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## Economics of probiotic feed supplementation to mulberry silkworm

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### Abstract

The silkworm larvae were fed on mulberry leaves treated with four different kinds of probiotics feed supplements viz., *spirulina*, Azolla, yeast and soy milk at five different concentrations (1, 2, 3, 4 and 5%) each was supplemented to silkworm hybrid, PM × CSR-2 from fourth instar onwards through mulberry leaves every day once in the morning till the spinning stage during both June-July and March-April rearings. Among the probiotics tested Azolla was found to be superior for effective rate of rearing, cocoon weight and B:C ratio on the day of 50 per cent spinning followed by soy milk and yeast in comparison with control (un supplementation) in both the rearings. Among the concentrations tried, two per cent concentration was found superior in both the rearings. The interaction of feed additives (probiotics) along with their concentrations indicated that Azolla at two per cent was superior for effective rate of rearing, cocoon weight and B:C ratio.

**Keywords:** Economics, Probiotics, feed supplementation, mulberry silkworm

### Introduction

Silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) is an important economic insect is being used as a tool to convert mulberry leaf protein into silk. The utilization of silkworm for the production of natural silk in the form of cocoons has been exploited worldwide since its discovery during 2700 BC. China, India and Korea are among the world's leading silk producing countries and the silk goods export earnings decreased over the years in our country due to global recession. The export earnings during 2016-17 were Rs. 2495.99 Crores. India is the second largest producer of silk in the world with an annual mulberry silk production of 20478 MT with an area of 2.23 lakh hectares of mulberry during 2016-17 (Anon, 2016) [3].

Nutrition plays an important role in improving the growth and development of the mulberry silkworm like other organisms. It is stated that silk production is dependent on the larval nutrition and nutritive value of mulberry leaves, which plays a very effective role in producing good quality cocoons (Legay, 1958) [12]. Significant seasonal variations occur in the nutritional value and composition of mulberry leaves depending on factors such as the weather, pest and diseases as well as agriculture practices. Enrichment of the quality of mulberry leaves by nutrient supplementation is one of the strategies by which silk productivity and its quality can be enhanced and maintained.

*Bombyx mori* L. larvae depends on mulberry leaves as the sole natural food and the quality of the mulberry leaves has a direct bearing on the normal growth and development of the larvae as well as quality of the cocoon (Horie *et al.*, 1967) [7]. Nutrition is the single most factor that influences the growth and development of *B. mori* (Laskar and Datta, 2000; Kanafi *et al.*, 2007) [11, 9]. The growth and development of larvae and subsequent cocoon production are greatly influenced by the nutritional quality of mulberry leaves (Masthan *et al.*, 2011) [15]. In addition to mulberry leaves, feed supplements are also given to silkworm to enhance economic characteristics (Jayapaul *et al.*, 2003, Sheeba *et al.*, 2006) [8, 22]. Nutritional supplements include vitamins, amino acids, proteins and probiotics when added to larval feed tend to increase nutritional efficiency and economic traits of silkworm (Singh *et al.*, 2005) [23].

Probiotics derived from Latin word pro means "for" and Greek biotic means "life" is "for life" it defined in many ways. The term 'probiotic' was first used by Lilly and Stillwell in 1965 to describe the 'substances secreted by one microorganism that stimulate the growth of another'. Then in 1989, Fuller modified the definition to 'a live microbial feed supplement, which beneficially affects the host animal by improving its microbial balance'. Currently, Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) endorsed by the International Scientific Association for Probiotics and Prebiotics, define probiotics as "live microorganisms, which, when administered in adequate

amounts, confer a health benefit on the host". Several researchers have reported about beneficial role played by use of probiotics in humans.

