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Probiotic supplementations to improve commercial characteristics, disease resistance and protein In the silkworm *Bombyx mori* L.

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Abstract

Generally the early Instars of silkworm larvae (I,II,III Instars) were more susceptible to bacterial infection than the IV and V Instar stages. Studies on bacterial pathogenicity were conducted on IV Instar mulberry silkworm *Bombyx mori*. Assessment of pathogenicity and identification of bacterium of *E.coli* were isolated from flacherie infected silkworm larvae. It is most common and deadly diseases in seri field. The present study showed that LD50 dose of *E.coli* infected to IV Instar larvae was 2.4×10^6 cells/ml and determined the LD50 value. When the inoculated worms were treated with the probionts *Bifidobacterium bifidum* and yeast at different concentrations. *Bifidobacterium bifidum* showed significant enhancement in larval weight, cocoon weight, shell weight, shell ratio, filament length, filament width, Denier and fibroin content. *Bifidobacterium bifidum* with a concentration of 6% was very effective. Mulberry leaves treated with *Bifidobacterium bifidum* (6%) fed larvae recorded a maximum shell ratio (11.87) filament length (956.33 ± 47.18) Denier (13.3%) and fibroin content (0.29 ± 0.03) Shell ratio in this treatment (6%) showed an increase of 11.87% whereas yeast treatment (6%) also increase of 13.8% and over the control, Various growth parameters like relative consumption rate (RCR) relative growth rate (RGR), weight gain, approximate digestibility (AD), efficiency of conversion of digested food (ECD) and efficiency of conversion of ingested food (ECI) were enhanced by the Amway supplementation. Determined the quantity of protein in *Bifidobacterium bifidum* (6%) 67.5%. whereas the yeast treatment (6%) had 28.57% over the control. Feed supplementation not only enhanced economic and nutritional parameters but also prevent bacterial infection in *B.mori*.

Keywords: Mulberry silkworm, Probiotic bacteria, LD 50, Pathogenicity, Economic parameters, Energy budget.

INTRODUCTION

In the farming system, Sericulture is an art and science of technology. The science and technology was inter related to each other. The science was implemented in the technology and developed in rural economy. The silk is discovered in china as one of the best material for clothing. The 6000 miles path of **Silk Road** starts from China India and reached to European countries. Sericulture occupies a unique position in India, especially in the southern states it has become an important and alternative cash crop.

In the world, India ranks second in raw silk production next to China. Karnataka state alone produces bulk of Indian raw silk (**Govindan and Devaiah, 1995**). In Iran more than 74000 families are related with sericulture industry (**Mavvjpour et al., (1996)**). The total annual production of raw silk in India was 15.74 thousand tones, of which mulberry raw silk output aggregated to about 13.97 thousand tones during 2003-2004. However the demand for raw silk production is higher than the current production. Thus, India has emerged as the largest silk consumer and in turn, largest importer of raw silk.

Sericulture occupies a unique position in Indian economy and assumes more importance in alleviating the problems of the poor in the rural areas. It is highly suitable in the context of diversification of farm enterprises and integration of farming system with other enterprises and has the capacity to generate attractive income.

The Silkworm is a Poikilotherm and it is a monophagous insect. It survives solely on mulberry leaves (*morus sp.*). The quality of the leaves has a profound superiority of silk produced by the *B.mori*. Silkworms respond very quickly to the environmental changes particularly to temperature and relative humidity (**Priyadharshini et al., 2008**). Higher or lower temperature and humidity, without proper ventilation and feeding adversely affect the physiological functions of the silkworms as a result of which they became highly susceptible to diseases. Four silkworm diseases are very common in India viz., grasserie (viral), flacherie (bacterial), muscardine (fungal) and pebrine (protozoan). Among these diseases, bacterial flacherie is considered as one of the serious diseases of silkworm rearing (**Sidhu and singh (1968)**) reported that 70% of diseases are caused by flacherie and this disease reduce the rate of crop production. **Pasteur (1970)** reported bacteria as etiological agents of silkworm flacherie. **Metalnikov and cubonie (1928)** found that *Serratia marcescens* is one of the causative organism of flacherie.

CHARACTERISTICS OF SILKWORM DISEASES

Silkworm diseases are generally divided into two broad categories: infectious and non-infectious. Infectious diseases are those caused by viruses, bacteria, fungi, protozoa and similar pathogenic micro-organisms which enter and harm the body of the silkworm. These diseases can be transmitted from infected larvae to

healthy ones. Those that are due to damage from arthropods, agricultural chemicals and mechanical injuries ones are termed non-infectious diseases.

Nutrition plays an important role in improving the growth and development of silkworm *Bombyx mori* like other organisms (**Legay J.M 1958**). As the silk production is dependent on larval nutrition and nutritive value of mulberry leaves play an effective role in producing good quality of cocoons.

