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Research Article

Effect of Sericin and Honey on Economical and Biological Traits of Silkworm, *Bombyx mori* (L.) (Lepidoptera: Bombycidae)

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NS, HMT and Azizullah designed the study. NS and HMT executed the experiments. HMT, MFB, MS, MAC and Azizullah analysed the data, wrote the manuscript. MS made tables and figures. HMT and Azizullah supervised the study.

Keywords
Bombyx mori, Sericulture, Silk sericin, Silk fibroin, Supplementation

Abstract | In this study the effect of sericin (5%) and honey (3%) on economical and biological traits of *Bombyx mori* (L.) (Lepidoptera: Bombycidae) was recorded. The 5th instar (day 1) mulberry silkworms were divided into four groups of 20 each: namely a control and three experimental groups. Plain (untreated) mulberry leaves were offered to the control group while experimental groups were fed on the mulberry leaves treated with exogenous nutrients alone (5% sericin, 3% honey) and in combination (5% sericin + 3% honey). Results showed that the larval weight of G3 (3% honey) (134.95%) was 40.38% higher than control (96.13%). The significant difference was noted in the larval weight in both control and experimental groups. Cocoon weight (1.34 ± 0.05), shell weight (0.39 ± 0.04g), pupal weight (1.03 ± 0.04g) and shell ratio (22.61 ± 2.83) were found higher in G2 (5% sericin) larvae compared to the control (G1). Similar trend was observed in G3 (3% honey) for cocoon width (1.67 ± 0.03cm), cocoon length (2.98 ± 0.05), filament length (1310.89 ± 15.58m) and percentage of fibroin (85.57 ± 0.28) (with less percentage of sericin (18.61 ± 0.28%). Furthermore, lowest (finer) denier (1.82 ± 0.07) was recorded in G2 larval group fed with 5% sericin. It is concluded from this study that 3% honey and 5% sericin (solitary) have constructive effect on the biological and economical traits of *B. mori*.

Novelty Statement | The study is first of its kind of analysis for feed supplements of natural products such as sericin and honey have improved the quality and quantity of silkworm and silk cacoons.

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Introduction

Silkworm, (*Bombyx mori* L.) belonging to Lepidoptera order is a monophagous and domesticated insect that is economically important. It is reared in controlled captivity for commercial silk thread production in tropical and subtropical regions in rural agro-industry (Rahmathulla, 2012; Soumya *et al*., 2017; Ruiz and Almanza, 2018; Xu *et al*., 2018; Zhang *et al*., 2019). It is noteworthy to say that silkworms have capacity to convert the plant proteins to the soft, fine and the lustrous silk through spinning (Shera *et al*., 2019). In addition to their agricultural and economic

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significance, silkworms are the standard organisms particularly for the biochemical, genomic and molecular genetics research (Goldsmith *et al*., 2005; Reumer *et al*., 2008). The silk cocoons usually contain sericin and fibroin the two main proteins nearly linked but have different properties and structural composition (Khyade and Yamanka, 2018).

Remarkably, silk sericin protein (Hydrophilic and adhesive) wraps around the fibroin and can be separated by the degumming (Padol *et al*., 2012). Importunately, *B. mori* silk (mulberry silk) is the most important and ubiquitous in distribution, with fascinating destination for textile industry, cosmetic industry, poultry industry, pharmaceutical industry and traditional medicines (Soumya *et al*., 2017; Buhroo *et al*., 2018; Ageitos *et al*., 2019). China is producing about 84% raw silk worldwide. However, India is categorized 2nd and contributes near to 15% worldwide raw silk production (Popescu, 2013).

Sericulture industry is crucially important to raise the poor rural community both economically and socially (Kim *et al*., 2010; Sreenivasa and Hiriyanna, 2014). Unfortunately, sericulture industry of Pakistan is not a priority of state as a cottage industry. There is a lack of trained persons and advanced research to improve and enhance the silkworms rearing activities in Pakistan. Lack of government interest, decrease in the mulberry areas and destruction of the Pakistani parent (P-1) silk seed has also contributed to decline in sericulture industry in Pakistan (Hyder, 2017). The cocoon's quality and quantity in sericulture mostly depends upon the healthy growth of larvae (Reddy *et al*., 2015). Leaves of the mulberry plant serve as a perfect diet and provide different nutrients for carrying the physiological functions in silkworm (Smitha and Rao, 2010).

