

Research Article

Influence of Different Honey and Protein Sources on Protein Content in Silkworm, *Bombyx Mori* L.

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Abstract

Experiments were carried out to assess the effect of honey from different bee species viz., *Apis cerana*, *A. florea*, *A. mellifera*, *A. dorsata* and *Trigona iridipennis*, and protein sources namely pollen, soya flour, red gram flour and horse gram flour on the protein content of mulberry silkworm, *B. mori* using different concentrations (1, 2, 3, 4, 5 and 6 %). The result revealed that *per os* application of different honey and protein sources to silkworm larvae starting from first day of fourth instar to spinning significantly altered the protein level. However, the maximum protein content in silk gland (118.96 mg/g), haemolymph (9.71 mg/ml), fat body (50.00 mg/g) and cocoon fibroin (78.38 %) were recorded in the larvae fed with *T. iridipennis* honey. Among the different concentrations tested, it was found that 5 per cent significantly enhanced the protein level over all other concentrations.

Keywords: *Bombyx mori*, honey, pollen, soya flour, red gram flour, protein content.

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Introduction

Silkworm, *B. mori*, a soft delicate sericigenous insect being monophagous in nature feeds exclusively on mulberry leaves to produce a long lustrous silk fibre, called as “*Queen of textiles*”. The larval growth & development, and subsequent quality as well as the quantity of raw silk, are significantly influenced by the nutritional content of mulberry leaves [1]. Though the silkworm nutrients are balanced in mulberry leaves, the quantity available is not sufficient for vigorous growth and development of silkworm larvae [2]. Apart from this, many-a-times the improper garden management practices and application of inadequate fertilizer dosages lead to poor leaf quality ultimately results in complete cocoon crop failure. This situation can be overcome by supplementing the mulberry leaves with essential nutrients [3] like amino acids, minerals, vitamins, etc. Honey which contains significant amount of proteins, enzymes, amino acids, minerals, trace elements, vitamins, aromatic compound and polyphenols [4], could be supplemented with mulberry leaves. Various studies have already been indicated that the application of honey as exogenous growth modulator increases the economic traits of mulberry silkworm [5-9]. But, the influence of honey from different bee species on silkworm biological traits particularly protein content is lacking. Hence, an attempt was made to assess the effect of different honey and protein sources on protein content of mulberry silkworm.

Materials and Methods

Disinfection

Silkworm rearing house and rearing appliances were thoroughly disinfected with 2.5 per cent Chlorine-di-oxide in 0.5 per cent slaked lime solution before the commencement of silkworm rearing. Then, the rearing house remain closed in air tight condition for one day for ensuring complete disinfection and opened to remove the chlorine smell [10].

Silkworm rearing

The rearing of Bivoltine silkworm, Double Hybrid (DH) was taken up during favourable period using V1 variety mulberry leaves harvested from three-years old plantation. The silkworm rearing was carried out under ambient ecological conditions with the temperature of $28\pm 1^\circ\text{C}$ and relative humidity of $80\pm 5\%$ in the method advocated by Krishnaswami [11]. The leaves were provided three times per day without starving the larvae. The bed cleaning was done using nylon net and the sufficient spacing was provided as age of the larvae increased. The bed disinfectant was dusted at 5 g/sq. ft. area to prevent diseases, immediately after every moult and the feed was given 45 - 60 minutes later [12]. The ripened larvae were mounted with Netrika and well-built cocoons were harvested six days after the spinning.

Administration of honey and protein sources

Different concentrations *viz.*, 1, 2, 3, 4, 5 and 6 per cent of honey and protein sources were prepared using distilled water. Weighed quantity of fresh mulberry leaves was separately sprayed with different concentrations of honey from five bee species namely *A. cerana*, *A. florea*, *A. mellifera*, *A. dorsata* & *T. iridipennis* (**Figure 1**), and four protein sources namely pollen, soya flour, horse gram flour and red gram flour (**Figure 2**). The larval batch fed on mulberry leaves sprayed with distilled water and control, without any treatment were also maintained for comparison. The treated leaves were shade dried and fed once from the first day of fourth instar to spinning. Each treatment was replicated thrice with 50 larvae per replication.