In recent years attempts have been made in sericulture with nutrient such as protein, vitamin, carbohydrates, amino acids, vitamins, hormones, and antibiotic 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 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 eco-friendly management of silkworm diseases. (**Subramanian et al., 2009**).

According to **Charles (2004)** lower animals do not have well developed humoral immunity and under such circumstances vaccine development may not be of much use and in these lower animals immunostimulation 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. Nowadays, 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 probiotics to prevent bacterial infection by enhancing disease resistance potential and energy budget, economic parameters and protein content in silkworm.

MATERIALS AND METHODS

The disease free eggs of LXCSR2 race purchased from Government sericulture farm Nanagaram, Tirunelveli district Tamilnadu was used for the present study.

SILKWORM REARING

According to **krishnaswami et al., 1978** the rearing operations were carried out in the present investigation. Silkworms were reared under standard recommended condition at 26±2°C temperature, 75% relative humidity. They were fed with MR₂ variety of Mulberry leaves.

PATHOGENICITY STUDY

The surface sterilized silkworms were homogenized aseptically. An aliquot of the homogenate was streaked on the nutrient agar plates and incubated one day at 36±2 °C. The bacterial colonies were observed, and an individual colony was separated. The individual colonies identified by selective media were subjected to biochemical test. The identified bacteria strain viz., *Serratia marcescens*, *E.coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis* were infect the flacherie diseases. *E.coli* bacteria were allowed to infect *B.mori* in order to find out their pathogenicity. The third, fourth and fifth Instar larvae were fed with high concentration of 1x10⁹ cells/ml of live bacteria through microbial injection method. *Bombyx mori* was found susceptible to the bacterium.

LD50 value was determined for the fourth Instar larvae by the method of Reed and Munch (**Woolf, 1968**). The original bacterial suspension was serially diluted in serial physiological saline from (2.4x10¹ to 2.4x10⁹). The load of bacterial cells was determined by serial dilution and plate counting techniques.

Nine bacterial dilutions (2.4x10¹ to 2.4x10⁹) with three replications were allowed to infect the worms. From each dilution, 0.2 ml of bacterial culture was injected to the upper portion of the skin. The lethal concentration for 50% mortality was calculated as per the Reed and Muench using the formula (**Savithri and Murali Mohan, 2003**).

$$LD50 = \frac{\text{Lower limit} + \frac{50\% \text{ of lower limit}}{\text{Upper limit} - \text{Lower limit}} \times (\text{log upper limit} - \text{log lower limit})}{1}$$

PREPARATION OF PROBIOTIC BACTERIA

In the present experiment, *Bifidobacterium bifidum* and yeast were given as 'Probiotic' to the silkworm through feed supplementation. The probiotic stock solution was prepared. From the stock solution, different concentration was prepared (2%, 4%, 6%) for the treatment.

ADMINISTRATION OF PROBIOTICS TO B.MORI

Silkworms were fed with untreated leaves until the end of III Instar stage. The fourth Instar larvae were divided into two sets for the treatment. One set treated with *Bifido bacterium bifidum* treatment groups and another one with Yeast treatment. In the course of rearing the worms were grouped into (7) batches with 25 larvae in each batch. The probiotic bacteria in different concentrations were sprayed on the mulberry leaves separately and the treated leaves were allowed to air dry for 15 minutes. The probiotic coated leaves were fed to first day of IV Instar larvae up to V Instar stage which were already infected by *E.coli*. The leaves were given for three times a day. The leaves of the control worms were sprayed with water and the water was dried before feeding. Three replications were maintained for each treatment. The

weights of the worms were measured by the electronic balance. The fecal matter and unfed leaves were removed from the bed daily.

The observations on economic parameters such as mature larval weight, cocoon weight, pupal weight, shell weight, shell percentage, filament length, width, denier, sericin and fibroin content were determined. Food consumption and growth parameters were measured on dry weight basis (**Waldbauer, 1968**).

OBSERVATION ON ECONOMIC CHARACTERS:

➤ **LARVAL WEIGHT:**

Larval weight was taken by using an electronic balance

➤ **COCOON CHARACTERS:**

The mature fifth instar larvae were picked up from rearing trays and released on Netrikas for spinning the cocoon. The cocoons were harvested after 4 to 5 days of spinning. Assessments of various cocoon parameters were made as follows.

➤ **COCOON WEIGHT:**

Ten randomly selected cocoons 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 pupae were weighed using an electronic balance.

➤ **SHELL WEIGHT:**

The shell weight of the cocoon, after removing the floss and pupa was weighed using an Electronic balance.

