Original Research Article

Effects of probiotic supplemented diet on growth performance of silkworm *Bombyx mori* and improved characteristics of cocoon and silk

ABSTRACT

Aims: The present work is to investigate the effect of probiotic such as normagut on the biochemical and commercial characteristics of silkworm *B. mori*.

Study design: In this study, probiotic diet was supplemented to the silk worm and the growth performance and biochemical properties were analyzed.

Place and Duration of Study: Experiments were performed at Muslim Arts College, Tamilnadu, India between June 2019 and May 2020.

Methodology: Freshly collected mulberry leaves supplemented with normagut at three different the concentration 1%, 2% and 3%. The feeding supplementation was starting from first day of third instar to last day of fifth instar at one day interval. 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 recorded. Maximum of haemolymph protein (58.12 µg/ml), trehalose (365.06 µg/ml) and of lipid (41.06 µg/ml) and also of 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.*. The economic traits of larval weight (3869.23 mg), cocoon weight (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: Thus the result of the study indicates the probiotics enhanced the commercial characteristics of cocoon and silk.

Keywords: probiotics; mulberry leaves; *Bombyx mori*; cocoon; silk

1. INTRODUCTION

The silkworm *B. mori* is an monophagous holometabalous and economic important insect. Nutrition plays an major role improving the growth and development of the mulberry

silkworm. Silk production also dependent upon the fortification of larval nutrition and nutritive value of mulberry leaves . Fortification of mulberry leaves with various supplementary compounds is one of the ways to improve overall development and disease resistance in *B.mori.* Probiotics are "live microorganism which when administered in adequate amounts confer a health benefit on 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 an increase host resistance and compete with pathogenic bacteria by producing organic and antibiotic substances [2]. *B.mori* silk parameters 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 in sericulture with nutrients, such as protein, vitamin carbohydrates amino acids vitamin, hormones and antibiotic etc for better performance good quality of cocoon [4]. The current research was carried out to highlight the effect of different concentration of commercial probiotic 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 is all the 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 probiotic potential of commercial and to analyze the growth performance and silk characters.

2. MATERIAL AND METHODS

2.1. Silkworm rearing technique

The disease free laying (PM × CSR2) of multivoltine silkworm,*B.mori* were procured from Nannaharam,Thirunelveli district. In the present investigation rearing operation were carried out according to (krishnaswami*et al..*,1978).Silkworms were reared under standard recommended condition at (26±2 º C) temperature 75% relative humidity.

2.2. Experimental designs

2.2.1. Preparation and supplementation of normagut

In the present experimental study, the *b,mori* larvae were supplemented with normagut at three different concentration through the foliar spray method.Commercially grade and easily available product of normagut was purchased from standard medical store,Trivandrum,Kerala. The probiotic normagut was obtainted in the tablet form. Silkworm were fed with untreated leaves until the end of third instar stage . Newlymoultedthirdinstar larvae were divided into four groups .Each group has reared 30 larvae, one group served as control and the others were used for experimental groupssuch as 1%,2%,and 3%. The freshly collected mulberry leaves were washed with tap water. The clean and dried leaves were dipped in 1%,2%,and 3%different concentration of preparedsoluion.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. Care was taken to ensures that all the larvae were fed on probiotic treated leaves. The treatment was given in to one day intervals.

2.2.2. Analytical parameters

The fifth instar larvae werewere 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 survibility were observed and also recorded cocoon and silk traits of length , weight, shell ratio, filament length, silk percentage and denier.