Figure 1 Honey from different bee species used for the experiments.



Soya flour

Red gram flour

Horse gram flour

Pollen powder

Figure 2 Different protein sources used for the experiments.

Estimation of silk gland, haemolymph and fat body protein

For the analysis of haemolymph protein, the haemolymph was collected in eppendorf tubes and immediately 1 mg phenylthiourea was added to prevent melanization and the samples were centrifuged at 10000 rpm for 10 minutes. The supernatant was removed and kept at -20°C for analysis. The silk gland was macerated with pestle and mortar in phosphate buffer (pH 7.0) and the supernatant was collected after centrifuging the content at 5000 rpm at 4°C for 5 minutes. For the analysis of fat body protein, the tissue protein was precipitated by the addition of 1 ml of 30 per cent trichloroacetic acid (TCA) followed by centrifugation at 3000 rpm for 30 minutes. It was repeated twice, and then the precipitate was dissolved in 1 ml of 0.1 N NaOH. The estimation of protein content was done by following standard procedure advocated by Bradford [13].

Estimation of sericin and fibroin in cocoon

The cocoon shell was treated with 2 per cent KOH at $70-80^{\circ}\text{C}$ for 5 minutes, constantly stirring till the cocoon became fluffy. The fluffy material was then washed thoroughly in tap water and treated with diluted acetic acid (1g/liter) to neutralize the alkali. After treatment another thorough wash in water was given and the fluff was dried at $90-100^{\circ}\text{C}$

in hot air oven. The weight of fibroin obtained after dissolution of sericin was recorded. The fibroin and sericin content in cocoon was calculated using the following formula [14].

$$\text{Fibroin (\%)} = \frac{\text{Weight of fibroin (g)}}{\text{Weight of cocoon shell (g)}} \times 100$$

$$\text{Sericin (\%)} = 100 - \text{Fibroin (\%)}$$

Statistical analysis

The data collected from the experiments were statistically analysed using Factorial Completely Randomised Design (FCRD) as described by Panse and Sukhatme [15].

Results and Discussion

The experimental results showed that there were significant changes in the protein content in silkworm due to supplementation of different honey and protein sources. The results are discussed in detail with the relevant supporting literature hereunder.

Silk gland protein

The rate of protein synthesis in the silk gland is treated as the index of silk productivity. The supplementation of honey and protein sources to silkworm pronounced gradual positive impact on silk gland protein upto 5 per cent concentration (**Table 1**). Among the different treatments, *T. iridipennis* honey recorded highest silk gland protein of 118.96 mg/g, which was found to be statistically superior over all other treatments. This was followed by soya flour (117.65 mg/g) and *A. florea* (117.11 mg/g) which did not differ statistically among themselves. The least silk gland protein of 111.37 mg/g was registered in the control.

Table 1 Influence of different honey and protein sources on silk gland protein (mg/g) of silkworm

Treatments	Concentrations						Mean
	1%	2%	3%	4%	5%	6%	
<i>A. cerana</i> honey	115.38	115.64	115.83	116.21	116.83	116.10	116.00
<i>A. florea</i> honey	116.57	116.78	116.91	117.54	117.71	117.13	117.11
<i>A. mellifera</i> honey	115.57	115.78	116.21	116.98	117.52	117.16	116.54
<i>A. dorsata</i> honey	115.81	115.97	116.17	116.41	116.93	116.47	116.29
<i>T. iridipennis</i> honey	118.38	118.59	118.83	119.03	119.81	119.13	118.96
Pollen powder	115.21	115.25	115.32	115.35	115.51	115.58	115.37
Soya flour	117.89	117.14	117.38	117.71	117.93	117.84	117.65
Horse gram flour	115.23	115.47	115.78	115.92	116.71	115.98	115.85
Red gram flour	115.21	115.38	115.71	115.91	116.18	116.01	115.73
Distilled water	113.23	113.41	113.73	113.89	113.97	113.91	113.69
Control	111.21	111.25	111.32	111.35	111.58	111.51	111.37
Mean	115.30	115.41	115.67	115.93	116.34	116.01	115.78
	SEd			CD (0.05)			
	T= 0.38			T= 0.87*			
	C= 0.03			C= 0.08*			
	TC= 0.54			TC= 1.05*			
Values of mean of three replications and pooled mean of two silkworm rearing.							
T - Treatment, C - Concentration, * Significant							

