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Studies on the efficacy of probiotic and vitamin on the growth and biochemical changes of silkworm *Bombyx mori* (L.)

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Abstract
Dietary need in food expending have direct effect on the comprehensive growth of silk worm and also rise the larval, pupae and cocoon weight with amount of silk formation. Cocoon characters, both numerics as well as subjective; depend chiefly on the quality and quantity of mulberry leaves. Utilization of health foods enriched mulberry leaves to impact the silkworm larval body weight and sway the silk output. Probiotics are matter which put up to intestinal microbial balance. The present study culminates the effect of supplements on the biochemical parameters of silkworm *B. mori*. The total soluble protein was evaluated by subsequent standard procedure with bovine serum albumin as the standard protein. The total protein, lipids and carbohydrates amount in the various parts of larvae rose when the larva was treated with shoot up concentration of sporlac. This study has been stipulated that the probiotics and vitamins reveal the existence of additional growth restorative and can be used to escalate the silk capitulate in mercantile silkworm nurture with credentials to sericulture.

Keywords: cocoon characters, nutritional requirement, probiotics, silk worm and vitamins

Introduction
Silkworm, *Bombyx mori* L. is utilizing a single kind of food that eats mulberry only .The quality of leaves provided to the worms for feeding has been ponder as the crucial element that outcome on fine cocoon proferring (Ravi Kumar, 1998). Even though the mulberry leaves are utter nourishment for silkworm, the enhancement of extra nutrients results plentiful (Rahmathulla *et al*., 2007) [27]. Nurture of silkworm is unique ingredient which almost singly augments load and variety of silkworm (Laskar and Datta, 2000) [20]. In current years, many researchers' strive have been build in sericulture with nutrient such as protein, vitamin, carbohydrates, amino acids, etc., for work on of aspect quality of cocoons (Sannapa *et al*., 2002) [32]. Healthy food includes vitamins, amino acids, proteins and probiotics when added to larval feed tend to raise nutritional productivity and fiscal traits of silkworm (Amala *et al*., 2011) [1]. Probiotics are picky fermented elements that lead to recast in the makeup and/or activity of the gastrointestinal microbiota, with resulting satisfaction for the host's welfare and fitness (Roberfroid, 2007) [29].
In current years, aims have been made in sericulture with nutrients such as protein, vitamins, carbohydrates, amino acids, vitamins, hormones and antibiotic etc for finer execution of high caliber of cocoons (Sannappa, 2002).
India is the second big promoter of silk in the world with a yearly mulberry silk making of 20,478 MT with an area of 2.23 lakh hectares of mulberry during 2016-17 (Anon, 2016) [2]. The silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) is a prime economic insect is being used as an aid to turn mulberry leaf protein into silk. Nourishment frolic a pivotal role in upgrade the growth and progress of the mulberry silkworm like the other organisms. It is stated that silk production is dependent on the larval nutrition and healthful value of mulberry leaves, which plays a very fruitful role in bring out preferable quality cocoons (Legay, 1958) [19]. In inclusion to mulberry leaves, feed supplements are also given to silkworm to intensify economic characteristics (Jeyapaul *et al.*, 2003, Sheeba *et al.*, 2006) [14, 33]. Nutritional additive include vitamins, amino acids, proteins and probiotics when added to larval feed tend to increase nutritional efficiency and economic attribute of silkworm (Etebari and Matindoost, 2005; Amalarani *et al.*, 2011, Singh *et al.*, 2005) [7, 1, 34, 37]. The present study has been focus to discern effective nutritional supplementary compound which one to mostly amplify the pupa and economic parameter with deem to food use by larvae and conclusive bang on the silk production.

Materials and Methods
The diseased free bivoltine hybrid (CSR_2XCSR_4) eggs of *Bombyx mori* silkworm were acquire from district sericulture office, Konam, During the first two instars, only soft leaves that have hold out ordinary size (topmost full blown leaf) were cater to the silkworms, after the methodology advocate by Krishnaswami (1979) [15] for infancy. After hatching, the worms were nourishing with MR_2, diversity of mulberry leaves.