The nutritional value of the mulberry leaves has a huge impact on the growth and development of larvae in addition to the production of the cocoons (Masthan *et al*., 2017). Mulberry leaves can be fortified with additional nutrients to produce high quality cocoons in greater quantity (Thangapandiyan and Daharanipriya, 2019). In recent years, sericulture has been made substantial and innovative advancements, due to the enrichment of mulberry leaves with various food supplements like horse gram flour (Nijagal *et al*., 2017), ascorbic acid (Quraiza *et al*., 2008), probiotics (Esaivani *et al*., 2014), bovine milk (Hossain *et al*., 2015) and Amway protein (Rani *et al*., 2011) to enhancing the economical and biological traits of silkworm. Moreover, honey comprises of carbohydrates, vitamins (B+C), metals, and proteins (Falco *et al*., 2003; Garcia *et al*., 2005; Ball, 2007). It improves the larval growth, cocoon production and silk quality and quantity in *B. mori* (Thulasi and Sivaprasad, 2015; Bhatti *et al*., 2019). In addition, sericin is used as an anti-oxidant, moisturizer, antibacterial, antitumor/anticancer, and as a barrier against ultraviolet radiation due to its distinctive composition, which includes glutamic acid (5%), threonine (6%), aspartic acid (17%), serine (25%) and polar groups (hydroxyl, amino and carboxyl) (Aramwit *et al*., 2009; Gimenes *et al*., 2014; Takechi *et al*., 2014).

Aim of current study was to enrich silkworm diet (mulberry leaves) with natural supplements/food additives (5% sericin, 3% honey, 5% sericin+3% honey) for enhancing the growth and the improvement of silk quality and quantity in silkworm. These food additives could possibly be used by silk grower to improve survival rate, growth of silkworm, yield and silk quality. It could play a significant role to alleviate poverty in the rural areas by providing various self-employment opportunities for the sericulture farmers (especially for women) which in turn increase the financial status of our country and reduces foreign exchange.

Materials and Methods

The Sericulture Wing of Punjab Forest Department, Govt. of Punjab, Lahore, facilitated me with the eggs of silkworm, *Bombyx mori* L. (Chinese HUAKAND2). The research was conducted at the Sericulture Wing laboratory, Department of Forestry, Ravi Road Lahore. The rearing room was disinfected with formalin (2%) prior to start of the experiment and kept air tight for 24 hours. The eggs were incubated under the optimum conditions of relative humidity (70-75 %), temperature (25-27°C) and photoperiod (16 hrs light: 8 hrs darkness). After 8 days, the eggs were hatched and after hatching, silkworms were put in the cardboard boxes (30cm × 30cm × 5cm) with the temperature of 25±1°C and humidity of 70-75% containing fresh, untreated and chopped mulberry leaves. Chopped tender mulberry leaves were offered to first two instars of silkworms whereas later three instars were fed on full grown leaves.

After fourth molt, fifth instars larvae were divided into four groups (G1, G2, G3 and G4) with 20 larvae each. G1 was taken as the control group while G2, G3 and G4 were experimental groups treated with 5% sericin, 3% honey and 5% sericin + 3% honey, respectively. Each food contained 6g, 8g, 10g of mulberry leaves for 1st, 2nd, 3rd days, respectively, maintaining 10g up to 8 days to the fifth instar. In a glass beaker, solution of 3% honey (3 ml honey + 97 ml water) acquired from the *Apis dorsata* (bees) was prepared. Silk sericin protein was extracted from the silk cocoons by degumming action under high temperature (120°C) and pressure (15 psi) for 2 hours. Thus, 5% sericin solution (95ml water + 5ml sericin) was prepared in another glass beaker.

Mulberry leaves (weighed) were placed for a few min into their respective experimental solutions and then air dried (approx. 15-20 min) on the labeled porous plastic plates below the ceiling fan before offering them to the larvae. The treated mulberry leaves were given on odd days (1, 3, 5 and 7 days) to silkworm larvae of the fifth instar. Conversely, untreated mulberry leaves were offered to the silkworms on the even days (2, 4 and 6 days) for feeding. Five meals were given to the larvae in a day starting at 9:00 am to 21:00 pm with a three-hour interval. Before the next feeding, unfed leaves and the fecal matter were regularly weighed and also removed from the trays providing hygienic environment for the healthy growth of larvae. Larval weights were recorded at the time of bed clearance daily. Percent increment in larval weight was also calculated. As the silkworms matured and cocoons formation began, each cocoon box was labelled with corresponding number of the larvae until they disappeared into the cocoon.

