



## RESEARCH ARTICLE

### STUDIES ON THE GROWTH PARAMETERS (LENGTH & WEIGHT) AND COCOON PRODUCTION IN *BOMBYX MORI*, FED ON MULBERRY LEAVES FORTIFIED WITH A PUTATIVE PROBIONT, *LACTOBACILLUS CASEI*

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#### ABSTRACT

The present work monitors the growth parameter and cocoon production in *Bombyx mori* fed on V1 mulberry leaves fortified with the putative probiotic bacterial, *Lactobacillus casei*. The growth of (length and weight) the bacteria supplemented silkworms of the all instars (II-V) increased significantly over the control animals. The maximum body length (55mm) observed was in the probiotic supplemented worms of the IV instar, while the control worms at IV instar could attain only 46mm. The body weight of probiont supplemented II instar larvae showed a three-fold increase (689mg) compared to the normally fed larvae (205mg). In the fifth instar larvae, the probiotic supplemented group recorded a very high body weight (3550.0mg) than the control group (2946.0mg). Weight of the cocoons from worms reared on probiotic bacteria was higher than that of the silkworms fed on mulberry leaves alone.

#### INTRODUCTION

Sericulture deals with large scale rearing of silk producing organisms. Sericulture industry of India solely depends on *Bombyx mori*, the mulberry silk moth. Over the years, Indian silk industry has developed into a multi-billion dollar business, bringing the much-needed foreign exchange to the country. Many developing countries of Asia-Pacific region have shown their interest in tapping the potential of sericulture. Recent approaches to improve the mulberry leaf quality and cocoon characters, include the application of VAM fungi and bacterial biofertilizers (Rama Rao et al., 2007) and the use of plant growth promoting rhizobacteria (Unni et al., 2008). Supplementing mulberry leaf powder with starch, phosphate, vitamin B complex, vitamin C, citric acid, ascorbic acid etc, has met with limited success (Pang-chuan, 1988). In the recent periods, the microbes *lactobacilli* and *Bifido bacteria* are widely used in probiotic therapy. They are the gram-bacteria producing lactic acid that constitute a major part of the normal intestinal microflora in animals and humans. However, the

efficacy of yeast as a nutrient supplement had not been tried in sericulture. Hence, an attempt has been made to trace the impact of probionts to prevent bacterial infection by enhancing disease resistance potential and energy budget, economic parameters and protein content in silkworm. Charles (2004) stated that the lower animals do not have well developed humoral immunity and under such circumstances, vaccine development may not be of much use and in the lower animals immuno stimulation could be achieved easily through Probiotics. Hence, the present attempt is a very good approach on *B.mori* to strengthen their immunity to resist the microbial pathogenic attack and to promote good yield. In recent years, many attempts have been made in sericulture with nutrient such as protein, vitamin, carbohydrates, amino acids, vitamins, hormones, etc. for better performance of good quality of cocoons, Sannappa (2002). Various researches have been carried out on the diet supplementation of mulberry leaves, which is fed to silkworms. Probiotics are the live microbial food supplements beneficially affecting host by improving the microbial balance and enhanced the rapid cellular growth and development (Fuller et al., 1993). The *Lactobacillus plantarum* is a Probiotic which improves the cocoon

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production of mulberry silkworm *Bombyx mori* (Singh et al., 2005). Certain probiotic bacteria inhibit the growth of microbes. *Streptomyces noursei* are probiotic microbes which prove the antibacterial activity and good ecofriendly management of silkworm diseases (Subramanian et al., 2009). Nutritional contribution and the symbiotic benefit offered by insect gut-dwelling bacteria (Dillon and Dillon, 2004; Yuan et al., 2015) is yet another area which can substantially modify and promote the health and silk production capacity of *Bombyx mori*, although this field has only attained limited attention in the sericulture research scenario, notwithstanding the view of many scientists to consider associated micro flora as potential pathogens or contaminants.

