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Effects of Probiotic Supplemented diet on Growth Performance of Silkworm *Bombyx mori* and Improved Characteristics of Cocoon and Silk

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SPA and SVD performed all experiments, analyzed the results and drafted manuscript. Author NY designed the experiment and edited manuscript. All authors read and approved final manuscript.

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Original Research Article

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ABSTRACT

Aims: The present work is to investigate the effect of probiotic namely normagut on the biochemical and commercial characteristics of silkworm, *B. mori*.
Study Design: Normagut treated mulberry leaves were supplemented at three different concentrations: 1%, 2% and 3%. It was fed to *B. mori* larvae and biochemical parameters were analyzed.
Place and Duration of Study: Experiments were performed at Muslim Arts College, Tamilnadu, India between June 2019 and May 2020.
Methodology: The feeding supplementation started from first day of third instar to last day of fifth instar for every 24 h. The research was performed with control and experimental groups of 3 replications of 30 larvae each.
Results: In our study, 2% concentration of probiotic normagut was very effective and maximum of fat body protein (31.18 µg/mg), glycogen (20.21 µg/mg) and lipid (255.31 µg/mg) was observed. Maximum of haemolymph protein (58.12 µg/ml), trehalose (365.06 µg/ml) and of lipid (41.06 µg/ml), free amino acid (34.32 µg/ml), protein (64.43 µg/mg) and 15.27 µg/mg of lipid was recorded in silk gland of *B. mori* ($p<0.001$). The economic traits of larval weight (3869.23 mg), cocoon weight

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(2131.36 mg), shell weight (463.13 mg), shell ratio (21.72%) and filament length (912.24 m) was recorded in 2% normagut treated group.
Conclusion: The probiotics enhanced the commercial characteristics of cocoon and silk.

Keywords: Probiotics; mulberry leaves; Bombyx mori; cocoon; silk.

1. INTRODUCTION

The silkworm *Bombyx mori* is a monophagous holometabalous and economic important insect. Nutrition plays a major role in improving the growth and development of the silkworm. Silk production is also dependent upon the fortification of larval nutrition and nutritive value of mulberry leaves. Fortification of mulberry leaves with supplementary diet is useful to improve disease resistance in *B. mori.* Probiotic organisms improved the growth and health benefits to the host [1]. The gut probiotic are involved in the digestive utilization of feeds and detoxification of metal link stimulation of non-specific immune system. They also promote the production of vitamins and increase host resistance and compete with pathogenic bacteria by producing organic and antibiotic substances [2]. *B. mori* silk parameters such as silk percentage, denier were increased quantitatively and qualitatively when the larvae treated with *Bacillus cereus*, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Lactobacillus casei* and *Lactobacillus plantarum* [3]. In recent years attempts have been made to improve sericulture with nutrients, such as protein, vitamin, carbohydrates, amino acids, hormones and antibiotic to improve the quality of cocoon [4]. Recent studies were carried out to highlight the effect of different concentrations of commercial probiotics on biochemical, and economical importance of parameters in *B. mori.* In recent times, the usage of probiotics is reported to produce beneficial effects in aquaculture [5], poultry [6] and pig nutrition [7]. The blue green algae *Spirulina* showed improved cocoon quantitative parameters (cocoon weight, shell weight, pupal weight, shell percentage and silk filament length) of silkworm [8]. The main objective of the present study is to investigate the potential of commercial probiotics by analysing the growth performance and silk characters.

2. MATERIALS AND METHODS

2.1 Silkworm Rearing

The disease free laying (PM × CSR2) of multivoltine silkworm, *B. mori* was procured from Nannaharam,Thirunelveli, India. In the present investigation rearing operation was carried out according to previous studies [4-6]. Silkworms were reared under standard recommended condition at (26±2 º C) temperature 75% relative humidity [5-6].

2.2 Experimental Designs

2.2.1 Preparation and supplementation of normagut

In the present study, the *B. mori* larvae were treated with normagut (Mega Lifesciences, Philippines) at three different concentrations through the foliar spray method [3]. Commercially grade normagut (probiotic) was purchased from Trivandrum, India. Normagut consists of *Saccharomyces boulardii*, gelatine, lactose, magnesium stearate, titanium dioxide, yellow iron oxide and chlorophyllin-copper-complex. The probiotic normagut was obtained in the tablet form. Silkworm was fed with untreated leaves until the end of third instar stage. Newly moulted third instar larvae were divided into four groups. Each group consisted of 30 larvae, one group served as control and the others were used for experimental groups including 1%, 2%,and 3% normagut concentrations. The freshly collected mulberry leaves were washed with tap water. A total of 20 g was fed initially and increased 40 g at the end of experimental period. The treated leaves were allowed to dry in air for 15 minutes. The probiotic enriched leaves were fed from first day of third instar larvae to till pupation. Three replications were maintained for each treatment. The probiotic feed was supplemented for every 24 h.