Cocoons were harvested, enumerated according to the number of its silkworm, weighed and the values of cocoon's weight were noted down. Pupal weights of control and experimental groups were weighed by using electronic balance (G and G electronic scale JJ223BC) for comparison. Shell weight (Cocoon weight - Pupal weight), and shell ratio (%) (Shell weight ÷ Cocoon weight ×100) were also measured, recorded, analyzed and compared to estimate the quantity of silk produced. Furthermore, vernier clipper was used to determine the width and length of the silk cocoons and their average values were compared with the control. Cocoons were dried at oven in 60°C for 8 hours. Dry cocoons were placed in hot water (70ºC) and brushed to obtain threads for reeling. Then silk reeled weight was measured in grams with digital electronic balance.

In the meantime, filament length was recorded by multiplying the number rotations of filament by the circumference of the jar. The reeled filament denier was obtained using Tanaka's formula (Size (dn) = Weight of silk filament (g)/ Length of filament (m) × 9000). In a weighing crucible, individual cocoons (Shell) were taken (shell) to obtain the sericin and fibroin content, to which KOH (20ml of 0.5 percent) was immersed for six hours. Sericin protein was extracted washing it twice in a boiling water for a few min, leaving behind protein filament i.e., fibroin. After that the vessel having fibroin protein was dried at 90°C for 24 hours. To evaluate the weight of fibroin and sericin, the following formulas were used.

Sericin Content (g) = Initial dry weight of the shell - Dry weight of the shell after alkali treatment

Fibroin Content (g) = Dry weight of the shell - Sericin content.

Statistical analysis

Shapiro-wilk test was used to assess the normality of variables. To compare the cocoon weight, cocoon width, cocoon length, shell weight, pupal weight, shell ratio (%), filament length, denier, percentage of sericin and fibroin among different groups (Control + experimental) One-way ANOVA followed by Tuckey's test for *post hoc* comparisons was used. The % increment of larval weight at different days among groups was analyzed by Repeated Measure ANOVA. SPSS (version 16) was used for all statistical analyses.

Results and Discussion

Results of Shapiro wilk test illustrate that our data was normally distributed (P>0.05 for cocoon weight, shell weight, pupal weight, shell ratio (%), cocoon length, cocoon width, filament length, denier, and the percentage of fibroin and sericin). The larval weights of different groups at different days differed significantly. It was inferred from the study that average weight increase in G3 (3% honey) larvae on the 7th day of the 5th instar was 134.95% of the prior weight of day 1, compared with 96.13% in control group (G1). The G3 larvae treated with 3% honey gained 40.38% higher weight than control group's larvae (Figure 1). The larval weights of different groups at different days were differed significantly ($F_{1, 10}$= 39.87; P < 0.001).

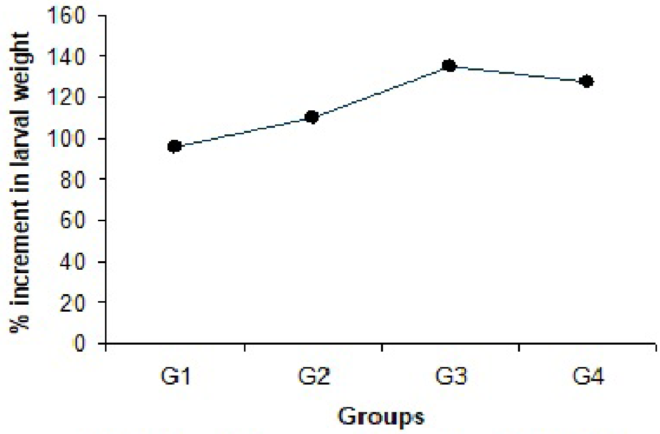

Figure 1: The % increment in larval weight of different groups.

The silkworms of the G3 and G2 matured earlier on the 8th day and started to form silk cocoons. However, in the control group, the larvae continued to feed till the 5 to 6 hours of 8th day and start cocoon formation. Table 1 show the percentage increment in larval weight of the experimental groups (G2, G3 and G4) and control group (G1) at different days. The cocoon weight of silkworms fed with 5% sericin (G2) was 44.09% higher than control group weight (Table 2). Likewise, the average cocoon width and pupal weight of G2 larvae was 16.78% and 35.52% higher than control group (Table 2). Shell weight was maximum (0.39g) in the larvae treated with 5% sericin (G2) against control (0.17g) (Table 2). However, non-significant differences were noted in the shell ratio (%) ($F_{3, 28}$= 1.27; P= 0.305) and cocoon length ($F_{3, 56}$= 2.03; P= 0.12) for the control and experimental groups (Table 2).