Irrespective of the treatments tested, 5 per cent concentration recorded maximum silk gland protein of 116.34 mg/g (**Figure 3**). This was followed by 6 (116.01 mg/g) and 4 (115.93 mg/g) per cent, which were found to be statistically on par with each other.

In the interaction between the treatments and concentrations, significantly highest silk gland protein of 119.81 mg/g was recorded in 5 per cent *T. iridipennis*, which was found to be statistically on par with 6 per cent *T. iridipennis* (119.13 mg/g). The next better treatments were 5 (117.93 mg/g) and 6 (117.84 mg/g) per cent soya flour. The lowest silk gland protein was observed in one per cent control (111.21 mg/g).



Figure 3 Silk gland of silkworm larvae fed with 5 % *T. iridipennis* honey

The present findings derive strength from the results of Madhavi *et al.* [9], who revealed that supplementation of 2 per cent honey significantly increased the silk gland protein content (116.7 mg/g) over the control (86.07 mg/g). This was further supported by Thulasi and Sivaprasad [7], who reported that the silk gland protein profiles grew significantly by 14.85 additional percentile points in the anterior silk gland, 8.68 additional percentile points in the middle silk gland and 15.17 additional percentile points in the posterior silk gland, compared to the control, when the fourth and fifth instar silkworm larvae were fed on mulberry leaves treated with honey at 2 per cent.

Haemolymph protein

The protein content of silkworm haemolymph was significantly enhanced with the supplementation of various honey and protein sources compared to control (**Table 2**). Among the various treatments, the highest haemolymph protein content was observed in *T. iridipennis* honey (9.71 mg/ml) which did not differ statistically from soya flour (9.59 mg/ml). This was followed by *A. florea*, *A. mellifera*, *A. dorsata* and *A. cerana*, which were found to be statistically on par with each other. The lowest haemolymph protein of 7.85 mg/ml was registered in the control.

Table 2 Influence of different honey and protein sources on haemolymph protein (mg/ml) of silkworm

Treatments	Concentrations						Mean
	1%	2%	3%	4%	5%	6%	
<i>A. cerana</i> honey	8.81	8.84	8.86	8.89	8.98	8.91	8.88
<i>A. florea</i> honey	8.85	8.88	8.89	8.95	9.21	9.02	8.97
<i>A. mellifera</i> honey	8.83	8.86	8.88	8.92	9.17	8.95	8.94
<i>A. dorsata</i> honey	8.83	8.86	8.88	8.92	8.96	8.93	8.90
<i>T. iridipennis</i> honey	9.58	9.64	9.71	9.76	9.81	9.77	9.71
Pollen powder	8.77	8.79	8.83	8.87	8.92	8.89	8.85
Soya flour	9.34	9.47	9.62	9.68	9.73	9.70	9.59
Horse gram flour	8.78	8.82	8.85	8.89	8.94	8.91	8.87
Red gram flour	8.73	8.75	8.81	8.83	8.88	8.85	8.81
Distilled water	7.82	7.83	7.84	7.87	7.91	7.89	7.86
Control	7.81	7.83	7.83	7.85	7.89	7.87	7.85
Mean	8.74	8.78	8.82	8.86	8.95	8.88	8.84
	SEd			CD (0.05)			
	T= 0.14			T= 0.28*			
	C= 0.02			C= 0.05*			
	TC= 0.31			TC= 0.63**			
Values of mean of three replications and pooled mean of two silkworm rearing.							
T - Treatment, C - Concentration, * Significant, ** Highly significant.							

