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RESEARCH ARTICLE

EFFECT OF SELECTIVE COMMERCIAL PRE AND PROBIOTIC SUPPLEMENTATION ON NUTRITIONAL INDICES AND COMMERCIAL CHARACTERISTICS OF SILKWORM *B.MORI* L.

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ABSTRACT

Mulberry leaf is the solo food and source of nutrition for the silkworm, *Bombyx mori* L. Fortification of mulberry leaves with supplementary compounds was found to increase the larval growth and post cocoon characteristics. Silk production is dependent on the larval nutrition and nutritive value of mulberry leaves. The use of commercial pre and probiotic bacteria in sericulture has tremendous scope. So to explore the effectiveness on nutritional and commercial characteristics of *B.mori* the study was carried out. Mulberry leaves supplemented with commercial pre and probiotics Flora-SB at the concentration of 1%, 3% and 5% were administered, starting with the first day, first feed of each instar. The research was performed with control and experimental groups of 3 replications of 50 larvae each. In order to have a detailed picture of the energy and economical parameters, daily measurement of dry matter ingested, faeces produced, biomass gained by the larvae, quantitative and qualitative characters of the cocoon were recorded. Pre and probiotics with a concentration of 3% was very effective and recorded maximum food consumption (1.836 ± 0.07g), assimilation (1.544 ± 0.05g), tissue growth (0.118 ± 0.002g), RGR (54.06 ± 1.84 per cent), cocoon weight (1.98 ± 0.09g), shell weight (0.37 ± 0.01g) and filament length (804.26 ± 23.12m). Probiotics are live microbial food supplement that beneficially affect an individual by improving intestinal microbial balance. Prebiotics are non- digestible dietary fiber that exert some biological effect by stimulating growth or bioactivity of beneficial microorganisms. Thus pre and probiotics together enhances nutritional efficacy of *B.mori* which inturn reflect on the quality improvement of cocoon characteristics.

INTRODUCTION

Nutrition plays a pivotal role in sericulture by improving the commercial characters of silkworm. Silkworm being a monophagous insect derives almost all the nutrients required for its growth from the mulberry leaf itself (Nasreen *et al*., 1999). The mulberry is the solo food and source of nutrition for the silkworm, *Bombyx mori* L. due to the presence of more protein, morin (Tribhuan and Mathur, 1989). Silkworm nutrition refers to the substances required by silkworm for its growth and metabolic functions, which are obtained from ingested food of mulberry / supplemented diet and the other nutritional components are synthesized through various biochemical pathways (Hamano *et al*., 1986; Zhang *et al*., 2002). The growth and development of larva and subsequent cocoon production are greatly influenced by nutritional quality of mulberry leaves. In recent years attempts have been made in sericulture with nutrient such as proteins, carbohydrates, amino acids, vitamins, sterols, hormones, antibiotics etc., for better performance and get higher yield with quantity and quality cocoon (Sannappa *et al*., 2002).

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Nutritional study on silkworm is an essential prerequestic for its proper commercial exploitation. Nutrition of silkworm is sole factor which almost individually augument quality and quantity of silk (Laskar and Datta, 2000). Although the mulberry leaves are complete diet for silkworm, the supplementation of extra nutrients along with mulberry leaves results higher yield (Rahmathulla *et al*., 2007). Supplementary nutrients are when added to normal food, increase the nutritional value of the food making it more useful (Krishnaswami *et al*., 1973). Effect of supplementary feed such as 'Serifeed' (Narayanaswamy and Ananthanarayanan, 2006 and Ananda kumar and Michael, 2011), Amway protein (Amala rani *et al*., 2011a), probiotics (Singh *et al*., 2005; Masthan *et al*., 2010 and 2011; Amala rani *et al*., 2011b., Bai and Bai, 2012) and pre and probiotics (Lakshmi Bai and Ramani Bai, 2011) on food consumption and utilization and its effect on quality and quantity of cocoon in silkworm had been well illustrated.