Among the aquatic plant species azolla is a most promising wonderful plant from the point of view of nutritive value ease of cultivation and productivity. The use of azolla as a feed resource for fish, swine, poultry, rabbit and cattle had been tested with favourable results (Castill *et al.*, 1981) [4]. Patil (2010) [18] tested the Azolla supplementation to fifth instar silkworms with favourable results on cocoon traits. Keeping in view, the importance of nutritional value of mulberry leaves, present study aims to economics of probiotic feed supplementation to mulberry silkworm.

### Material and Methods

The present study was undertaken to know economics of probiotic feed supplementation to mulberry silkworm, *Bombyx mori* L. at the Department of Agricultural Entomology, College of Agriculture, University of Agricultural Sciences, Raichur, Karnataka. The experiment was carried out during 2016-17 at Raichur, which is situated in the North Eastern Dry Zone (Zone-II) of Karnataka between 16° 15' N latitude and 77° 20' E longitude with an altitude of 389 m above the mean sea level.

### Preparation of probiotic concentrations solution used for the study

Azolla was collected freshly, brought to the laboratory, washed thoroughly with clean tap water and subsequently with distilled water. Azolla was weighed to 1000 grams for extraction using 1000 ml distilled water. Initially Azolla was grinded with 100 ml of distilled water (out of 1000 ml) and sieved, the residue was mixed with 100 ml distilled water and grinded and sieved, and procedure was repeated 4-5 times and finally the filtrate volume was made up with the remaining distilled water and treated as stock solution. From this stock solution different concentrations were prepared *viz.*, one, two, three, four and five per cent and the same was used for feed supplementation as done by Vijaykumar *et al.*, 2016 [24]. *Spirulina* tablets were purchased from Raichur pharmaceutical shop and prepared 500 ml *spirulina* solution of different concentrations *viz.*, one, two, three, four and five per cent. Soy milk was purchased from the super market of Raichur and from that stock, different concentrations of the study was prepared and Baker's yeast (powder form) was purchased from the super market of Raichur and prepared the required concentrations of yeast solution appropriately used for feed supplementation.

### Method of supplementation

Different concentrations of probiotic supplements of half litre solution was prepared and taken in separate containers every alternate day treatment wise. The harvested mulberry leaves were dipped in probiotic solutions separately treatment and concentration wise and then fed to silkworms once daily (morning feed) treatment and concentration wise from fourth instar first day onwards till they reached spinning stage. Spinning larvae were separately mounted on netrikas for cocoon construction treatment and replication wise. Observations were made on larval weight a day before spinning, fifth instar and total larval duration; effective rate of rearing and fresh weight of silk glands on the day of 50 per cent spinning.

### Results and Discussion

Probiotics are the live microbial food supplements beneficially affecting host by improving the microbial balance and enhanced the rapid cellular growth and development (Fuller, 1993) [6]. There are no commercial probiotics formulations specifically designed for sericulture though they are available in for human, aquaculture and veterinary medicinal use. Singh *et al.*, 2005 [23] observed improvement in larval body weight, cocoon weight, shell weight and pupation percentage of silkworm larvae when fed on mulberry leaves treated with *Lactobacillus plantarum*. In the present study, the effect of Azolla, soya milk, yeast and *spirulina* on mulberry silkworm through leaf fortification have been investigated. It is well known that probiotics contain nutrient supplements such as proteins, carbohydrates, amino acids, vitamins, sterols, hormones, antibiotics etc. and are known to influence the growth and development as well as health of humans, poultry, fish and cattle. Attempts have been made by several authors to know the effect of probiotics under different concentrations in isolation on silkworms and in the present study the effect of different probiotics on mulberry silkworms under different concentrations has been studied for effective rate of rearing, cocoon weight and economics.

Supplementation of probiotics had the positive impact on ERR as compared to control in both the rearings. Maximum ERR of (95.73% during June-July rearing) and (92.87% during March-April rearing) was recorded in case of silkworms fed with Azolla supplemented leaves. The next best ones were absolute control and soya milk (94.33 and 93.33% for June-July rearing) and (91.67 and 90.47% for March-April rearing) respectively. Among the five concentrations tested, one to four per cent concentrations gave significantly higher ERR (92.67 to 93.17% in June-July rearing) and (90.00 to 90.61% for March-April rearing) (Table 1).

