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Studies on Fortification of Mulberry Leaves with Probiotics for Improvement of Silk Quality

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Abstract

Nutrition plays an important role in improving the growth and development of silkworm Bombyx mori. Silk production is mainly dependent on larval growth and nutritive value of mulberry leaves. The purpose of this study is to investigate the impact of application of various food probiotics on mulberry leaf and using it for improvement of silk quality and quantity. Previous studies showed some promising results with certain microorganisms such as Spirulina and Saccharomyces cerevisiae etc. on cocoon quantity parameters. However in the present study an attempt is made to know the impact of food probiotics on not only on cocoon quantity but also the type of silk that is produced and its sercin and fibroin content. Total four different food probiotics organisms were given to the silkworm Bombyx mori (Bivolatain race) through the fortification of mulberry leaf *Moras alba*. At the end of silkworm rearing silk quality parameters such as sercin and fibroin were estimated in addition to these cocoon quantitative parameters like Cocoon weight, shell weight, pupal weight, shell percentage and silk filament length of silk worm Bombyx mori. Data was collected and subjected to the statistical analysis. The results showed that blue green algae Spirulina and *Saccharomyces cerevisiae* yielded better fibroin content indicating the good quality silk when compared to lactobacillus acidophilus and *Lactobacillus sporogens*.

Keywords: Sericulture, Probiotics, Spirulina, Yeast, lactobacillus, sercin, fibroin

INTRODUCTION

Silkworm, Bombyx mori L. is monophagus. It eats only mulberry only due to the presence of a chemical Morin [1]. The growth and development of larva, and subsequent cocoon production are greatly influenced by nutritional quality of mulberry leaves. Efforts are made to study the effect of fortification of nutrient supplements such as proteins, carbohydrates, amino acids, vitamins, sterols, hormones, antibiotics etc. for better performance and get higher yield, quantity and quality of cocoons[2]. There are no commercial probiotics formulations specifically designed for sericulture though they are available in for human, aquaculture and veterinary medicinal use[3,4].Probiotics microorganisms are to be nonpathogenic and non toxigenic, and to retain viability during storage and survive passage through the stomach and small bowel[5] . Singh et al., 2005[6] observed improvement in larval body weight, cocoon weight, shell weight and pupation percentage of silkworm larvae when fed on mulberry leaves treated with Lactobacillus plantaram. However there are no studies on the colonization of *L.plantarum* in silkworm, gut. Spirulina and yeast were used as probiotics in experimental studies of silkworm [7,8]. But their studies were limited to cocoon quantity but not on silk quality In the present investigation an attempt is made to study the effect of four different probiotics such as , Blue green algae Spirulina, Yeast *Saccharomyces cerevisiae*, *Lactobacillus acidophilus* and *Lactobacillus sporogens* on the quantitative as well as qualitative cocoon parameters viz., cocoon weight, shell weight, pupal weight, shell percentage and silk filament length, sercin and fibroin contents.

MATERIALS AND METHODS

Rearing

Rearing of silk worms were conducted at Moshe Farms at S.C. colony, Kaligiri village of Kaligiri Mandal of Sri Potti Sriramulu Nellore district during the year 2013-2014.The silkworm belonging to cross breed (NB_4D_2)of *Bombyx mori* (L) have been reared in Farm ($28^o \pm 2^oC$, 80 to 85% RH) by following rack method described by Krishnaswamy[9] (1978).

Material:

V_1 variety mulberry leaves (Moras alba) were used for the present experimentation. Blue green algae (Spirulina) was procured from the Department of Biotechnology, D.R.W. College, Gudur and the yeast was purchased in local bakery. Standard culture of Lactobacillus *acidophilus and* Lactobacillus sporogens procured from National Collection of Industrial Micro-organisms (NCIM 2083), National Chemical Laboratory, Pune was cultured in MRS broth medium. The broth culture of *L. acidophilus* and Lactobacillus sporogens (10^6 cfu/ml) was used for this experiment.