Oral administration of foliage of mulberry and eri silkworm supplemented with cyanobacteria, enhanced larval and shell weight subsequently commercial characters of cocoon (Kumar *et al*., 2009; Masthan *et al*., 2011; Sampath *et al*., 2013; Kumar and Bala subramaniyan, 2013). The silk protein fibroin is fibrous in nature, forming the main silk filament content, while

sericin is a sticky coating substance between the layers of fibroin. Thus the quality of cocoons depends on sericin and fibroin (Singhvi and Bose, 1991). The quality of raw silk is determined by size deviation, neatness and cleanness of the silk fibre. The size deviation of raw silk is dependent on the fibre character (Suresh kumar *et al*., 2002).

Many research activities were initiated to find out a suitable supplementary feed additives to improve the health and cocoon production. Probiotics or bio remediators are gaining more popularity as eco friendly supplementary feed to *B.mori*. The FAO/WHO (2001) defines probiotics as 'Live micro organisms which when administered in adequate amounts confer a health benefit on the host'. Probiotic bacteria have many beneficial effects. In the course of their proliferation and survival in the gastro intestinal tract, probiotics produce metabolites such as lactic acid, antibiotic like substances called bacteriocins that suppress the growth of putrefactive micro organisms. Their metabolic activities also help in the pre digestion of food components and the production of vitamins B and improve the bio availability of minerals (Wood, 1992).

Bacteria from genus *Pediococcus, Leucomostoc* and *Lactobacillus* are found to be beneficial to *B.mori* larvae. Mulberry leaf supplemented with *Spirulina* as fed to *B.mori* orally, found to be effective in enhancing the larval and cocoon characters (Kumar *et al*., 2009; Masthan *et al*., 2011; Kumar and Balasubramaniyan, 2013). Improvement in growth and economic characters were noticed when probiotics *Lactobacillus plantarum* (Singh *et al*., 2005), *L.acidophilus* (Masthan *et al*., 2010) and *Bifido Bacterium bifidum* (Amala rani *et al*., 2011b) supplemented with *B.mori* larvae. The efficacy of *Enterobacter cloaca* (20 µl) (Ramani Bai, 2010) and pre and probiotic Bifilac (Lakshmi Bai and Ramani Bai, 2011) on cocoon characters had been studied in *B.mori*. Prebiots are non- absorbable carbohydrate substrates which stimulate the growth of probiotics. When application of probiotics supplemented with prebiotics, it promotes the component of the normal intestinal microflora and evince a health benefit to the host (Isalauri *et al*., 2011).

Scientific work on the effect of pre and probiotics in *B.mori* was scantly. Therefore current research was carried out to highlight the effect of different concentration of commercial pre and probiotic Flora - SB on nutritional and cocoon parameters in *B.mori*.

MATERIALS AND METHODS

Rearing conditions and experimental design

Fresh disease free layings (DFLs) of PM x CSR_2 multivoltine race was purchased from Government Sericulture Farm, Konam, Kanyakumari District, Tamil Nadu, India.

The temperature in the rearing chamber was maintained at 28 ± 2ºC and the RH was at 73±5 %. All the rearing operations were carried out according to Krishnaswami *et al*. (1973). The larvae were divided into control and experimental groups with 3 replications of 50 larvae each and fed with same quantity and quality of mulberry leaves. Fresh mulberry leaves were cleaned with sterile cloth, weighed quantity of leaves were sprayed with pre and probiotics using a hand automizer. The pre and probiotics sprayed leaves were allowed to dry in shade for 15 min prior to feeding. Treated leaves were given as the first feed for the day beginning from the day of each instar.

Feeding and pre and probiotic supplement preparation

In view of the consumption levels equal and known quantity of leaf was offered to all silkworm batches on a daily basis. For the present experiment commercial pre and probiotics selected for supplementation was, Flora-SB. Flora-SB sachet (1gm) contains, *Lactobacillus acidophilus* – 0.4 billion, *L. rhamnosus* – 0.4 billion, *Bifidobacterium longum* – 0.4 billion, *B.bifidum* – 0.25 billion, *Saccharomyces boulardii* – 0.05 billion and Fructo oligo saccharides – 100 mg.

The commercial pre and probiotics Flora - SB was dissolved in luke warm water and kept 12 hours for fermentation and prepared at the concentration of 1%, 3% and 5% by dissolving 0.1, 0.3 and 0.5 g in 10 ml luke warm distilled water. Fresh mulberry leaves were cleaned with a sterile cloth. The pre and probiotics sprayed fresh leaves were allowed to dry in shade for 15 minutes prior to feeding. Treated leaves were given as the first feed of the day beginning from the first day of each instar. Control worms were given normal leaves.