➤ **SHELL RATIO:**

The Shell ratio was calculated using the following formula and expressed in percentage.

$$\text{Shell Ratio} = \frac{\text{Shell Weight}}{\text{Cocoon Weight}} \times 100$$

➤ **SILK CHARACTERS:**

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.

$$\text{Silk filament length} = \frac{\text{Number of rotations in epprouvette}}{\text{Cocoon}} \times \frac{9}{8}$$

➤ **RENDITTA:**

CSTRI have given certain constants that can be used for estimating the renditta from the shell ratio. The constants suggested by them are given below,

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

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

➤ **DENIER:**

Denier is the unit, used to denote the thickness of silk filament. It is the weight of 9,000m length of silk expressed in gms. The value of denier varies from 1.7 to 2.8. It is calculated by using the formula

$$\text{Denier} = \frac{\text{Weight of the filament (g)}}{\text{Length of the filament (m)}} \times 9000$$

Filament denier is used to estimate the number of cocoons required to reel the silk of a specific denier. Filament denier is measured using an epprouvette and a denier scale.

SERICIN AND FIBROIN CONTENTS OF THE COCOON:

Individual cocoons were taken in a weighing crucible to which 20ml of 0.5% percent KOH was added and allowed to remain soaked for 6 hours. The protein sericin was 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 sericin were determined by the following 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

The protein content of the haemolymph of V Instar larvae of control and probiont treated were estimated by adopting lowry's method.

RESULTS

In the present investigation, pathogenicity of the bacteria E.coli was investigated. LD 50 doses of the bacterial isolate to IV Instar larvae of B.mori was calculated 48 hours after bacterial administration (Table I). The present study showed that LD50 dose for E.coli to IV Instar larvae was 2.4x10⁶ cells/ml. Generally it is reported that the early Instars of silkworm larvae (I,II,III Instars) were

more susceptible to bacterial infection than the IV and V Instar stages (**Savithri and Murali Mohan, 2003**). In the present study the larvae tested were in IV Instar stages, so the LD50 doses of bacterial pathogens were little higher.

The symptoms observed in the larvae after pathogen administration are in general agreement with earlier report (loss of appetite, sluggishness, development of flaccid and discoloration, rapid pulsation in dorsal vessel, wriggling movement, vomiting brown fluid, excretion of soft faeces, lifting of heads, spasm, paralysis and sudden collapse (**Singh et al,1994, Savithri and Murali Mohan, 2003**))

Gururaj et al., (1999) had reported that the pathogenic microbial infection on B.mori induced a shift in metabolic profiles and the activities of enzymes like amylase, invertase, and trehalose and protease. Bacterial flacherie also inflicted the abnormal multiplication of bacteria in the larval gut lumen as reported by **Ratna sen et al., (2003)** and interfered with gut physiology causing poor feed intake. The poor food intake, bio-chemical changes, bacterial toxins and histopathological changes, in gut epithelium had affected the energy budget in the worms. The break down in energy budget was reflected in the weight of the larva and pupa, shell and cocoon weight, filament length and sericin and fibroin contents. Hence it is imperative to develop management strategies. Of the different management techniques, the enhancement of immunity by administrating suitable immune modulating agents was studied in the present investigation.

COMMERCIAL CHARACTERISTICS

The commercial characteristics of the silkworms were enhanced by the probiotic supplements, when compared to the worms that was not provided with supplements.

a) **PUPAL WEIGHT:**

The mean weight of the pupa was maximum (12.23±0.66) in 6% of Bifidobacterium bifidum treatment. It was followed by 4% treatment of B.bifidum (11.4 ±0.45). The pupal weight of the control was only (9.98 ±0.01). In yeast treatment worms the pupal weight was maximum (11.32 ±0.44) in 6% concentration (Table II).

b) **SHELL RATIO:**

The shell ratio is an important commercial characteristics of B.mori. When compared to control, the shell ratio had increased 2.75%, 8.88% and 11.87% in 2%, 4%, and 6% treatment of B. bifidum respectively (Table III). The shell ratio had increased to 4.81%, 10.12% and 13.8% in 2%, 4% and 6% treatment of yeast respectively. This was in accordance with the results of previous workers.

c) **FILAMENT LENGTH:**

The filament length was maximum, (956.33±47.18m) in B.bifidum 6% treated worms (Table III) and it showed an increase of 24.3% when compared to the control. Silkworm larvae treated

with 4% dilution of *B.bifidum* showed a filament length ($858\pm 70.02\text{m}$) and it was 11.57% more than the control. The filament length of worms treated with 4% and 6% of yeast are ($848\pm 64.62\text{m}$) ($884\pm 82.48\text{m}$) respectively. The percentage of increase was 10.27 and 14.95. This suggests that the supplements have an ability to increase the length of the filament.

d) FILAMENT WIDTH:

The width of the silk filament also increased significantly on treating the worms with the probionts *B.bifidum* and yeast.

e) DENIER:

There was not much difference in the denier value of worms treated with 2% dilution of yeast. However 4% and 6% of yeast treatment showed an increase of 5.17% and 9.28% than the control. The worms treated with 2% , 4% and 6% dilution of *B.bifidum* treatment showed an increase of 4.08%, 7.21% and 13.3% than the control.

f) FIBROIN AND SERICIN:

The silk protein fibroin and sericin were also increased significantly in the treated worms. The probionts *B.bifidum* and yeast not only gave resistance to the bacterial pathogen but they also increased the commercial characteristics significantly. *B. bifidum* was found to be more effective than yeast. Worms treated with *B.bifidum* at 6% dilution showed a maximum fibroin content (0.29 ± 0.03) and sericin (0.14 ± 0.04) whereas in control it was 0.21 ± 0.04 and 0.04 ± 0.03 respectively (TableII).

g) RENDITTA:

Significant change in Renditta value was also observed in treated worms.

ENERGY BUDGET:

The energy budget revealed that there is a tremendous increase in the weight of the treated larvae when compared with the control (Table IV). In the IV Instar

stage maximum larval weight (52.93 ± 0.93) was noticed in silkworms fed with *Bifidobacterium bifidum* treated mulberry leaves when compared with the control (38.8 ± 2.25). In the V Instar stage also maximum weight was observed in larvae fed with *B.bifidum* treated mulberry leaves at 6% dilution ($91.4\pm 0.95\text{mg}$) when compared with the control ($76.5\pm 1.80\text{mg}$).

The weight was maximum in IV Instar larvae fed with yeast at 6% dilution treated mulberry leaves ($52.46\pm 0.87\text{mg}$). In control worm the body weight was only ($39.14 \pm 2.45\text{mg/animal/day}$). In the V Instar stage the larval weight was ($84.54\pm 0.37 \text{mg}$) when fed with yeast at 4% dilution and ($88.67\pm 0.67\text{mg}$) when fed with yeast at 6% dilution. Whereas the larval weight for the control was ($75.34\pm 1.90 \text{mg dry wt/animal/day}$)

Growth Index, Approximate Digestibility, Efficiency of Conversion of Digested food and Efficiency of Conversion of Ingested food were increased when the worms were administered with *B.bifidum* at 2%, 4% and 6% dilution and yeast at 2%, 4% and 6% dilution. The result was highly significant at $P<0.05$.

The total haemolymph protein in control worms treated with mulberry leaves and experimental worms treated with mulberry leaf enriched with probionts *B.bifidum* at 6% dilution showed significant variations in protein profile (Table IV). The increment in the protein content of V Instar stage of probiont treated larvae was found to be high. An analysis of the protein increment of V Instar stage showed that *B.bifidum* 6% treated larvae showed a maximum increase of 67.5% over the control. In yeast 6% treated larvae the increase in haemolymph protein level was 28.5%.

Table: I
Mortality of the fifth Instar larvae of silkworm *Bombyx mori* due to *E.coli*

No of bacterial cells cfu/ml	Initial Number	No of bacterial cells cfu/ml	Survival	Dead Ratio	Survival Ratio	Mortality	Cumulative Mortality
10 ⁹	25	25	0	97	0	97/97	100.00
10 ⁸	25	22	3	72	3	72/75	96.00
10 ⁷	25	18	7	50	10	50/60	83.33
10 ⁶	25	12	13	32	23	32/55	58.18
10 ⁵	25	12	13	20	36	20/56	35.71
10 ⁴	25	8	17	8	53	8/61	13.11
10 ³	25	0	25	0	78	0/78	0.00

Mortality above 50%-50

$$\begin{aligned}
 LD50 &= \frac{\text{Mortality above 50\% - Mortality below 50\%}}{\text{Dilution above 50\% - Proportionate distance}} \\
 &= \frac{58.18 - 50}{22.47} \\
 &= \frac{8.18}{22.47} = 0.36 \\
 &= \text{Dilution above 50\% - Proportionate distance} \\
 &= 6 + 0.36 = 6.36
 \end{aligned}$$

$$\text{Antilog } 6.36 = 2.4 \times 10^6$$

$$LD50 = 2.4 \times 10^6$$

Table :II

IMPACT OF PROBIOTIC BACTERIA ON COMMERCIAL CHARACTERISTICS OF SILKWORM *BOMBYX MORI*

Probiotic treatment	Different dosages	Pupal weight	Sericin	Fibroin	Renditta
	Control	10.36±0.55	0.05±0.02	0.25±0.04	10.11±0.57
	2%	11.06±0.75	0.06±0.04	0.26±0.04	9.87±0.27
Bifidobacterium	4%	11.4±0.45	0.08±0.03	0.27±0.02	10.85±0.57
bifidum	6%	12.23±0.66	0.14±0.04	0.29±0.03	9.87±0.27
	Control	9.98±0.01	0.04± 0.03	0.21±0.04	11.25±0.07
Yeast	2%	10.11±0.22	0.05±0.02	0.22±0.03	10.85±0.57
	4%	10.45±0.36	0.07±0.03	0.24±0.02	9.87±0.27
	6%	11.32±0.44	0.011±0.04	0.25±0.04	9.25±0.07