Among the various concentrations, maximum haemolymph protein of 8.95 mg/ml was observed in 5 per cent, which was found to be statistically superior over all other concentrations (**Figure 4**). This was followed by 6 (8.88 mg/ml) and 4 (8.86 mg/ml) per cent, both of which showed statistical parity with each other. The least haemolymph protein of 8.74 mg/ml was observed in one per cent concentration.

In the interaction between the treatments and concentrations, maximum haemolymph protein of 9.81 mg/ml was recorded in 5 per cent *T. iridipennis* honey, which was found to be on par with 6 per cent *T. iridipennis* honey (9.77 mg/ml), 5 (9.73 mg/ml) and 6 (9.70 mg/ml) per cent soya flour. Minimum haemolymph protein of 7.81 mg/ml was found in one per cent control.

This was strengthened by Sivaprasad and Thulasi [6], who reported that silkworm supplemented with honey significantly enhanced the haemolymph protein content to 9.52 mg/ml from 8.81 mg/g in the control. Hassan *et al.* [5] observed that oral application of camphor honey at 4 and 5 per cent concentrations to silkworm larvae on first day of fifth instar significantly increased the protein content to 28.43 mg/g and 38.43 mg/g, respectively from 15.10 mg/g in the control. This finding can also be corroborated with the present observations. Further, the present observation of enhancement in haemolymph protein due to soya flour was supported by Thilsath *et al.* [16], who reported that supplementation of plant protein (soya flour) significantly increased the haemolymph protein in silkworm.



Figure 4 Silkworm larvae fed with 5 % *T. iridipennis* honey

Fat body protein

Per os administration of different honey and protein sources to silkworm larvae exerted a significant positive influence on the fat body protein (**Table 3**). Among the various treatments, highest fat body protein was observed in *T. iridipennis* honey (50.00 mg/g) which was found to be statistically superior over all other honey and protein sources. Here, though all the other treatments were statistically found to be on par with each other, they showed statistical superiority over the control.

Table 3 Influence of different honey and protein sources on fat body protein (mg/g) of silkworm

Treatments	Concentrations						
	1%	2%	3%	4%	5%	6%	Mean
<i>A. cerana</i> honey	48.51	48.57	48.67	48.73	48.81	48.75	48.67
<i>A. florea</i> honey	48.71	48.85	48.95	49.13	49.38	49.27	49.05
<i>A. mellifera</i> honey	48.78	48.89	48.97	49.09	49.21	49.14	49.01
<i>A. dorsata</i> honey	48.47	48.59	48.73	48.85	48.97	48.86	48.75
<i>T. iridipennis</i> honey	49.38	49.63	49.78	50.08	51.01	50.13	50.00
Pollen powder	48.41	48.53	48.71	48.79	48.85	48.83	48.69
Soya flour	48.81	48.94	48.99	49.10	49.31	49.23	49.06
Horse gram flour	48.42	48.55	48.71	48.83	48.98	48.87	48.73
Red gram flour	48.52	48.59	48.67	48.74	48.85	48.81	48.70
Distilled water	45.72	45.81	45.92	46.07	46.12	46.09	45.96
Control	45.23	45.61	45.79	45.81	46.08	45.93	45.74
Mean	48.32	48.47	48.59	48.71	48.92	48.77	48.63
	SEd			CD (0.05)			
	T= 0.45			T= 0.90*			
	C= 0.10			C= 0.20*			
	TC= 1.89			TC= 3.76*			
Values of mean of three replications and pooled mean of two silkworm rearing.							
T - Treatment, C - Concentration, * Significant							

Among the different concentrations tested, 5 per cent recorded highest fat body protein of 48.92 mg/g, which did not differ from 6 per cent (48.77 mg/g). The next better concentrations were in the order of 4, 3, 2 and 1 per cent, which was found to be statistically on par with each other and showed statistical superiority over the control.