Determination of nutritional indices

The healthy larvae were counted daily in each treatment of three replications and the unequal weak / unhealthy larvae if any, were replaced by healthy ones of the same age from the reserve stock. The left over leaves and the excreta were dried in a hot air oven at 80ºC, and the values were recorded. However initial and final weight of the larvae were recorded to determine the larval growth. Food consumption, utilization and other nutritional indices such as approximate digestibility (AD), efficiency of converting leaf ingested (ECI) and leaf digested (ECD), consumption rate (RCR), growth rate (RGR) and coefficient of metabolism (COM) were calculated as suggested by Waldbauer (1968) and Kaushal *et al*. (1988). The nutritional efficiency parameters were calculated by the following formulae;

Food consumption = Initial weight of leaves — weight of unconsumed leaves (in g dry weight / day / animal)
Assimilation = Food consumed - weight of litter (in g dry weight / day / animal)
Tissue growth = Final body weight - Initial body weight of larvae (in g dry weight / day / animal)

$$\text{AD (in \%)} = \frac{\text{Assimilation}}{\text{Consumption}} \times 100$$

$$\text{RCR (in \%)} = \frac{\text{Food consumed}}{\text{Fresh leaf supplied}} \times 100$$

$$\text{RGR (in \%)} = \frac{\text{Weight gain of the larva during feeding period}}{\text{Duration of feeding period} \times \text{Mean weight of larva during (days) feeding period}} \times 100$$

$$\text{ECI to larva (in \%)} = \frac{\text{Tissue growth}}{\text{Consumption}} \times 100$$

$$\text{ECD to larva (in \%)} = \frac{\text{Tissue growth}}{\text{Assimilation}} \times 100$$

$$\text{COM (in \%)} = \frac{\text{Assimilation - Tissue growth}}{\text{Assimilation}} \times 100$$

Cocoon Characters

The matured larvae were mounted using bamboo mountages and the cocoon were harvested on the 5th day and used for calculation of commercial parameters, such as cocoon length and weight, pupal weight, shell weight and shell ratio percentage. Length, weight and diameter of the whole cocoon were obtained as per Nirwani and Kaliwal (1996).

Shell ratio was calculated by the formula,

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

Quantitative analysis of sericin and fibroin

For quantitative analysis of sericin and fibroin, individually weighed cocoon was soaked in 20 ml of 0.5% KOH for 6hrs. Then the protein sericin was removed by washing in boiled distilled H_2O twice, leaving behind the other protein filament fibroin. The crucible fibroin content was oven dried at 90°C for 24 hours. The weight of fibroin and sericin was determined by the formulae;

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.

From each treatment 10 cocoons were selected for estimating filament length and size deviation. The fresh cocoons were dried in a hot air oven for a period of 5 hours by the temperature profile as following 100°C (1 hour), 90°C (1 hour), 80°C (1 hour), 70°C (1 hour) and 60°C (1 hour).

Qualitative analysis of cocoon

The cocoons were cooled and brought to a reeling temperature of 40°C by sprinkling cold water. The cocoons were individually reeled in epprouvettes having one metre circumference. The reeling was carried out at a speed between 120 to 150 metres/ min.

$$\text{Filament length (m)} = \frac{\text{Number of rotations in epprouvette}}{\text{Cocoon}} \times \frac{9}{8}$$

$$\text{Denier} = \frac{\text{Conditioned weight of raw silk(g)}}{\text{Total length of raw silk (m)}} \times 9000$$

$$\text{Raw silk percentage (\%)} = \frac{\text{Weight of raw silk reeled}}{\text{Weight of cocoon}}$$

$$\text{Elongation (\%)} = \frac{\text{Elongation length}}{\text{Original length}} \times 100$$

Reelability = The percentage ratio of unbroken filament to the whole filament length represents the reelability of the cocoons.

$$\text{Renditta} = \frac{\text{Constant}}{\text{Shell ratio}}$$

Neatness

Silk filament neatness of different treatments were carefully examined with the help of a hand magnifying lens and needle. The portions of filament where the defects observed was again examined under a low power of the microscope and recorded the number of loops.

Statistical analysis

The data were analysed using Students t-test and foundout the presence and absence of significance (Khan and Khanum, 1994).