Treatments

Four different feed additives (probiotics) like *Spirulina*, Yeast (*Saccharomyces ciriviceae*), *Lactobacillus acidophilus* and *Lactobacillus sporogens* were selected for the present study. 300ppm of each feed additive were prepared by disperse the feed additive in clean UV irradiated tap water. Freshly harvested mulberry leaves were soaked. Healthy and uninjured II instar larvae were selected for experiment and they were divided in to five batches. First batch of silk worms were fed with Mulberry leaves and treated as control. Second batch of Silk worms were with *Spirulina* 300ppm third batch with Yeast, Fourth batch with *Lactobacillus acidophilus,* and fifth batch with *Lactobacillus sporogens*. During experimentation the cocoon quantitative parameters like cocoon weight (grams), shell weight (centigrams), pupal weight (grams), silk filament length (meters) were recorded[10] (Nirwani and Kaliwal, 1996). Shell ratio (percentage) was also computed. Sercin and Fibroin were estimated by the method given below.

Methods

ECONOMIC CHARACTERS

LARVAL WEIGHT: Larval weight was taken by using an electronic balance in grams.

COCOON CHARACTERS: The mature fifth instar larvae were picked up from rearing trays and kept on Chandrika for spinning the cocoons. The cocoons were harvested after 5 days of spinning. Assessments of various cocoon parameters were made as follows.

COCOON WEIGHT: Ten randomly selected cocoons (five male and five female) were taken and weighed using an Electronic balance. The weight was expressed in grams.

PUPAL WEIGHT: After removing the floss, the cocoons were cut open and the pupae were taken out without causing any damage to them. Then the ten pupae (five male and five female) were weighed using an electronic balance.

SHELL WEIGHT: Ten shell weight (five male and five female) of the cocoon, after removing the floss and pupa were weighed using an electronic balance.

SHELL %: The Shell ratio will be calculated using the following formula and expressed in percentage.

Shell % = Shell weight x 100 / Cocoon weight

SILK CHARACTERS

FILAMENT LENGTH: Cocoons from each replication were stifled in boiling water and threads from individual cocoons were reeled using an epprouvette and observed for their silk characters such as silk filament length and silk filament weight.

RENDITTA: For estimating the vendetta from the shell %.the following constants were used.

- 165 for cocoon with shell % of 14-16%
- 150 for cocoon with shell % of 17-20%
- 133 for cocoon with shell % of 21-23%

Renditta = Constant /Shell ratio

DENIER: Denier was the unit, used to denote the thickness of silk filament. It is the weight of 9,000m length of silk expressed in grams. Filament denier is measured using an epprouvette and a denier scale. The value of denier varies from 1.7 to 2.8. It is calculated by using the formula.

Denier = Weight of filament(g) X 9000 Length of filament

SERICIN AND FIBROIN CONTENTS OF THE COCOON:

Individual cocoons were taken in a weighing crucible to which 20m1 of 0.5% percent KOH was added and allowed to remain soaked for 6 hours. The protein sercin was be removed by washing in boiling distilled water twice, leaving behind the protein filament, fibroin. Then the crucible containing fibroin was oven dried at 90°C for 24 hours. The weight of fibroin and sercin was determined by the following formulae.

Sercin Content (g) = Initial dry weight of the shell - Dry weight of the shell after alkali treatment

Fibroin Content (g) = Dry weight of the shell - Sercin content

Statistical analysis

The data were subjected to statistical analysis of variance for identifying significant differences among the treatments using standard method.

RESULTS

The data regarding the use of cocoon characters and silk quality was presented in Table.

Table: Effect of various Probiotics on the on the cocoon and silk characters of Bombyx mori.

(Values are mean of ten observations. ± Standard deviation; +/- indicate percent increase or decrease over control)

S.No	Parameter		Control	Spirulina treated	Yeast treated	Lactobacillus acidophilus Treatment	Lactobacillus sporogenes Treatment
1	Cocoon weight	Mean	1.65	1.87	1.81	1.74	1.7
		S.D.	±0.002	±0.003	±0.004	±0.02	±0.02
		%change over control		13.333	9.697	5.455	3.03
2	Pupal weight	Mean	1.35	1.42	1.4	1.39	1.39
		S.D.	±0.01	±0.01	±0.011	±0.009	±0.008
		%change over control		5.185	3.704	2.963	2.963
3	Shell weight	Mean	0.3	0.45	0.41	0.35	0.31
		S.D.	±0.001	±0.002	±0.003	±0.002	±0.004
		%change over control		50	36.667	16.667	3.333
4	Cocoon/shell ratio	Mean	18.182	24.064	22.652	20.115	18.235
		S.D.	±0.01	±0.002	±0.003	±0.002	±0.004
		%change over control		32.351	24.585	10.631	0.291
5	Thread per revolution	Mean	1.59	1.79	1.65	1.62	1.59
		S.D.	±0.01	±0.01	±0.011	±0.009	±0.008
		%change over control		12.579	3.774	1.887	0
6	Filament length	Mean	810.9	912.9	841.5	826.2	815
		S.D.	±22	±67	±33	±35.	±41
		%change over control		12.579	3.774	1.887	0.506
7	Filament weight	Mean	0.246	0.296	0.226	0.22	0.25
		S.D.	±0.01	±0.010	±0.011	±0.009	±0.008
		%change over control		20.325	-8.13	-10.569	1.626
8	Denier	Mean	2.73	2.918	2.877	2.832	2.775
		S.D.	±0.1	±0.25	±0.12	±0.02	±0.1
		%change over control		6.886	5.385	3.736	1.648