Incubation
The DFLs (eggs) were incubated at temperature of 25^0c. Relative humidity of 80-85% was maintained. At the end of incubation period a blue spot was developed on each and every fertilized egg. The eggs at this stage were kept in dark box for static conceive of all the eggs at a time at a next day. In the dark condition, the untimely maturing embryos of silkworm are prevented from maturing and hence, hatching. The DFLs were kept in dark box for 24 hours.

Brushing
Brushing refers to separation of the rearing bed. On the next day after keeping in dark the DFLs were suddenly exposed to diffused light. The newly hatched young caterpillars were separated from the egg shell by brushing. The pieces of mulberry leaves were spread on the surface of egg card along with newly hatched young Caterpillars were separated from egg shell.

Feeding the Larval Instars
Feeding must satisfy the appetite and nutritional requirements of the larvae. The appetite deals with amount and frequency of feeding for each larval instar. Nutritional requirements deal with the quality of mulberry leaves. The growth required quality and quantity of mulberry leaves were harvested for each larval instars of silkworm, *Bombyx mori* tender and succulent leaves were chopped and provided to newly hatched larvae, the ants. Tender and mature leaves were chopped and given to the young age silkworm (1, II and III instars larvae). The late age silkworms (IV and V instar larvae) were supplied with mature leaves. For early instars the size of the chopped leaves was in creased along with increase in the age, late instars were fed with entire leaves.

Maintenance of Humidity and Temperature
During the rearing attention was paid to maintain the optimum environmental conditions constant in the rearing house. Humidity and temperature affect both the growth of larvae and quality of cocoons produced. Humidity has influence over the physiological activities of *Bombyx mori*. Constant humidity was maintained as per the requirements of the instar as indicated. During summer when the humidity was low and temperature was high, Khus curtains were used and water was sprinkled on them time to time. Probiotic tablet and Vitamin capsule were procured ram Bawa Medicals, Nagercoil Another second moult, the third instar larvae were cut up into four groups. Each set consists of 10 replicates of 50 worms every one. The first group was stated normal feedings 4-5 times a day and handle as the control. The abide group was ponder as the hypothetical batch and was treated with different concentrations (2%, 4%, and 6%) of Probiotic and Vitamin was prepared for the treatment. Raw mulberry leaves were marinate with every one concentration and the withered in air for 15 minutes. Handled leaves of various concentrations were fed to III, IV and V instar larvae, once daily.

Estimation of protein -Method Lowry *et al* (1951)
The sample was bring out by inclusion of 1ml of 30 percent trichloroacetie acid (TCA) solution lead centrifugation at 1000rpmfor 30 minutes. It was replicate twice and the precipitate was dissolved in 1 ml of 0.1 sodium hydroxide A known a liquid of this solution was then mixed with 5ml of alkaline copper reagent (20 percent sodium carbonate preparation in 0.I N sodium hydroxide hold in sodium potassium tartarate and one percent copper sulphate). After 10 min, 0.5 ml of Folin Ciocalteu's reagent was put on to the tube and the tubes were shivering completely. Then the tubes were kept for 20 minutes for colour forming. The readings were taken on the UV spectrophotometer at 650 nm. Bovine serum serves as the standard. The total protein amount of silk gland, fat body and muscles were expressed as mg/g and haemolymph as mg/ml.

Carbohydrate estimation (Schiefter *et. al*., 1950)
One gram of sample was ground well with 2ml of 10 % trichloro acetic acid and 8ml of distilled water. The homogenate was possessed and centrifuged at 4000rpm for 15 minutes. After centrifuged, 1 ml of supernatant was taken and added 4 ml of freshly prepared anthrone reagent. The tubes were covered with aluminium foil and kept in boiling water bath for 10mins. After cooling at room temperature the optical density of the sample was recorded at 620 nm wave lengths. Glucose solution gives out as the standard. The carbohydrate of silk gland, fat body and muscles were indicating as mg/g, and haemolymph as mg/ml.

Estimation of Lipid (Method Folch *et. al*. (1957)
Samples were integrated with apt volume of chloroform: methanol mixture. The homogenate was then quantitatively fetched to a 50 ml separating funnel and then an equal volume of chloroform was added. The two solvents were separate by the inclusion of distilled water. After the funnel was shaken, the mixture was permit to perch overnight. The lower chloroform layer hold lipid was void. The lipid sample was kept in vacuity desiccators until constant weight was acquired. The lipid of silk gland, fat body and muscles were manifest as mg/g and haemolymph as mg/ml.