The interaction effect between treatments and concentrations showed that maximum fat body protein was observed in 5 per cent *T. iridipennis* honey (51.01 mg/g) followed by 6 (50.13 mg/g) and 4 (50.08 mg/g) per cent *T. iridipennis* honey. Minimum fat body protein content was recorded in control at 1 per cent (45.23 mg/g).

The present observations are strengthened with the findings of Sivaprasad and Thulasi [6], who observed that oral supplementation of honey at 1 per cent to silkworm larvae from fourth instar to spinning once daily significantly improved the protein content of fat body (49.58 mg/g) compared to control (41.73 mg/g).

Fibroin

Fibroin, the internal core of the silk filament is a main silk protein secreted in the posterior region of the silk gland. The quantity of fibroin is directly correlated with the raw silk quality. Oral supplementation of honey and protein sources elucidated a significant positive response in the fibroin content of cocoon (**Table 4**). Among the various treatments, *T. iridipennis* honey recorded the highest fibroin content (78.38 %). This was followed by soya flour (77.34 %), which was found to be on par with *A. florea* honey (76.74 %) and *A. mellifera* honey (76.55 %). The next better treatments were *A. dorsata* honey (76.20 %) and *A. cerana* honey (75.92 %) and pollen (75.86 %). Silkworm fed with untreated mulberry leaves recorded the lowest fibroin content (70.06 %).

Table 4 Influence of different honey and protein sources on fibroin (%) of silkworm

Treatments	Concentrations						
	1%	2%	3%	4%	5%	6%	Mean
<i>A. cerana</i> honey	75.09	75.58	75.86	76.22	76.56	76.21	75.92
<i>A. florea</i> honey	75.97	76.19	76.72	76.75	77.64	77.18	76.74
<i>A. mellifera</i> honey	75.67	75.96	76.29	76.85	77.53	77.01	76.55
<i>A. dorsata</i> honey	75.32	75.85	76.13	76.40	77.11	76.37	76.20
<i>T. iridipennis</i> honey	77.58	77.87	78.31	78.57	79.38	78.55	78.38
Pollen powder	75.37	75.46	75.63	75.97	76.69	76.02	75.86
Soya flour	76.30	76.62	77.23	77.81	78.22	77.84	77.34
Horse gram flour	74.48	74.50	74.71	75.09	75.37	75.28	74.91
Red gram flour	73.48	73.67	74.08	74.35	75.64	74.89	74.35
Distilled water	71.14	71.23	71.38	71.47	71.95	71.76	71.49
Control	69.81	69.87	69.93	69.98	70.74	70.04	70.06
Mean	74.56	74.80	75.12	75.41	76.08	75.56	75.25
	SEd			CD (0.05)			
	T= 0.51			T= 1.01*			
	C= 0.03			C= 0.07*			
	TC= 0.75			TC= 1.50*			
Values of mean of three replications and pooled mean of two silkworm rearing.							
T - Treatment, C - Concentration, * Significant							

Among the concentrations evaluated, 5 per cent recorded significantly maximum fibroin content of (76.08 %) over all other concentrations (**Figure 5**). This was followed by 6 (75.56 %), 4 (75.41 %) and 3 (75.12 %) per cent. Minimum fibroin content was observed in 1 per cent (74.56 %).

In the interaction between treatments and concentrations, significantly more fibroin content was found in 5 per cent *T. iridipennis* honey (79.38 %). However, this was found to be statistically on par with 4 (78.57 %), 6 (78.55 %) and 3 (78.31 %) per cent *T. iridipennis* honey, and 5 per cent soya flour (78.22 %). The lowest fibroin content was recorded in control at 1 per cent (69.81 %).

This observation falls more or less in line with the findings of Alagumanikumar and Prema [8], who observed that the cross breed silkworm (PM X CSR2) fed on mulberry leaves enriched with honey had significantly improved the fibroin content. The present finding of soya flour performing next to *T. iridipennis* got strengthened with the result of Thilsath *et al.* [16] (2016), who reported the highest fibroin content when the silkworm was fed on 7 per cent soya flour treated mulberry leaves (79.30 %) compared to control (71.10 %).