9	No. Of cocoon reeled	Mean	440	490	480	470	465
		S.D.	±12	±21	±8	±11	±13
		%change over control		11.364	9.091	6.818	5.682
10	Realability	Mean	44	49	48	47	46.5
		S.D.	±0.02	±0.023	±0.022	±0.012	±0.017
		%change over control		11.364	9.091	6.818	5.682
11	Sercin content	Mean	0.39	0.33	0.35	0.37	0.38
		S.D.	±0.002	±0.001	±0.001	±0.002	±0.002
		%change over control		-15.385	-10.26	-5.128	-2.564
12	Sercin %	Mean	28.9	23.239	25.000	26.619	27.338
		S.D.	±1.02	±1.44	±1.06	±0.99	±0.89
		%change over control		-19.588	-13.495	-7.893	-5.405
13	Fibroin %	Mean	71.1	76.761	75.000	73.381	72.662
		S.D.	±2.3	±2.1	±1.67	±2.89	±1.99
		%change over control		7.962	5.485	3.208	2.197

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	13	1421.338	109.3337	58660.88
Column 2	13	1585.038	121.926	74138.26
Column 3	13	1500.875	115.4519	64527.25
Column 4	13	1471.837	113.2182	62132.54
Column 5	13	1453.13	111.7792	60546.46

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1191.616	4	297.904	0.004655	0.999956	2.525215
Within Groups	3840065	60	64001.08			
Total	3841256	64				

From the above table we can deduce that all most all ccocoon charecters and silk quality was superior in Spirulina treatment followed by yeast Sacchiromysis cerviceae.

DISCUSSION

The digestive system is home to many types of bacteria. They help keep the intestines healthy and assist in digesting food. They are also believed to help the immune system. These friendly organisms also help fight bacteria. In probiotics therapy, live microbial feed supplements are improving the intestinal microbial balance of host. These non-pathogenic bacteria play a key role in enhancing resistance to colonization by exogenous potentially pathogenic organism. Many bacterial strains have been evaluated for ability to normalize the properties of abnormal native micro flora and reinforce various aspects of intestinal defense. Pathogen growth in the intestines. Probiotics can stabilize the structure in the intestinal barrier and maintain rigidity in the tight junctions between epithelial cells. Probiotics can also stimulate the body's innate defense mechanisms, as with the increased production of the antimicrobial peptide 'defenses'' in the intestines.

Lactobacillus plantarum in vitro model demonstrated its ability to prevent adherence of a pathogenic strain, as well as increased the expression of protective proteins. Colonized probiotics ferment dietary fiber, and in doing so can induce pH and other chemical changes in the intestinal lumen (cavity) that also affect the inhibition of pathogen growth. Additionally, short-chain fatty acids are released as a byproduct of bacterial fermentation display anti-inflammatory properties in the epithelial (intestinal lining) cells.

Various live microorganism (probiotics) have been demonstrated to modify the composition of the micro flora, restore the microbial balance and therefore have the potential to provide health benefits when normal intestinal flora is disturbed due to diarrhea, food toxification etc. Probiotics prevent infections due to competition for binding sites and available substrates, lowering lumina. PH, production of 'bactericins' and production of other antibacterial substances enhancement of intestinal motility and up gradation of genes mediating innate immunity.

CONCLUSION

Fortification of mulberry leaves by using supplementary nutrient and feeding to the silkworms is a useful modern technique to increase economic value of cocoon[11,12]. Fortification of food with certain vitamins successfully tried as a prophylactic measure in silkworm[13].The present investigation concluded that two probiotics namely Spirulina and Yeast significantly promotes the cocoon characters and silk quality, when compared to lactobacillus acidophilus and Lactobacillus sporogens.

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