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Table 1: Measurement of the weight gain (g) between the control and experimental groups of the larvae.

S. No.	Groups	Treatments	Initial weight (g) (av.)	Total weight on day 1 (g)	Total weight gain on day 7 (g)	Average weight gain on day 7 (g)	Average increase in weight on day 7 from day1(g)	Weight gain in percent (days 1-7)
1	G1	Control (untreated)	1	20.18	39.58	2.07	1.07	96.13
2	G2	Sericin (5%)	1.13	22.61	45.05	2.25	1.12	109.74
3	G3	Honey (3%)	0.96	18.71	43.96	2.16	1.22	134.95
4	G4	Sericin + Honey (5%+3%)	1	20.03	43.24	2.21	1.21	127.24

Table 2: Mean values of quantitative and qualitative characters of cocoons under different combinations of natural food supplements.

Groups	Cocoon weight (g)	Pupal weight (g)	Shell weight (g)	Shell ratio (%)	Percentage of Sericin	Percentage of Fibroin	Cocoon length (cm)	Cocoon width (cm)	Denier
G1 control	$0.93^{a} \pm 0.06$	$0.76^{a} \pm 0.04$	$0.17^{a} \pm 0.02$	$17.85^{NS} \pm 1.84$	$21.45^{b} \pm 0.44$	$78.55^{a} \pm 0.44$	$2.81^{NS} \pm 0.06$	$1.43^{a} \pm 0.06$	$2.66^{b} \pm 0.04$
G2 sericin (5%)	$1.34^{b} \pm 0.05$	$1.03^{b} \pm 0.05$	$0.39^{b} \pm 0.04$	$22.61^{NS} \pm 2.82$	$20.02^{ab} \pm 0.34$	$79.99^{ab} \pm 0.39$	$2.97^{NS} \pm 0.05$	$1.53^{ab} \pm 0.04$	$1.82^{a} \pm 0.07$
G3 honey (3%)	$1.2^{b} \pm 0.05$	$0.94^{b} \pm 0.05$	$0.26^{ab} \pm 0.01$	$22^{NS} \pm 1.23$	$18.43^{a} \pm 0.28$	$81.57^{b} \pm 0.28$	$2.98^{NS} \pm 0.05$	$1.67^{b} \pm 0.03$	$2.86^{b} \pm 0.06$
G4 sericin (5%) + honey (3%)	$1.22^{b} \pm 0.06$	$0.97^{b} \pm 0.05$	$0.25^{ab} \pm 0.02$	$20.69^{NS} \pm 1.63$	$20.21^{ab} \pm 0.87$	$79.79^{ab} \pm 0.87$	$2.89^{NS} \pm 0.04$	$1.49^{a} \pm 0.04$	$2.01^{a} \pm 0.04$
One-way ANOVA	F 3.28= 8.94; P<0.001	F 3.28 = 5.13; P=0.006	F3.28= 4.57; P= 0.10	F3.28= 1.27; P= 0.305	F3.8= 5.18; P=0.03	F3.8= 5.18; P= 0.028	F 3.56= 2.03; P= 0.12	F 3.56= 4.65; P= 0.006	F3.8= 85.3; P<0.001

Note: The values in the columns with different superscripts are statistically different.

The administration of mulberry leaves fortified with 5% sericin, 3% honey, 5% sericin + 3% honey to silkworm showed significant differences of filament length of each cocoon. The filament length of G3 (3% honey) was 11% higher than control. Figure 2 show the comparison on mean (±SD) silk filament length (m) in the control and experimental groups. The percentage of silk sericin and silk fibroin varied significantly among control and experimental groups. The percentage of silk fibroin was recorded 3.84% higher with lower percentage of silk sericin (18.43) in the larvae fed with 3% honey than control (Table 2). By hand rubbing, cocoon stiffness in the G2 (5% sericin) was felt and deemed to be comparatively stronger than control group (G1).