Probiotic formulations are commercially used for humans (Brigidi et al., 2001), and in aquaculture (Douillet and Langton, 1994), while there are no reports on probiotic formulations specifically designed for silkworms. Although Singh et al. (2005) observed improvement in larval body weight, cocoon weight, shell weight and pupation percentage of silkworm larvae when fed on mulberry leaves fortified with a veterinary probiotic formulation containing *Lactobacillus plantarum*, the extent of colonization of *L. plantarum* in the gut of silkworm was not ascertained. The present study deals with the growth parameters, length and weight of the various instars of the larvae of *Bombyx mori*, sustained on mulberry leaves fortified with a putative probiotic, *Lactobacillus casei* a resident bacterium of the silk worm gut.

## MATERIALS AND METHODS

### Mulberry leaves

Mulberry leaves selected for the present study belonged to Mysore hybrid variety V1 (irrigational varieties). These were raised in the Zoology Department garden, Periyar E.V.R. College, Tiruchirappalli -23. Fresh leaves were collected daily and fed to the silkworms, *ad libitum*, till the end of the experiment.

### *Bombyx mori* Larvae

The silk growth *Bombyx mori* (Lepidoptera) *L* × *CSR2*, (Local female and a hybrid multivoltine variety of male) was used in this study. Second instar larvae were obtained from the Tamilnadu State Government Silk Rearing Centre, Manikandam, Tiruchirappalli and were maintained in bamboo rearing trays lined with newspaper sheets and covered by a wire mesh-lid. The trays were kept on moistened gunny bags to maintain cool, humid conditions (27±2°C, relative humidity: 70±5%).

### Experimental protocol

Mulberry leaves were fed to the *Bombyx mori* larvae as per the rearing methods suggested by Krishnaswami et al. (1978; 1991). Experimental as well as control groups had three replications consisting of 100 larvae each. Mulberry leaves of equal weight and approximately of the same number were used for feeding *B. mori* larvae in control and experimental groups. Experimental groups were fed with mulberry leaves, coated

with a suspension of the probiotic (*Lactobacillus casei*) and partially dried at room temperature. The control group of *B. mori* larvae were fed with mulberry leaves without bacterial coating.

### Feed coating

*Lactobacillus casei*, a prominent bacterium residing in the intestine of *Bombyx mori* (personal observation) was mass cultured in nutrient broth and harvested by refrigerated centrifugation. Appropriate amount of the putative probiotic *Lactobacillus casei* (10<sup>-6</sup> cfu) was taken and mixed thoroughly with molten 'China grass' (food grade) at about 50°C and coated rapidly on both the sides of mulberry leaves with a sterile paint-brush. The coated leaves were cut into appropriate sizes prior to feeding the larvae at the various instars.

### Growth measurements

Length of the larvae was measured in mm, with the help of dividers when the larvae were in a relaxed state, taking utmost-care, not to disturb them. Weight (mg) of the larvae was measured on a sensitive, digital electronic balance.

### Shell ratio (SR)

Randomly selected twenty cocoons from the each experimental group were weighed in grams with an electronic balance, then the cocoons were gently opened with the help of a sharp knife and the empty shells (free from pupae) were weighed in grams. The percentage of shell ratio (SR) was calculated using the following formula.

$$\text{Shell ratio} = \frac{\text{Shell weight}}{\text{Cocoon weight}} \times 100$$

## RESULTS AND DISCUSSION

Size increase of *Bombyx mori* larvae (III-V<sup>1</sup> instars) and the cocoons are given in Fig.1. The body length showed significant variations in all instars of silkworm, between the probiotic bacteria supplemented and the control animals. II instar larvae of probiotic supplemented animals recorded 26.90mm length, whereas control animals recorded 20.50mm length. Similarly in III instar larvae the length increased to 47.80mm in the probiotic supplemented group, and in the control group length was 34.90mm only. In IV instar 55.35mm in experiment (control 45.80mm) and the V instar shows 49.80mm (control 47.80mm). The probiotic coated mulberry leaves could bring about greater increase in length, although this increase was of a lesser, compared to the trend in other instars (Table 1). The amount and nature of food consumed by a larvae influence its growth rate, development and the probability of survival (Slansky and Scriber, 1985). A fundamental shift in the microbial profile in silkworm larval gut as a result of microbial feed supplementation is beneficial to the host which in turn may significantly contribute to increased silk production. Food intake is also regulated by the physical nature of food and also the presence of phagostimulants (Dadd, 1970). *B. mori* reared on mulberry leaves supplemented with minerals, oral protein