RESULTS

The observations recorded on the effect of supplementing different concentrations of pre and probiotic Flora - SB on the nutritional parameters of *B.mori* were statistically analysed and presented in Table 1. Mean food consumption of fourth and fifth instar control *B.mori* larvae was 0.512 ± 0.02 and 1.602 ± 0.04g respectively. Food consumption of fourth (0.558 ± 0.01g) and fifth (1.836 ± 0.07g) was to be highest under the concentration of 3% pre and probiotics. Mulberry leaves foliated with 3% concentration of pre and probiotic significantly increased the food consumption by 14.60 per cent in the fifth instar, over control larvae. It is observed that the assimilation was significantly increased by 22.92 per cent in 3% concentration treated *B.mori* larvae followed by 21.25 per cent (5% concentration) of fifth instar, over the control larvae. The highest mean tissue growth of fourth and fifth instar of 3% pre and probiotic treated larvae was 0.118 ± 0.002 and 0.366 ±0.004g. The percent change over was significantly increased by 21.59 per cent in the fifth instar of 3% pre and probiotic treated larvae. The lowest (78.40 ± 2.92 per cent) and highest (84.94 ± 3.89 per cent) mean value of AD was observed in the fifth instar of control and 5% pre and probiotic treated larvae respectively. ECD was higher in the fourth instar (28.35 ± 1.12 per cent) larvae treated with 5% concentration. The treatment groups with 5% concentration have significantly increased by 16.18 per cent when compared with control (24.40 ± 0.92 per cent) groups. The same trend was noticed with ECI, and significantly increased by 16.46 per cent in the fourth instar. Relative consumption rate increased maximum in the fifth instar (96.49 ± 4.72 per cent) larvae, fed with 5% pre and probiotic. But the average RGR was highest in the fourth instar (19.82 ± 0.64 per cent) larvae, under 3% concentration, followed by 5% (19.21 ± 0.62 per cent) 1% (16.45 ± 0.41 per cent) and control (15.48 ± 1.02 per cent). RGR of fourth instar

larvae under the treatment of 3% concentration increased significantly by 28.03 per cent against its respective control. However, COM did not evince significant difference among the different treatments.

indicated that significant differences of fibroin content were observed with 3% and 5% treated groups.

Table 1. Effect of Flora-SB on energy parameters of silkworm, B. mori larvae

Treatments (concentrations)	Instars	Food consumption (g)	Assimilation (g)	Tissue growth (g)	AD (%)	ECD (%)	ECI (%)	RCR (%)	RGR (%)	COM (%)
Control	IV	0.512± 0.02	0.381±0.04	0.093±0.001	74.41±1.41	24.40±0.92	18.16±1.41	76.13±1.21	15.48±1.02	75.59±2.22
	V	1.602±0.04	1.256±0.03	0.301±0.007	78.40±2.92	23.96±1.05	18.79±0.89	83.09±3.65	45.50±2.21	76.03±3.84
1%	IV	0.530±0.01 (3.51)	0.398±0.02 (4.46)	0.106*±0.001 (13.97)	75.09±3.71 (0.91)	26.63*±1.02 (9.13)	20.00*±0.43 (10.13)	82.80±3.65 (8.76)	16.45±0.41 (6.26)	73.36±2.71 (-2.95)
	V	1.772*±0.08 (10.61)	1.404*±0.07 (11.78)	0.341*±0.003 (13.28)	79.23±4.55 (1.05)	24.28±0.78 (1.33)	19.24±0.68 (2.39)	89.41±4.47 (7.60)	49.04±1.25 (7.78)	75.71±2.53 (2.18)
3%	IV	0.558±0.01 (8.98)	0.439*±0.07 (15.22)	0.118*±0.002 (26.88)	78.67±3.42 (5.72)	26.87*±1.04 (10.12)	21.14*±0.84 (16.40)	86.12*±1.36 (13.12)	19.82*±0.64 (28.03)	73.12±2.35 (-3.26)
	V	1.836*±0.07 (14.60)	1.544*±0.05 (22.92)	0.366*±0.004 (21.59)	84.09±4.45 (7.25)	23.70±1.11 (-1.08)	19.93±0.68 (6.06)	94.99*±4.14 (14.32)	54.06*±1.84 (18.81)	76.29±2.61 (0.34)
5%	IV	0.539±0.02 (2.70)	0.402±0.02 (5.51)	0.114*±0.002 (22.58)	74.58±4.25 (0.22)	28.35*±1.12 (16.18)	21.15*±0.43 (16.46)	83.25*±3.81 (9.35)	19.21*±0.62 (24.09)	71.64±3.21 (-5.22)
	V	1.793*±0.05 (11.92)	1.523*±0.06 (21.25)	0.354*±0.004 (17.60)	84.94±3.89 (8.34)	23.24±1.03 (-0.08)	19.74±0.72 (5.05)	96.49*±4.72 (16.12)	53.59*±2.33 (17.78)	76.75±3.26 (0.94)