Values are presented as MEAN±SD (10 cocoons for each parameter)

Table: III

REELING PERFORMANCE OF SILKWORM *BOMBYX MORI* FED WITH MULBERRY LEAVES COATED WITH PROBIOTIC BACTERIA IN DIFFERENT CONCENTRAIONS

Treatments	Different Dosages (%)	Shell Ratio	Filament length	Filament Width	Denier
	Control	16.01	769±37.72	2.10±0.08	32.33±0.5
	2%	16.45 (2.75%)	814±21.12 (5.85%)	2.1±0.09	33.65±2.51
Bifidobacterium	4%	17.44 (8.88%)	858±70.02 (11.57%)	1.76±0.25	30.66±4.16 (7.21%)
bifidum	6%	17.91 (11.87%)	956.33±47.18 (24.3%)	2.53±0.10	31.66±3.21 (13.29%)
	2%	16.78 (4.81%)	805.33±39.06 (4.68%)	2.52±0.09	32.33±1.15
Yeast	4%	17.63 (10.12%)	848±64.62 (10.27%)	2.41±0.14	34±1 (5.17%)
	6%	18.22 (13.8%)	884±82.48 (14.95%)	2.32±0.04	36.33±0.5 (9.28%)

Significant at P< 0.05

Table IV

Influence of Probiotic bacteria on the Energy Budget of *Bombyx mori.L* (X±S.D)

Treatments	Stadium	Different Concentration	Weight of the larvae (mg dry wt/animal/day) (X±SD)	Growth Index (%)	A.D (%)	ECD (%)	ECI (%)
Bifido Bacterium bifidum	IV	2%	48.16±2.15	0.83	93.56	67.4	55.76
		4%	50.5±0.50	0.98	94.79	66.7	58
		6%	52.93±0.93	0.99	95.44	68.34	59.35
		Control	38.8±2.25	0.66	90.34	61.71	50
	V	2%	82.2±0.98	2.12	95.08	78.5	64
		4%	87.9±1.73	2.45	95.57	79	66.76
		6%	91.4±0.95	2.56	96.19	82.2	75
		Control	76.5±1.80	2.28	93.58	70	62.34
Yeast	IV	2%	40.34±1.67	0.59	90.14	67.34	66.79
		4%	45.63±0.53	0.76	92.23	65.54	68
		6%	52.46±0.87	0.80	94.67	69	68.54
		Control	39.14±2.45	0.58	91.1	66.67	64.45
	V	2%	80.34±0.99	2.11	93.32	76.4	63
		4%	84.54±0.37	2.14	94.45	77	65.11
		6%	88.67±0.67	2.16	95.67	78.5	74.32
		Control	75.34±1.90	2.05	92.23	71	61.78