supplementation, cereal flours, medicinal extracts, plant growth hormones were reported to yield only limited success (Singh, 1997). There was a considerable increase on the energy budget of silkworm like food consumption, Relative Consumption Rate, Growth Index, Approximate Digestibility (AD), Efficiency of Conversion of Ingested food (ECI) and Efficiency of conversion of Digested food (ECD). The order of efficiency of the two probiotics on the nutritional status and commercial characteristics of *B.mori* was yeast < *Bifidobacterium bifidum*. Feed supplementation not only enhanced economic and nutritional parameters but also prevent bacterial infection in *B.mori*, Amala Rani *et al.* (2011). Singh *et al.* (2005) observed the improvement in larval body weight, cocoon weight, shell weight and pupation percentage of silkworm larvae, when fed on mulberry leaves treated with a commercial probiotic formulation containing *Lactobacillus plantarum*. However, the extent of colonization of *L. plantarum* in the gut of silkworm was not established. *Streptomyces noursei* cultures were used in a similar way by Subramaniam *et al.* (2009) and they readily colonized the gut of silkworm when applied as a probiotic formulation, as the microbe was originally isolated from an indigenous silkworm breed.

In the V instar *Bombyx mori* L. larvae were fed mulberry leaves enriched with different concentrations of (1%, 2% and 3%) *Ocimum sanctum* L. They were good growth promoting effect on silkworm, which helps to enhance the commercial qualities of silk and can be used in sericulture for yield improvement (Padma Sree *et al.*, 2015). Thilagavathi, *et al.*, (2013), reported that many factors that influence the success of production of silk. In recent years, some antibiotic agents have been used for growth enhancement of silkworm larvae and improvement of silk production. The use of antibiotic *amoxicillin* enhanced the cocoon weight, the cocoon shell and the length of the thread. From the above study, it is evident that the scope to improvise the silk output by supplementing the silkworms with the selected and efficient antibiotics, so that industry will be economically viable Waktole Sori Gobena and R.N. Bhaskar (2015).

Nutrition plays a pivotal role in sericulture. It improves the growth, development, health; feed consumption and conversion of silkworm thereby improving the commercial trait likes improve the nutritional intake, growth, disease resistance and tolerance of silkworm ultimately improving the cocoon production also. Narayanaswamy and Ananthanarayanan (2006) reported that the mean body weight of V instar larvae showed an increase over control when fed on 'seri-feed' treated leaves. Kamioka *et al.* (1971) observed that the larval growth and cocoon quality could be improved to an extent when mulberry leaves were coated with soyabean flour. Nirmala *et al.* (2001) showed that protease activity increased in the gut of *Bombyx mori* larvae reared on soy protein. Similar results were obtained by supplementation of different nutrients including proteins (Horie and Watanabe, 1983; Sarkar *et al.*, 1992). Body weight of silkworm *Bombyx mori* was evaluated from second instar to fifth instar larvae. After probiotic supplementation, in the second instar larvae, a three fold increase was observed in body weight (689mg) than the normally fed one (205mg). Gradual increase was observed in III and IV instar silkworms after probiotic bacteria supplementation. Student 't'-test revealed that there was significant variation between the probiotic supplemented and control animals (Table-1). In the fifth instar larvae, the probiotic-supplemented group recorded a very high body weight (3550.0mg) than the control group (2946.0mg). Comparable weight increment could also be observed in silkworms of other bivoltine races, fed on mulberry leaves grown with various types of animal wastes as manure (Kerenhap *et al.*, 2007). This observation underscore the need for studies so designed as to analyze the synergetic effects of probiotics, nutritionally enriched mulberry leaves and other feed supplements like soy proteins, vitamins and minerals, on the growth of silkworm larvae and cocoon quality.

**Length-weight relation**

The correlation coefficients were all close to unity, indicating thus the high level of correlation between larval length and weight in all the instars.