Percent deviation over control values in parentheses
* Significant
t – test values significant at $p \leq 0.05$

The effect of pre and probiotic Flora - SB on cocoon quantitative characters viz., cocoon length, breadth, weight, shell weight, shell ratio, sericin content and fibroin content observed in the present investigations are summarized in Table 2.

Highest mean fibroin content was 0.30 ± 0.01g with 3% whereas its counter control was 0.25 ± 0.01g. The effect of 3% concentrations of pre and probiotic Flora- SB was consistent overall cocoon characters except shell ratio.

Table 2 Influence of selective commercial pre and probiotics on cocoon quantitative characters of silkworm, *B. mori*

Treatments	Concentrations	Cocoon length (cm)	Cocoon breadth (cm)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)	Sericin content (g)	Fibroin content (g)
Control		3.2±0.14	2.0±0.09	1.55±0.06	0.31±0.01	20.00±0.87	0.06±0.002	0.25±0.01
Flora.SB	1%	3.4±0.08 (6.25)	1.9±0.01 (-5.00)	1.80*±0.08 (16.12)	0.34*±0.01 (9.67)	18.89±0.86 (-5.55)	0.06±0.001 (0.00)	0.28*±0.01 (12.00)
	3%	3.6*±0.12 (12.50)	2.1±0.08 (5.00)	1.98*±0.09 (27.74)	0.37*±0.01 (19.35)	20.22*±0.64 (1.10)	0.07*±0.004 (16.66)	0.30*±0.01 (20.00)
	5%	3.5*±0.16 (9.37)	2.0±0.05 (0.00)	1.90*±0.07 (22.58)	0.35*±0.02 (12.90)	18.42±0.75 (-7.90)	0.06±0.003 (0.00)	0.29*±0.01 (16.00)

Percent deviation over control values in parentheses.
* Significant t – test values significant at $p \leq 0.05$

The cocoon length and breadth of control silkworm, *B.mori* was 3.2 ± 0.14cm and 2.0 ± 0.09cm respectively, whereas the highest mean cocoon length (3.6 ± 0.12cm) and breadth (2.1 ± 0.08cm) were found under 3% concentration of pre and probiotic administered larvae. Mulberry leaves foliated with 3% concentration, significantly increased the cocoon weight by 27.74 percent over control (1.55 ± 0.06g). The maximum mean shell weight registered was 0.37 ± 0.01g when supplemented with 3% concentration and this was significantly increased by 19.35 per cent to that of control (0.31 ± 0.01g). As far as sericin content concern positive trend was observed in 3% concentration treated groups. The result of the experiment

Table 3 shows variations in the qualitative traits of cocoon, when larvae were fed with pre and probiotic supplemented mulberry leaves. The experimental results indicated significant variations between control and treated groups. The mean filament length reached maximum at 3% concentration (804.26 ± 23.12 m) supplemented larvae. The treatments with 3% concentration enhance filament length significantly by 15.84 per cent when compared with control (694.28 ± 22.12 m). Significant reduction in the case of filament size (13.54 per cent) and renditta (10.85 per cent) was registered with 3% concentration pre and probiotic fed larvae when compared with control.