2.2.2 Analytical parameters

The fifth instar larvae were randomly selected and analysed the biochemical components of protein, glycogen, free aminoacids, lipids and trehalose.

2.2.3 Morphological and other characters of fifth instar larvae

Quantitative traits of larval length, weight and larval survival rate were observed and also

recorded cocoon and silk traits of length, weight, shell ratio, filament length, silk percentage and denier.

Length: The length of larvae and cocoon was measured using a vernier caliber with 1C = 0.01mm and expressed in centimeter.

Weight: The weight of larvae, cocoon, shell and silk was measured by using an electronic balance and expressed as milligrams.

Survivability: The percentage of larval survivability was calculated by using the following formula.

$$\text{Survivability percentage} = \frac{\text{Number of larvae reached final instar}}{\text{Total number of larvae reared}} \times 100$$

Shell ratio: The shell ratio of each cocoon was calculated by the following formula.

$$\text{Shell ratio} = \frac{\text{Single cocoon shell weight (mg)}}{\text{Single cocoon weight (mg)}} \times 100$$

Silk percentage: The silk percentage was calculated by the following formula.

$$\text{Silk percentage} = \frac{\text{Weight of raw silk reeled}}{\text{Weight of cocoon shell}} \times 100$$

Denier: The denier was calculated by the following formula

$$\text{Denier} = \frac{\text{Weight of reeled silk}}{\text{Length of reeled silk}} \times 9000$$

2.4 Statistical analysis

One way analysis of variance was performed and the "p" value <0.05 was considered as significant.

3. RESULTS

3.1 Biochemical Composition of Larvae

In the present study, fortification of mulberry leaves with normagut at different concentration on biochemical components of *B. mori* larvae was analysed. The biochemical components of protein, glycogen and lipid from the fat body were observed and the result was described in Table 1 ($p<0.001$). The biochemical components were improved in the *B. mori* larvae treated with 2% normagut ($p<0.001$). The protein, trehalose and lipid content of treated and untreated control were described in Table 2. Maximum level of protein, trehalose and lipid content was observed at 2% normagut, followed by 1%, and 2%, respectively. The increased level of free aminoacid, protein and lipid was observed in the experimental animal treated with 2% normagut and the result was described in Table 3 ($p<0.001$).

3.2 Analysis of Economic Parameters

The economic parameters of *B. mori* larvae, cocoon and silk were analysed and the result was tabulated. The weight and length of larvae and percentage survival rate were observed. Maximum larvae weight, length and percentage survival rate were detected at 2% normagut treated group (Table 4) ($p<0.001$). The cocoon weight, length, shell weight and shell ratio were maximum at 2% normagut treated *B. mori* larvae (Table 5) (<0.001). At 2% normagut concentration, *B. mori* cocoon showed improved filament weight, length, maximum silk percentage and denier concentration than 1% and 3% dosages (Table 6) ($p<0.01$).

4. DISCUSSION

In the silkworm *B. mori* larva, the fifth instar stage is most active feeding period, during this stage the larva accumulate the large quantity of biomolecules reserves in body tissues. Silkworm use these biomolecules for cocoon spinning, metamorphosis and reproduction [9]. In the present study, all the biochemical constituents were increased at 2% normagut treated group followed by 1% and 3% treated group. Similar result has been reported previously by Bai [10] and reported increased haemolymph glucose content in the *B. mori* larvae supplemented with 3% bifilac. Glycogen, trehalose and other nitrogenous compounds are the main haemolymph constituents have been reported to be crucial during growth, development and in maintenance of diapauses in insects [11]. Bai and Bai [12] have been conducted the studies on the commercially available probiotic, Darolac and reported the population of *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium longum, Saccharomyces boulardii* in combination with a neutraceutical agent produced significant beneficial

Table 1. Biochemical characters of fat body of *B. mori* larva fed with probiotic diet

Experimental Groups	Concentration	Protein	Glycogen	Lipid
	Control	21.43±3.53	12.54±2.31	184.23±9.23
	1%	27.12±5.22 (26.55)	17.32±1.45 (38.11)	231.25±8.12 (25.52)
Normagut	2%	31.18±4.12 (45.49)	20.21±2.54 (61.16)	255.31±10.35 (38.58)
	3%	26.27±2.07 (22.58)	18.26±1.09 (45.61)	243.13±8.11 (31.97)

Table 2. Biochemical characters of haemolymph of *B. mori* larva fed with probiotic diet

Experimental Groups	Concentration	Protein	Trehalose	Lipid
	Control	46.23±2.13	304.34±3.51	23.54±2.16
	1%	53.42± 3.14 (15.55)	335.46±2.19 (10.22)	32.65±3.45 (38.70)
Normagut	2%	58.12.± 1.07 (25.71)	365.06±3.07 (19.95)	41.06±5.28 (74.42)
	3%	48.15.36±2.04 (4.15)	358.41±3.25 (17.76)	29.32±3.52 (24.55)