**Table 1. Mean, standard deviation and 't' values for length and weight measurements of the various instars of *Bombyx mori* fed by *L. casei***

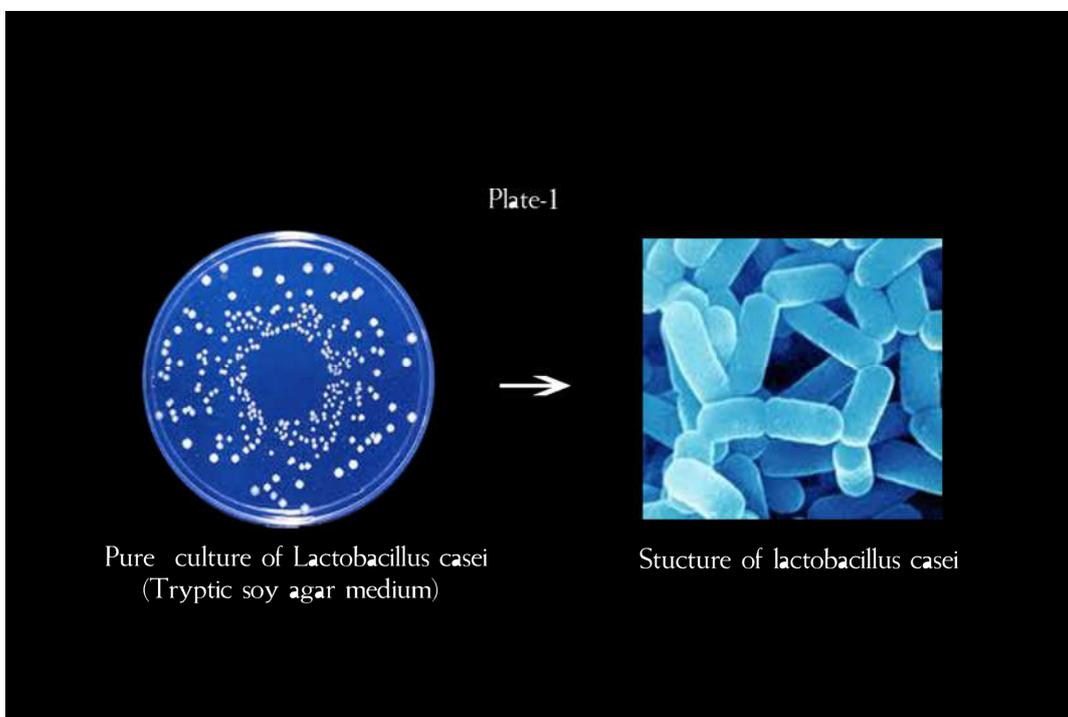
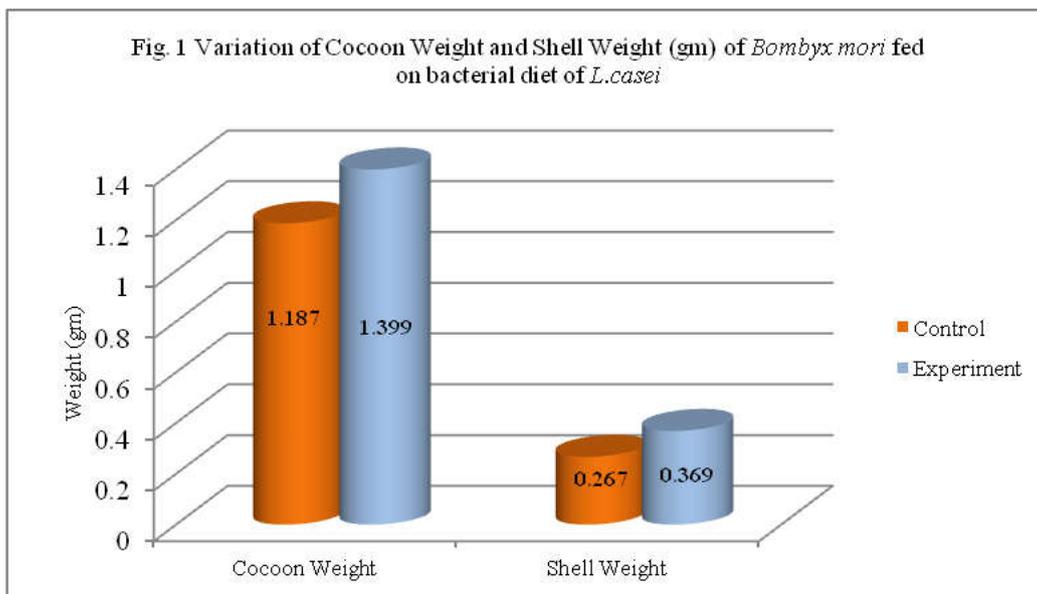
Instars	Length(mm) Mean ± SD		't' values	Significance	Weight(mg) Mean ± SD		't' values	Significance
	Control	Experiment			Control	Experiment		
II	20.5±1.760	26.90±2.918	8.31	P<0.001	0205.0±017.917	0689.0±024.165	72.017	P<0.001
III	34.9±2.863	47.80±4.640	10.576	P<0.001	1165.0±056.522	1575.5±139.750	12.173	P<0.001
IV	45.8±2.783	55.35±2.758	10.899	P<0.001	2276.0±101.846	2944.5±116.933	19.277	P<0.001
V	47.8±2.166	49.80±2.706	2.706	P<0.05	2946.0±082.615	3550.0±127.380	17.938	P<0.001