Table: 3 Influence of selective commercial pre and probiotics on cocoon qualitative characters of silkworm, *B. mori*

Treatments	Concentrations	Filament length (m)	Denier	Renditta	Reelability (%)	Raw silk (%)	Elongation (%)	Neatness (points)
Control	-	694.28±22.12	2.51±0.13	9.21±0.39	78.30±4.21	10.97±0.43	14.52±0.63	80.71±3.23
Flora - SB	1%	718.42±21.33 (3.40)	2.32±0.14 (-7.56)	9.61±0.31 (6.51)	79.13±2.62 (1.06)	13.03*±0.61 (18.79)	15.87*±0.74 (9.29)	80.84±2.47 (2.16)
	3%	804.26*±23.12 (15.84)	2.17*±0.09 (-13.54)	8.21*±0.23 (-10.85)	83.16±2.65 (6.20)	15.23*±0.52 (38.83)	16.74*±0.57 (15.28)	83.27±2.46 (3.17)
	5%	743.51±13.47 (7.09)	2.29±0.06 (-8.76)	9.42±0.17 (2.28)	80.42±3.52 (2.70)	14.86*±0.43 (35.46)	16.43*±0.23 (13.15)	81.92±3.48 (1.49)

Percent deviation over control values in parentheses
* Significant t – test values significant at $p \leq 0.05$

However, pre and probiotic Flora - SB had no significant effect on reelability and neatness, but raw silk and elongation significantly increased by 38.83 and 15.28 per cent respectively, when larvae fed with 3% concentration treated mulberry leaves.

DISCUSSION

B.mori like other Lepidopteran insect is highly specialized for rapid growth, primarily achieved by higher rate of food consumption. In the present investigation, food consumption significantly increased in the fifth instar larvae, when exposed to different concentration of pre and probiotic Flora - SB. Maximum (1.836 ± 0.07g) mean increase of food consumption was registered with 3% concentration of pre and probiotic supplemented larvae. This may be due to the nutritional supplementation as reported earlier (Amala rani *et al*., 2011a and b; Balasundaram *et al*., 2013). Food consumption has a direct relevance on the weight of larvae, cocoon, pupae and shell (Shiva kumar, 1995). The independent parameters of consumption and productivity vary depending on the type of nutrition and silkworm breeds (Rema devi *et al*., 1992). In the present study, it has observed that the food consumption and utilization have found to be lowered in the fourth instar than the subsequent stage of growth and development. In general, the present results are in agreement with the observations of earlier workers (Balasundaram *et al*., 2008; Rath, 2010; Lakshmi Bai and Ramani Bai, 2011). In the present work, assimilation, tissue growth and approximate digestibility of different concentration of pre and probiotics treated larvae showed an increasing trend when compared to the control. Among the different concentration, 3% concentration reported significant increase. In the present study, in general it has been observed that the assimilation and AD have found to be significantly increased by 22.02 and 8.34 per cent in the fifth instar when compared to the control. The present results are in agreement with the observations of Amala rani *et al*. (2011a) and Ganesh prabu *et al*. (2012). According to Nath *et al*. (1990) the food consumption index (CI) and approximate digestibility (AD) varied depends upon the quality of leaf and age of the larva. Magadam *et al*. (1996) investigated that when ingesta increases, the total digesta increases. Food digestion and digestibility and larval growth inter related and the rate of digestion in silkworm increases with the advance of instar, which is highest about 65% in the fifth instar (Ueda, 1982).

Further, it has been revealed from the present study that the tissue weight was significantly increased in the fourth instar. Similar trend was noticed with ECD, ECI and RGR. In the present study, ECD, ECI and RGR declined with the advancement of larval development, while corresponding RCR increased which corroborated with the earlier findings (Waldbauer, 1968; Rath, 2010 and Amala rani *et al*., 2011a) which maintained the larval growth. The decline in ECD, ECI and COM might be due to the shorter residence time of food in the gut in younger larvae (Perez *et al*., 2000). Rath (2010) revealed that 95 - 96% of the total food intake of different larval instars was ingested during the last two instars, which confirms the result of the present findings. The decline in ECD, ECI, RGR and COM with age in *B.mori* might be due to the consumption of a higher proportion of indigestible fibre during the final (V) instar. Oral administration of dietary pre and probiotics in rabbit fish has shown the production of digestive enzymes such as amylase, protease and lipase which enrich the concentration of intestinal enzymes (El - Dakar *et al*., 2007; Lee and Lee, 1990) and promote faster digestion. According to Irianto and Austin (2002) probiotics may produce vitamins and detoxify the compounds in the diets or breakdown the digestible compounds, which may lead to the nutritional improvement and stimulate appetite. Gibson and Robert froid (1995) emphasized that prebiotics along with probiotic microbes promoting or stimulating bacteria in the intestinal tract, inturn improving the host intestinal balance.