Table 3. Biochemical characters of silk gland of *B. mori* larva fed with probiotic diet

Experimental Groups	Concentration	Free aminoacid	Protein	Lipid
	Control	28.16±2.12	54.21±2.64	12.45±2.56
	1%	31.09±1.32 (10.40)	61.33±3.31 (13.13)	14.42±1.45 (15.82)
Normagut	2%	34.32±2.07 (21.87)	64.43±5.22 (18.85)	15.27±1.64 (22.65)
	3%	32.43±2.05 (15.16)	59.05±4.36 (8.92)	13.08±2.45 (5.06)

Table 4. Effect of probiotic diet on the growth and survival of silkworm *B. mori* larvae

Experimental Groups	Concentration	Larval weight(mg)	Larval length(cm)	Survival rate (%)
	Control	3229.23±15.34	5.4±0.3	89.23±1.32
	1%	3453.05±12.62 (6.93)	6.1±0.6 (12.96)	91.35±1.13 (2.37)
Normagut	2%	3869.23. ±9.34 (19.81)	6.7±0.4 (24.07)	94.27±2.93 (5.64)
	3%	3542.42±8.43 (9.69)	5.9±0.2 (9.25)	93.04±1.35 (4.26)

Table 5. Effect of probiotic diet on the growth parameters of *B. mori* cocoon

Experimental Groups	Concentration	Cocoon weight (mg)	Cocoon length (cm)	Shell weight (mg)	Shell ratio (%)
	Control	1943.26±9.45	3.1±0.4	384.32±12.32	19.77± 1.26
	1%	2138.57±10.54 (10.05)	3.6±0.5 (16.12)	443.47±16.47 (15.39)	20.73±2.02 (4.85)
Normagut	2%	2231.36±11.04 (14.82)	3.8±0.7 (22.58)	463.13±10.32 (20.50)	21.75±1.49 (10.01)
	3%	2117.45±9.36 (8.96)	3.4±0.3 (9.677)	429.11±9.43 (11.65)	20.26±2.43 (2.47)

Table 6. Effect of probiotics on the physical properties of silk from *B. mori*

Experimental Groups	Concentration	Filament weight (mg)	filament length (m)	Silk percentage (%)	Denier
Normagut	Control	312.54±10.24	866.42±17.53	81.32±2.42	3.24±0.56
	1%	378.13±12.32 (20.98)	996.29±21.54 (14.98)	85.26±3.41 (4.84)	3.41±0.12 (5.24)
	2%	402.23±8.43 (28.69)	912.24±19.53 (5.28)	86.85±1.32 (6.80)	3.96±0.43 (22.22)
	3%	365.34±14.09 (16.89)	861.43±18.65 (-0.57)	85.13±1.65 (4.68)	3.81±0.65 (17.59)

effect in the silkworm.The result of the present study shows that larval weight, length and survival rate were significantly increased in normagut treated group when compared to control group, similar to those by Masthan et al. [13] who reported that the effect of probiotics bacteria *Lacidophilus* on increasing larval length and weight. Lakshmibai and Ramanibai [14] have been reported that the larval weight was significantly increased when the *B. mori* treated with pre and probiotic bifilac at 5% concentration. Thus the administration of probiotic is a beneficial effect on economical parameters of silkworm *B. mori.* The cocoon and silk parameters were also increased in the experimental group when compared to control. Masthan et al. [15] have been reported the effective dose of *Spirulina* and yeast supplementation (300 ppm) for the growth of *B. mori.* Saravanan et al. [16] indicated the improved growth of cocoon when the *B. mori* was supplemented with 7.5% of *Vigna unguiculata*. The probiotic *Lactobacillus* improved the cocoon production of mulberry silkworm *Bombyx mori*. Rahul et al. [17] have been performed to analyze the efficacy of *Lactobacillus rhamnosus* ATTC 9595 and *Tlactobacillus acidophillus* ATCC 4356 in improving the economic parameters of *B. mori*. The result of the present study influenced economical importance of silkworm *B. mori*.

5. CONCLUSION

Nutrients play an important role in improving the growth and development of silkworm *B. mori.* The fortification of mulberry leaves with nutrients is a useful technique to increase the economic value of cocoon and silk. The enrichment of mulberry leaves with nutrients such as pre and probiotics, antibiotics, vitamins, amino acids are one of the strategies by which cocoon and silk productivity can be increased and the quality can be enhanced. Current research studies focused the beneficial role of probiotics because probiotics play a major role to reduce disease and increasing the economic parameters of *B. mori*. Normagut capsule contains the probiotics as active ingredients. Normagut capsule works by increasing the bowel mass and promoting growth of good bacteria, stimulating the growth of gut friendly bacteria and inhibiting the digestive enzymes involved in acid secretion.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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