2.2.3.1. Length

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

2.2.3.2. Weight

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

2.2.3.3. Survivability

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

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

2.2.3.4. Shell ratio

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

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

2.2.3.5. Silk percentage

The silk percentage was calculated by the following formula

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

2.2.3.6. Denier

The denier was calculated by the following formula

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

3. RESULTS

3.1. Biochemical composition of larvae

In the present experimentastudy investigate to fortification of mulberry leaves with normagut at different concentration on biochemical and economic parameters of *b.mori* larvae were analysed and recorded. The biochemical componentsof fat body (protein,

glycogen and lipidµg/mg), Haemolymph (protein,trehalose and lipidµg/ml) and silkgland (protein and lipidµg/mg) were analysed and the results are presented in tables. In control group , the fat body protein, glycogen and lipid content was observed in (21.43±3.53), (12.54±2.31), (184.23±9.23) respectively (Table 1). Maximum of (31.18± 4.12)protein , (20.21±2.54)glycogen,(255.31±10.35)lipid contentwas recorded when *b.mori* larvae were fortified with normagut at 2% concenterated group. The haemolymph content of protein, trehalose and lipid in untreated group (46.23±9.47),(304.34±3.51),(23.54±2.16) was respectively (Table 2). Maximum (58.12±1.07)protein,(365.06±3.07) trehalose and (41.06±5.28) lipid was observed and recorded in 2% followed by 1% and 3%probiotic group.Maximum, silk gland free amino acid(34.32±2.07),protein (64.43±5.22)and lipid(15.27±1.64) content was also increased when *b.mori* larvae fortified with 2% concentration of normagut group (Table 3).

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

Experimental Groups	Concentration	Protein	Glycogen	Lipid
Normagut	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)
	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
Normagut	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)
	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
Normagut	1%	31.09±1.32 (10.40)	61.33±3.31 (13.13)	14.42±1.45 (15.82)
	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)

3.2. Analysis of economic parameters

The economic parameters of *b.mori* larvae, cocoon and silk were analysed and recorded. The larval parameters of weight, length and percentage of survaibility was observed and recorded. In control, larval weight (3229.23±15.34) mg,length (5.4±0.3)cm and survival rate (89.23±1.32)% was recorded. Maximum larval weight of (3869.23±18.08)mg, larval length (6.7±0.4)cm and percentage of survaibility (94.27±2.93) was obtained in 2% normaguttreated group (Table 4). The maximum (3.8±0.7)cm ofcocoon length , (2231.36±11.04mg)cocoon weight,(463.13±10.32mg) shell weight and (21.75±1.49) shell ratio (Table 5) and also the silk traits of filament weight was (402.23±8.43mg), filament length (912.24±19.53m), silk percentage (86.85±1.32)and denier(3.96±0.43) was recordedwhen the larvae treated with 2% followed by 1% and 3% of normagut fortified group (Table 6).

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 (%)
Normagut	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)
	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(%)
Normagut	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)
	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 on the physical properties of silk from *B. mori*

Experimental Groups	Concentration	Filament weight (mg)	filament length(m)	Silk percentage(%)	Denier
	Control	312.54±10.24	866.42±17.53	81.32±2.42	3.24±0.56
Normagut	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)

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 and the biomolecule is used for cocoon spinning, metamorphosis and reproduction [9]. In the present study reported, 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] that haemolymph glucose content was increased in the *B. mori* larvae supplemented with 3% bifilac. Glycogen, trehalose and other nitrogenous compounds are main haemolymph constituents have been reported to be crucial during growth, development and in maintenance of diapauses in insect [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 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 result has been obtained in Masthan *et al.* [13] reported the effect of probiotics bacteria *Lacidophilus* on increasing larval length and weight. Lakshmibai and Ramanibai [14] have been reported, the larval weight was significantly increased when the *B. mori* treated with pre and probiotic bifilac at 5% concentration. Thus the administration 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 300 ppm *Spirulina* and yeast supplementation the qualitative and quantitative traits of *B. mori.* Saravanan *et al.* [16] indicated, the significantly increased in cocoon parameters when the *b.mori* supplemented with 7.5% of vigna unguiculata. The probiotic lactobacillus improve the cocoon production of mulberry silkworm *Bombyx mori*. Rahul *et al.* [17] have been performed to efficacy of successful proven *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*.

4. CONCLUSION

Nutrients play an important role in improving the growth and development of silkworm *bombyx mori.* The fortification of mulberry leaves with nutrients is a useful modern 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 that the probiotics contains beneficial bacteria, it play a major role in reduce the 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.

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