Present results are in line with the results of Vijaykumar *et al.* (2016) [24] who reported that the use of aqueous solution of Azolla at 50 per cent and 25 per cent concentrations had the maximum ERR of 97.16 per cent and 96.33 per cent, respectively. The lowest ERR was found in absolute control (85.27%); Anitha *et al.* (2015) [2] found Dorlac at two per cent had higher ERR in eri silkworms (95.00%) when compared to control (85.00%); Sampath *et al.* (2013) [21] reported that eri silkworms fed with *Anabaena variabilis* at 500 ppm concentration showed significantly maximum ERR (71.95%) as compared to control (39.78%) and Rekha and Neelu (2010) [20] observed maximum ERR of 90.02 per cent when supplemented with soybean and in absolute control the same was minimum (82.55%). The increased ERR observed in the present study may be due to less incidence of diseases in the treated silkworms and this might be due to influence of feed additives containing protein, amino acids, carbohydrates and minerals which act as building blocks of tissues making them robust and thus imparted healthiness to larvae. Further, due to better growth and development on account of nutritious feed received during silkworm rearing. The lowest ERR in *spirulina* might be due to increased concentration of *spirulina* supplementation to mulberry leaves, resulted in poor feeding and reduced survival rate.

The larvae of PM × CSR-2 fed on mulberry leaves supplemented with probiotics exhibited better cocoon weight and cocoon shell weight as compared to control.

Cocoon weight was significantly higher in azolla (9.50 g for June-July rearing) and (8.69 g for March-April rearing)

followed by soya milk, yeast and *spirulina* (7.25 to 8.14 g for June-July rearing) and (6.42 to 7.22 g for March-April rearing) in order. Among the concentrations tested, two per cent gave significantly higher cocoon weight (7.88 g and 1.36 g for June-July rearing) (7.00 g for March-April rearing) respectively (Table 2).

The cocoon weight depends on the rearing conditions, quality and quantity of the mulberry leaves supplied during rearing. The cocoon weight is important commercial character indicate the approximate quantity of raw silk output. The increased cocoon was attributed to daily feeding of mulberry leaves supplemented with probiotics to fourth instar and fifth instar silkworms. Also, the feed supplement ingredients involved in the synthesis of silk proteins and nucleic acids such as RNA and DNA in the silk gland cells there by improves the silk content in the cocoon shell (Ananda Kumar and Sandhya (2011) [11]. Feeding silkworms with leaf supplemented with a proteinaceous source can enhance the cocoon and shell weights has been reckoned in earlier works of Vijayakumar *et al.* (2016) [24] cocoon and shell weights were found in mulberry leaves supplemented with azolla at 50 per cent concentration (16.78 g) over control (13.27 g). In the present study, soya milk stood next to azolla and the previous workers also have found the beneficial effects of soya products on silkworms and accordingly Mane and Patil (1998) [14] reported that extra foliation of soybean flour at 4g/sq. feet bed area on mulberry leaves during fifth instar *B. mori* larvae (NB<sub>18</sub>) recorded significantly higher cocoon weight (2.39 g) Matsura (1994) [16] reported the higher cocoon weight on soybean meal (3.13 g) and Nalini *et al.* (1994) [17] noticed that dusting of defatted soybean flour at ratio of 12.5 g/kg mulberry leaves, once during third, fourth and fifth day of final instar of *Bombyx mori* L. increased cocoon yield.

