded that diet supplements in silkworm feed can improve the sericulture farming and results in generation of more revenue and uplifting the economy of a country.

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