A.D = Approximate Digestibility

ECD = Efficiency of Conversion of Digested food

ECI = Efficiency of Conversion of Ingested food

Significant at $P < 0.05$

Fig 1: Shell ratio of control and inoculated and probiont treated larvae

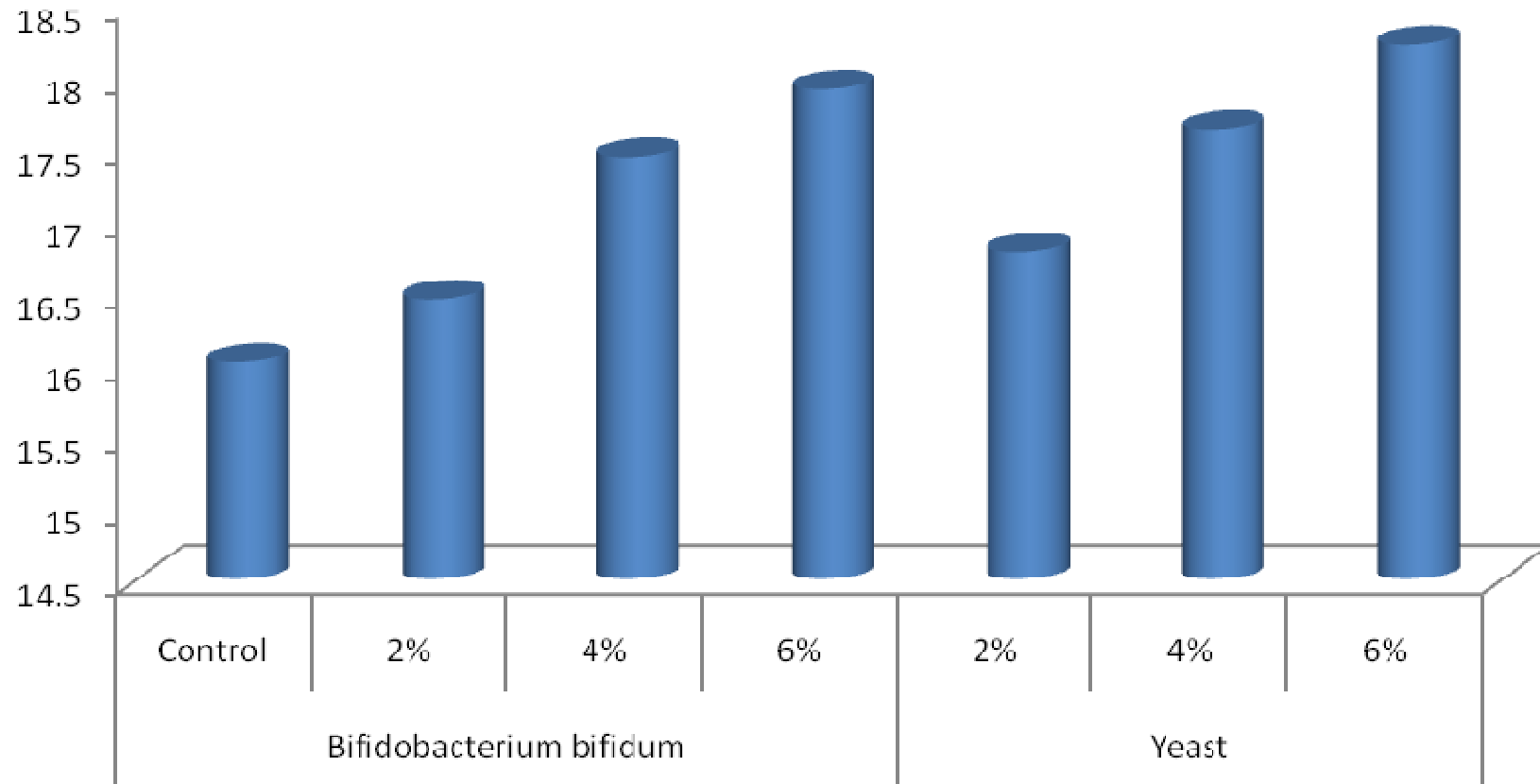
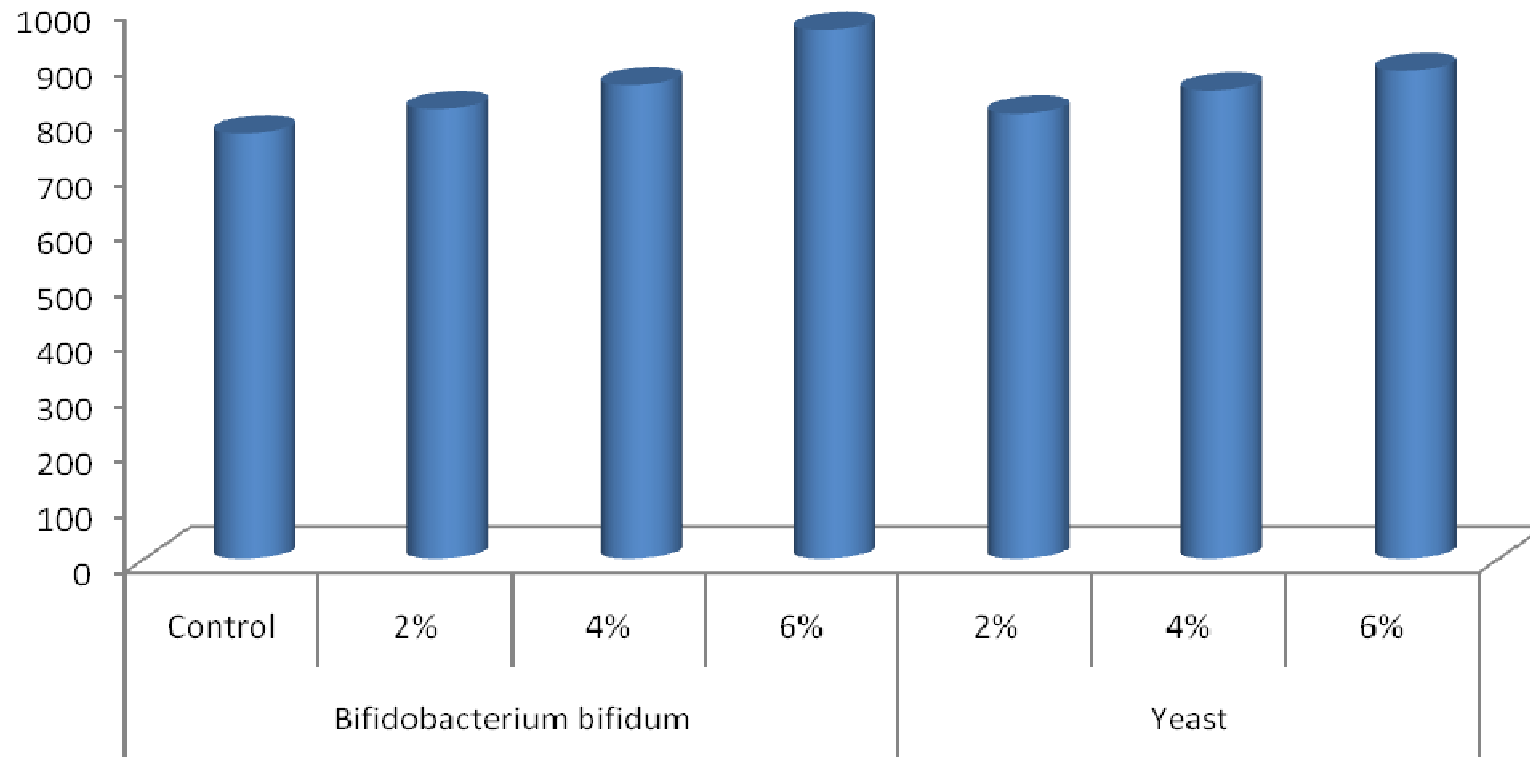