Similarly, Rahul *et al.* (2017) [19] observed the beneficial effects of yeast at 0.5 and 1 per cent concentration on mulberry silkworm cocoon and shell weights over control and Anitha *et al.* (2015) [2] recorded that the probiotic Darloc at two per cent concentration gave significant increase of cocoon weight (4.61 g) when compared with control (3.99 g) in eri silkworms. The results pertaining to yeast in the present study was supported by earlier works of Esaivani *et al.* (2014) [5] where *Saccharomyces cerevisiae* treated MR2 mulberry leaves when fed to fifth instar *B. mori* larvae produced maximum cocoons and (35.12 g/10) in five per cent concentration. Kumar *et al.* (2009) [10] observed that feeding of *spirulina* at 300 ppm during fifth instar of CSR<sub>2</sub>×CSR<sub>4</sub> increased the cocoon weight (1.08 g).

Benefit cost ratio was worked out for the probiotic feed supplements considering the cost of the probiotics, common cost of rearing and sale price of the cocoons. Higher net returns of Rs. 13,778 was noticed in the batch of silkworms fed on the leaves supplemented with azolla followed by soya milk (Rs.9, 592), spirulina (Rs.6, 164), distilled water (Rs.5, 523) and yeast (Rs.4, 328). The benefit cost ratio was highest in azolla (2.34) followed by soya milk (1.93) (Table3).

It is concluded that the mulberry silkworm hybrid, PM × CSR-2 reared on mulberry leaves supplemented with two per cent Azolla once every day in the morning from fourth instar till spinning stage exhibited superiority for all the quantitative traits in both the rearings followed by soya milk, yeast and *spirulina*. Hence, probiotics as a feed supplements at two per cent concentration may be conveniently utilized to enhance the silk production after large scale trials. The benefit cost ratio of probiotic feed supplements indicated that the B:C ratio was highest in Azolla (2.34) followed by soya milk (1.93) as compared to other treatments.

**Table 1:** Effect of probiotic feed supplementation to mulberry silkworm, PM × CSR-2 on cocoon weight

Treatments	Cocoon weight (g/5 cocoon)													
	March - April rearing							June - July rearing						
	Concentration					Treatment mean	% ↑ over Ab. control	Concentration					Treatment mean	% ↑ over Ab. control
	1%	2%	3%	4%	5%			1%	2%	3%	4%	5%		
<i>Spirulina</i>	7.43	6.91	6.41	5.92	5.40	6.42	25.88	8.28	7.81	7.26	6.62	6.27	7.25	18.66
Azolla	8.03	9.59	9.09	8.60	8.12	8.69	70.39	9.32	10.30	9.79	9.29	8.81	9.50	55.48
Yeast	6.81	6.36	6.86	7.50	7.98	7.09	39.02	6.52	7.08	7.58	8.06	8.58	7.56	23.73
Soya milk	8.22	7.72	7.23	6.76	6.21	7.22	41.57	9.36	8.82	8.31	7.35	6.81	8.14	33.22
Water control	6.31	6.31	6.31	6.31	6.31	6.31	23.73	7.21	7.21	7.21	7.21	7.21	7.21	18.00
Absolute control	5.10	5.10	5.10	5.10	5.10	5.10	-	6.11	6.11	6.11	6.11	6.11	6.11	-
Concentration mean	6.98	7.00	6.83	6.70	6.52	6.81	-	7.80	7.88	7.71	7.44	7.31	7.63	-
	S.Em ±					CD @ 1%		S.Em ±					CD @ 1%	
Treatment (A)	0.02					0.06		0.03					0.09	
Concentration (B)	0.02					0.07		0.03					0.07	
Interaction (A×B)	0.06					0.16		0.07					0.20	