\*No of analysis - 3

**Table 2. Regression Equation and Correlation Coefficient (r) for Length (mm)-Weight (mg) relationship of various instars of *Bombyx mori*, (L). larvae under normal and *Lactobacillus casei* supplemented feeding**

Instars	Control and Experiment	Regression Equation Log w=log a+b log L	Correlation Coefficient (r)
II	Control	W= 27.79 ±8.64 L	0.85
	Experiment	W = 486.83±7.53 L	0.91
III	Control	W = 548.9±17.6 L	0.89
	Experiment	W = 202.7± 28.71 L	0.95
IV	Control	W =787.5±32.5 L	0.88
	Experiment	W= 645.53±41.55 L	0.97
V	Control	W= 1475.5±30.76 L	0.81
	Experiment	W =1322.5±44.82 L	0.95

L – Length W – Weight



Regression equations for length-weight relationship in the instars III-V showed progressively increasing relative growth coefficients ('b'), for probiotic supplemented worms. ANOVA indicates that the variation in the length and weight significant ( $p < 0.001$ ). Which proved that under the influence of the probiotic, the weight increment was way ahead of corresponding growth in length (Table-2).

#### Cocoon weight

The impact of probiotic *L. casei* (plate-1) leaf coating of mulberry leaves was clearly evident in the cocoon weight of the silkworm *Bombyx mori*. In feed supplemented silkworm cocoons weighed 1.399g more on the average and 1.187g in control. Similarly an increase was observed in shell weight of probiotic supplemented silkworm *Bombyx mori* was 0.369g

and 0.267g in control (fig.1). The percentage of total cocoon production and shell ratio (SR) showed an increase in bacteria-supplemented groups, recently it has been reported that oral supplementation with potassium permanganate, potassium and magnesium chloride and their synergic effects resulted in a significant increase in the fat body and haemolymph protein, and consequently the weight of the silk gland, cocoon weight and shell weight significantly increased (Goudar and Kaliwal, 2001 and Bhattacharaya and Kaliwal, 2004; 2005 a, b, c and d). When the inoculated worms were treated with the probiotics *Bifidobacterium bifidum* and yeast at different concentrations the commercial characteristics of the silkworms such as cocoon characteristics (cocoon weight, pupal weight, shell weight, shell ratio) and silk characters (filament length, filament width, denier, sericin and fibroin content) were enhanced. The study of Kochi and Kaliwal (2006) showed an

increase in the protein content of fat body and haemolymph which might possibly be due to the stimulatory effect of the feed supplemented with the mineral, potassium bromide. The present work concluded that the probiotic *L.casei* coated of mulberry leaves was clearly evident in growth parameter length and weight improvement and increases the cocoon weight of the silkworm *Bombyx mori*.

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