Figure 5 Cocoons produced by larvae fed with 5 % *T. iridipennis* honey

Sericin

Sericin is a gummy protein which goes as waste during the silk reeling process. Hence, the superior quality silk should have less amount of sericin. The *per os* application honey and protein sources positively altered the sericin content in cocoon (Table 5). Significantly lowest sericin content was obtained when silkworm was supplemented with *T. iridipennis* honey (21.62 %) over all other treatments. This was followed by soya flour (22.66 %), which was found to be in parity with *A. florea* honey (23.26 %) and *A. mellifera* honey (23.45 %). The highest sericin content was observed in control (29.94 %).

Among the different concentrations studied, 5 per cent registered the lowest sericin content of (23.92 %), which did not differ from the concentrations 6 (24.44 %) and 4 (24.59 %). Sericin content was significantly more in 2 per cent (25.20 %).

The interaction between treatments and concentrations showed that the least sericin content was observed in 5 per cent *T. iridipennis* honey (20.62 %) and 4 per cent *T. iridipennis* honey (21.43 %) which did not differ from 6 per cent *T. iridipennis* honey (21.45 %). The next better supplements were 3 per cent *T. iridipennis* honey (21.69 %), 5 per cent soya flour (21.78 %) and 2 per cent *T. iridipennis* honey (22.13 %). Significantly highest sericin content was observed in control at 1 per cent (30.19 %).

The present observations were supported with the findings of Alagumanikumaran and Prema [8], who revealed that mulberry leaves treated with honey fed silkworm exhibited the lowest sericin content. The lowest sericin content was registered in the silkworm when fed on mulberry leaves treated at 7 per cent soya flour [16]. This finding can also be corroborated with the present result.

Table 5 Influence of different honey and protein sources on sericin (%) of silkworm

Treatments	Concentrations						
	1%	2%	3%	4%	5%	6%	Mean
<i>A. cerana</i> honey	24.91	24.42	24.14	23.78	23.44	23.79	24.08
<i>A. florea</i> honey	24.03	23.81	23.28	23.25	22.35	22.82	23.26
<i>A. mellifera</i> honey	24.33	24.04	23.71	23.15	22.47	22.99	23.45
<i>A. dorsata</i> honey	24.68	24.15	23.87	23.60	22.89	23.63	23.80
<i>T. iridipennis</i> honey	22.42	22.13	21.69	21.43	20.62	21.45	21.62
Pollen powder	24.63	24.54	24.37	24.03	23.31	23.98	24.14
Soya flour	23.70	23.38	22.77	22.19	21.78	22.16	22.66
Horse gram flour	25.52	25.5	25.29	24.91	24.63	24.72	25.09
Red gram flour	26.52	26.33	25.92	25.65	24.36	25.11	25.65
Distilled water	28.89	28.77	28.62	28.53	28.05	28.24	28.52
Control	30.19	30.13	30.07	30.02	29.26	29.96	29.94
Mean	25.44	25.20	24.88	24.59	23.92	24.44	24.75
	SEd			CD (0.05)			
	T= 0.52			T= 1.03*			
	C= 0.43			C= 0.85*			
	TC= 0.70			TC= 1.40**			
Values of mean of three replications and pooled mean of two silkworm rearing.							
T - Treatment, C - Concentration, * Significant, ** Highly significant							

Conclusion

It is concluded from the present experiments that supplementation of silkworm with different honey and protein sources significantly enhanced the protein content in *B. mori*. Among the different sources and concentrations tested, it was revealed that *T. iridipennis* honey at five per cent significantly improved the protein content in silk gland, haemolymph and fat body in silkworm which could be advocated for large scale field evaluation.

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Publication History	
Received	02.06.2021
Revised	09.08.2021
Accepted	17.08.2021
Online	30.09.2021