**Note:** % ↑ over Ab. control denotes per cent increase over absolute control.

**Table 2:** Effect of probiotic feed supplementation to mulberry silk worm, PM × CSR-2 on effective rate of rearing

Treatments	Effective rate of rearing (%)													
	March - April rearing							June - July rearing						
	Concentration					Treatment mean	% ↑↓ over Ab. control	Concentration					Treatment mean	% ↑↓ over Ab. control
	1%	2%	3%	4%	5%			1%	2%	3%	4%	5%		
<i>Spirulina</i>	90.67 (72.23)*	90.00 (71.57)	89.33 (70.94)	88.00 (69.73)	87.33 (69.15)	89.07 (70.72)	-2.84	92.33 (73.93)	91.33 (72.88)	90.67 (72.23)	89.33 (70.94)	88.00 (69.73)	90.33 (71.94)	-4.24
Azolla	93.00 (74.66)	94.67 (76.66)	93.67 (75.43)	92.00 (73.59)	91.00 (72.56)	92.87 (74.59)	1.31	95.33 (77.54)	97.33 (80.64)	96.33 (78.98)	95.00 (77.08)	94.67 (76.66)	95.73 (78.18)	1.48
Yeast	87.67 (69.44)	88.33 (70.03)	89.67 (71.25)	91.67 (73.23)	92.00 (73.57)	89.87 (71.50)	-1.96	90.67 (72.23)	91.00 (72.56)	92.33 (73.93)	93.67 (75.43)	94.00 (75.85)	92.33 (74.00)	-2.12
Soya milk	92.67	91.33	90.67	89.00	88.67	90.47	-1.31	95.00	94.00	93.33	92.67	91.67	93.33	-1.06

	(74.30)	(72.88)	(72.22)	(70.63)	(70.33)	(72.07)		(77.08)	(75.82)	(75.05)	(74.30)	(73.23)	(75.10)	
Water control	87.67 (69.46)	87.67 (69.46)	87.67 (69.46)	87.67 (69.46)	87.67 (69.46)	87.67 (69.46)	-4.36	91.00 (72.56)	91.00 (72.56)	91.00 (72.56)	91.00 (72.56)	91.00 (72.56)	91.00 (72.56)	-3.53
Absolute control	91.67 (73.23)	91.67 (73.23)	91.67 (73.23)	91.67 (73.23)	91.67 (73.23)	91.67 (73.23)	-	94.33 (76.24)	94.33 (76.24)	94.33 (76.24)	94.33 (76.24)	94.33 (76.24)	94.33 (76.24)	-
Concentration mean	90.56 (72.22)	90.61 (72.31)	90.44 (72.09)	90.00 (71.64)	89.72 (71.38)	90.27 (71.93)	-	93.11 (74.93)	93.17 (75.12)	93.00 (74.84)	92.67 (74.42)	92.28 (74.05)	92.84 (74.67)	-
	S. Em ±			CD @ 1%				S. Em ±			CD @ 1%			
Treatment (A)	0.28			0.73				0.29			0.78			
Concentration (B)	0.25			0.67				0.26			0.70			
Interaction (A×B)	0.62			1.64				0.65			1.73			

**Note:** % ↑↓ over Ab. control denotes per cent increase or decrease over absolute control.

\* Figures in the parentheses are arc sin transformed values

**Table 3:** Cost economics of probiotic feed supplementation to mulberry silkworm hybrid, PM×CSR-2

Treatment	Cocoon yield 100 dfl (kg)	Gross returns (Rs.)	Common cost (Rs.)	Treatment cost (Rs.)	Total cost (Rs.)	Net Returns (Rs.)	B:C Ratio (Rs.)
<i>Spirulina</i>	57.05	17,114	10,200	750	10,950	6,164	1.56
Azolla	80.23	24,068	10,200	100	10,290	13,778	2.34
Yeast	51.53	15,458	10,200	930	11,130	4,328	1.39
Soya milk	66.31	19,894	10,200	102	10,302	9,592	1.93
Water control	52.51	15,753	10,200	30	10,230	5,523	1.54
Absolute control	46.09	13,828	10,200	0	10,200	3,628	1.36

Price of cocoon Rs. 300/kg

*Spirulina* Rs.1200/kg

Azolla Rs.100/kg

Yeast Rs.1500/kg

Soya milk Rs.120/lit

Water Rs.1/lit

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