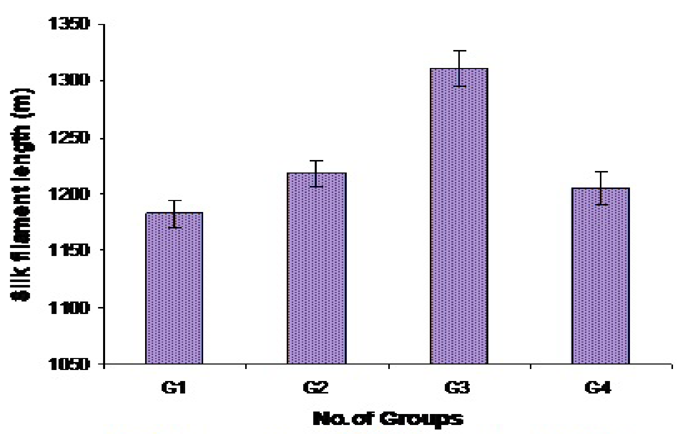

Figure 2: Comparison of silk filament length (m) among different groups.

Silkworms fed with 5% sericin coated mulberry leaves showed best results of denier. Significantly denier size was higher in control (2.66 ± 0.04) than 5% sericin (1.82 ± 0.07*)* (Table 2).

Results of ANOVA followed by Tukey's test are given in Table 2. Silkworm larvae exhibited better cocoon-silk traits when fed with 5% sericin and 3% honey fortified mulberry leaves individually and in combination (5% sericin + 3% honey) compared to control which fed only with plain mulberry leaves.

In the present study, mulberry leaves supplemented with exogenous nutrients (5% sericin, 3% honey, 5% sericin + 3% honey) were offered to 5th instar larvae. We found highly significant increment in the larval weight, filament length, cocoon width, and percentage of fibroin with lower percentage of silk sericin in the larvae fed with 3% honey (G3) against the control group (G1). Cocoon length was also higher in G3 larval group. Honey's effect on the larval development is due to its dietary minerals and vitamins (B+C) (Kavitha *et al*., 2012). Bhatti *et al.* (2019) reported that honey (2% in distilled water) can stimulate larval growth and improve the silk cocoon's production. The consumption rate of G3 larvae was observed to be higher than G1 (control). The higher rate of silkworm consumption may be attributed to the various enzymes found in the honey that may have had a beneficial effect on the weight and feeding behaviour of the G3 (3% honey)

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(El-Akkad *et al*., 2008).

Thulasi and Sivaprasad (2015) reported that honey plays a major role in metabolism, and growth of the *B. mori* and tended to be tissue-specific and concentration dependent. Moreover, the experiment was conducted in autumn season, when less succulent, and hard mulberry leaves are ripen; however, if this experiment was performed in spring season, when mulberry leaves are fresh, and climate is more suitable for larval development, the cocoons production might have been better than the current results.

In the current study, higher pupal weight, cocoon weight, shell weight, and shell ratio were registered in the G2 larvae treated with merely sericin (5%) as against the control (G1). It might be due to the predominantly polar amino acid (inclusive of threonine (6%), glutamic acid (5 %), serine (25 %) and aspartic acid (17 %) and polar groups (amino, hydroxyl and carboxyl) present in the sericin that might have affected on the cocoon performance and the silk yield positively (Padamwar and Pawar, 2004; Wu *et al*., 2007; Aramwit *et al*., 2009; Gimenes *et al*., 2014; Takechi *et al*., 2014). The larvae and pupae that were treated with sericin were fairly healthy, motile and robust. Treated silkworms were also noted to have matured earlier as they started to form cocoons on average 7 to 8 hours before control group's larvae (untreated) (Bhatti *et al*., 2019). The findings of our research are close to previous studies conducted to improve the larval growth, and silk cocoon production of silkworm (Javed and Gondal, 2002; Manjula *et al*., 2011; Lattala *et al*., 2014; Khyade and Mullis, 2018; Wani *et al*., 2018; Thangapandiyan and Dharanipriya, 2019). This strategy of rearing of the silkworms can be readily embraced by the silkworm rearing communities, due to greater economic opportunities and negligible increases in rearing costs. In addition, this study may play a significant role in the country's revival and expansion of silk cottage industry.

Conclusions and Recommendations

It is inferred from the study that honey (3%) and sericin (3%) elicits favorable response in improving the economical (cocoon width, cocoon length, cocoon weight, shell weight, shell ratio (%), denier, filament length, silk fibroin and silk sericin content) and biological characters (fresh weights of each of larva, silk gland, pupa and moth) of silkworm.

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Conflict of interest

The authors have declared no conflict of interest.

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