Fig2: Filament length of control and inoculated and probiont treated larvae



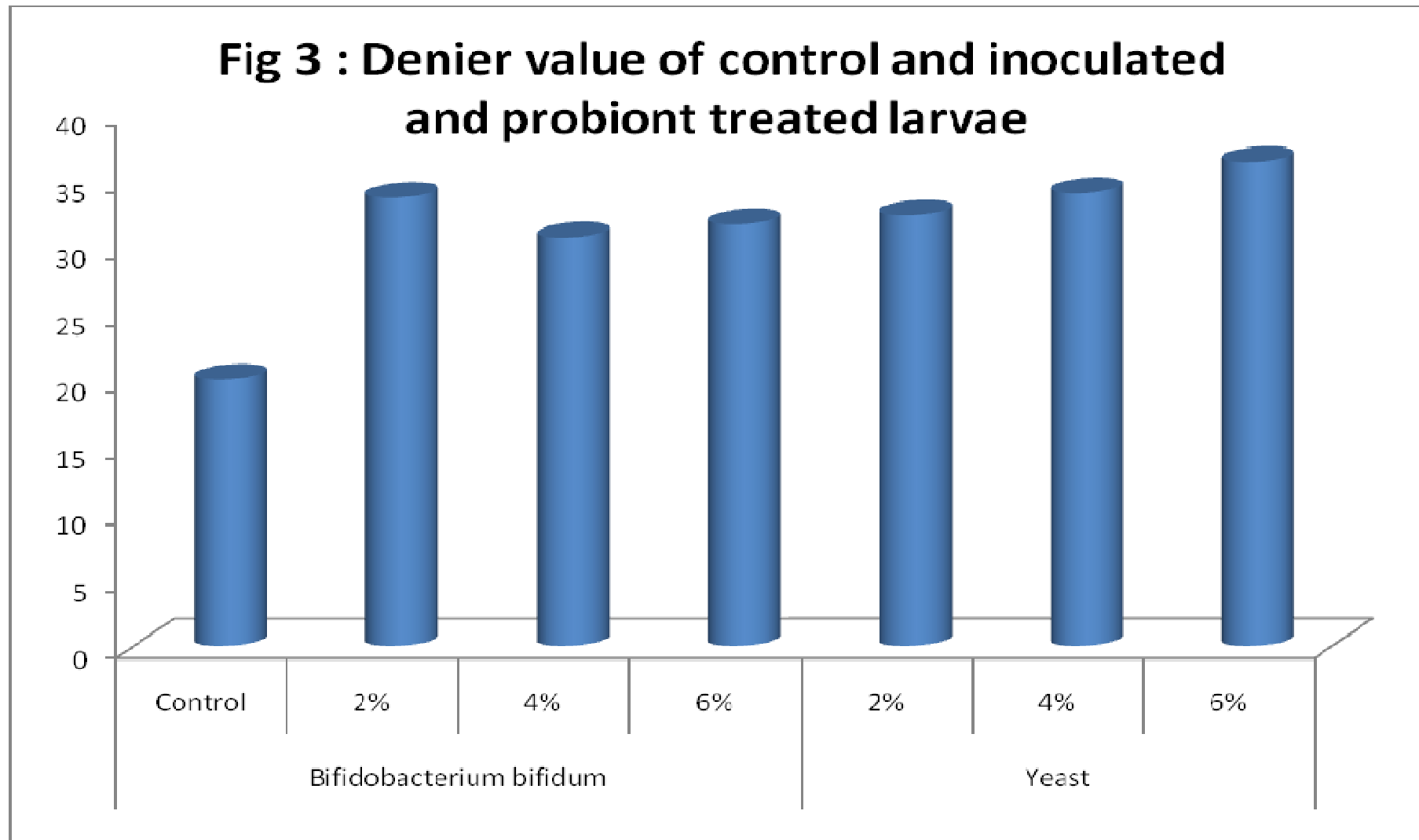
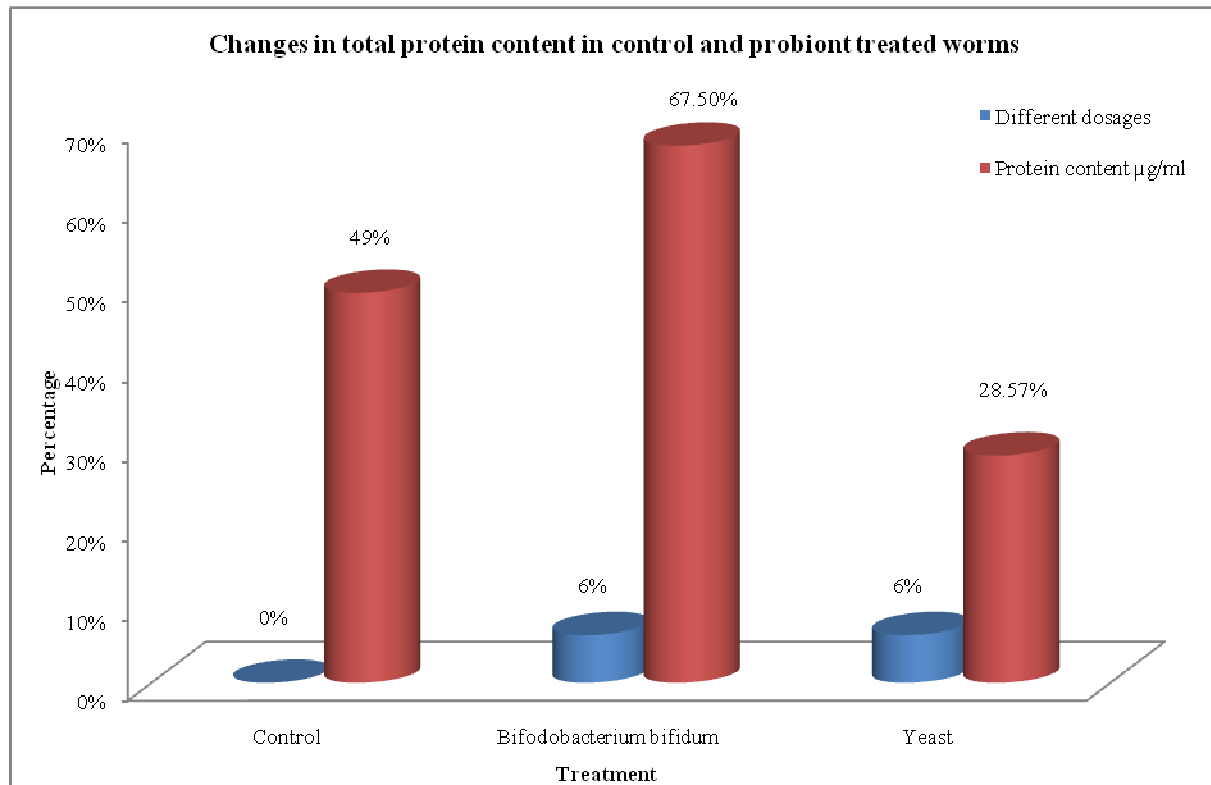


Table V

Changes in total protein content in control and probiont treated worms

Treatment	Different dosages	Protein content µg/ml
	Control	49
Bifodobacterium bifidum	6%	87 (67.5%)
Yeast	6%	63 (28.57%)



CONCLUSION

Sericulture is an agro based industry. Due to the operation of food chain and food web relationships in the natural ecosystems, silkworms have an antagonistic relationship with pathogens. The main disease reported in Tirunelveli District is bacterial disease and the most common bacterial disease is flacherie. In the present study the bacterium *E.coli* was inoculated to the silkworms and the LD50 value was determined. 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.

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*. There was a significant variation in protein profile of the control worms and experimental worms fed with mulberry leaves treated with the probiotics. When compared to control the protein content was high in the treated worms. The present study insists the upgradation of the immunity of silkworms rather than giving control measures for a disease.

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