In the present study, the important cocoon parameters significantly increased in all the treated groups but cocoon shell ratio decreased. Highly significant increase in cocoon weight, (27.74 per cent) was registered at 3% concentration as against the control. Previously it was reported that enrichment of mulberry leaves by vitamin (Etebari, 2002 and 2005; Ganesh prabu *et al*., 2012; Balasundaram *et al.,* 2013) and Amway protein (Amala rani, 2011a) could increase cocoon weight and shell weight significantly. The specific dose of *Spirulina* with 300 ppm concentration (Venkadesh kumar *et al*., 2009), *Spirulina* and yeast with 300 ppm concentration (Masthan *et al.,* 2011) and 1:1 ratio of probiotic and a neutraceutical combination (Bai and Bai, 2012) as feed supplementation to *B.mori* found to be effective in increasing single cocoon weight, shell weight, and silk filament length. Similar results were obtained when eri silkworm *Samia cynthia ricini*, Boisduval were treated with *Spirulina* (Jaya prakash *et al*., 2005). However, in the present experiment, pre and probiotics

did not improve the shell ratio and sericin content. Enrichment of mulberry leaves by pre and probiotics could increase leaf quality, which reflect on the quantitative performance of cocoon significantly, but it cannot be expected that always there is a positive correlation between the supplementation and biological performance. In the present result, it is clear that supplementing the diet with pre and probiotics does not have significant influence on the shell ratio and sericin content. The results of the present study are in agreement with Radjabi (2010), that supplementation with amino acids asparagine and alanine does not have any positive impact on the economical traits significantly.

The results of the present study indicated that high significant differences between the treatment groups in respect of qualitative characters. It has been revealed from the present study that the length of the filament and other important price fixing qualities of the silk fiber found to be maximum at 3% concentration of pre and probiotics administered groups. The results confirmed the earlier findings in the same insect, with supplementation of antibiotic Amoxicillin (Thilagavathi *et al*., 2013), Amway protein (Amalarani *et al*., 2011a), Probiotic *B.bifidum* and yeast (Amalarani *et al*., 2011b) and commercial pre and probiotic (Lakshmi Bai and Ramani Bai, 2011). Anandakumar and Michael (2011) emphasized that the supplementation of 'SERIFEED' enhanced filament length and weight significantly. Fortification of mulberry leaves with glycine (Babu, 1994), Soybean flour (Sundarraj *et al*., 2000) and 10% Amway protein (Amala rani *et al*., 2011a) significantly enhance finer denier in *B.mori*. In the present study, commercial fiber qualities like reeling and raw silk percentage changed in different treatments.

Previously, it was reported that enrichment of mulberry leaves by folic acid (Nirwani and Kaliwal, 1996) and probiont *E.coli* isolated from *P.americana* (Ramani Bai, 2010) brings positive impact on the silk quality characters of *B.mori*. Sukumar (1983) has reported enhancing effect on silk yield using mulberry phylloplane yeast *Sporobolomyces roesus*. Narayanan *et al*. (1969) indicated that neatness is the important character by which the quality as well as excellence of silk fiber is judged. In the present study, similar observations may be attributed due to increased efficiency of digestion and assimilation of food materials, leading increased protein synthesis and subsequent accumulation of storage protein in the body on account of activity of probiotic microbial flora in the gut of host. The quality of the leaves has a profound superiority role in the silk production of *B.mori*. Enrichment of mulberry leaves by nutrient supplementation is one of the strategies by which cocoon and silk productivity can be increased and quality can be enhanced and maintained. Pre and probiotics supplemented in this study, beneficially affect the host by improving its intestinal microbial balance, thus inhibiting pathogens and enhance larval biomass and healthiness which inturn reflects on the qualitative and quantitative improvement of cocoon characters.

Conclusion

The commercial pre and probiotic administration has significantly induce food consumption, utilization and superior cocoon characters by maintaining a balance in the gut flora. Thus it is inferred that silkworm larvae fed with commercial pre and probiotics beneficially influence the energy and economic parameters of *B.mori*, which can be exploited in commercial cocoon production.

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