Economic Parameters
The mature fifth instar larvae were lift up from nurture trays and liberate on chantrika for swing the cocoon.

Larval Weight
Larval weight was taken by using digital balance on the fifth instar.

Cocoon weight
Five cocoons were taken and contemplate using digital balance and weight was indicating in mg.

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 digital balance.

Shell weight
The shell of the cocoon is obtained after detach the floss and the cocoon was weighed using an electronic balance.

Shell ratio

Shell ratio was intended using the formula.

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

Filament length
Cocoon from each copy were move in boiling water and threads from independent cocoons were totter and measured expressed in meter.

Statistical Analysis
Data were fetched by means * SD. P values < 0.05 were consider as statistically notable.

Result and Discussion
The Probiotic and Vitamin were administered to silkworm at different concentration and the effect was studied on the growth, chemical and economic parameter of the larvae at different stages. Protein amount of silk gland of *B.mori* larvae fed with Probiotic and Vitamin is presented in 'Table 1. Protein content of silk gland increased to 19.10+0.65mg/g with 4 per cent and 18.30+0.40 mg/g with 2 per cent concentrat1on of Probiotic and Vitamin, respectively and decreased to 17.30+0.58mg/g with 2 per cent and 14.75+0.82 mg/g with 6 per cent concentration of probiotic and vitamin, respectively. When compare to control 16.10+0.45 mg/g. In Haemolymph maximum19.90+0.48 mg/ml was observed 4 per cent and 19.54+0.74mg/ml with 2per cent concentration of probiotic and vitamin, respectively. Protein content was reduced 18.55+0.74 mg/ml, when larvae fed with 6 percentand17.64+0.45 mg/ml with 6 per cent concentration of probiotic and vitamin, respectively. When compared to control 18.60+0.80 mg/ml. The maximum 22.45+0.75 mg/g amount of protein content muscles were observed, when the larvae fed with 4 per cent and 21.40+0.65 mg/g with 2% concentration of Probiotic and vitamin respectively and was Cent of vitamin. The minimum $0.94^{\pm}$ 0.06 mg/gm of lipid was observed in muscles of B. m1ori, when the larvae nourish with 6 per cent of vitamin. The haemolymph and muscles content of muscles were $2.32^{\pm}$0.31mg/ml and $03.15^{\pm}$0.32 mg/g increased, when larvae fed with 4 percent of vitamin and 2 per cent of Probiotic, respectively, The minimum amount of haemolymph and muscles content of muscles were $1.81^{\pm}$0.09 mg/ml and $2.10^{\pm}$0.49 mg/g. respectively at 6 per cent Probiotic.

Larval weight at V th instar
The mean body weight of control was 1540+35.50 mg. When treated with probiotic at 2, 4 and 6 per cent of concentration the calculated body weight was 1600 ± 56.00 mg, 1680 ± 62.20 mg and1590 ± 50.60 mg, respectively. Similarly the mean body weight of larvae was 1740 ± 48.75 mg, 1560 ± 82.50 mg and $1510^{\pm}75.10$ mg at 2, 4 and 6 percent concentration of Vitamin (Table 4).

Cocoon weight
Regarding the cocoon weight the control has 1350=48.40 mg and the larvae treated with Probiotic have $1365^{\pm}\ 46.00\ mg$, $1445^{\pm}$58.54 mgand$1400^{\pm}$ 46.60 mg at 2, 4 and 6 per cent concentration. Vitamin treated larvae showed $1520 \pm 50.40\ mg, 1340 \pm 26.75\ mg$ (Table 4).

Pupa and Shell weight
The pupa and shell weight of control *B. mori* larvae was 1141 ± 28.90 mg and 208 ± 22.90mg, respectively. The maximum 1264 ± 30.54 mg and 256 ± 30.10 mg pupa and shell weight was observed, when the larvae nourish with 2 per cent of vitamin. The minimum 1140 ± 22.80 mg and 190 ± 35.20 mg pupa and shell weight were observed, when the larvae nourish with 4 and 6 per cent of vitamin, respectively.

Shell Ratio

When shell ratio was intended for the control cocoons showed a Probiotic treated cocoon was 15.40 ± 0.80mg. The shell ratio of 2, 4 and 6 % probiotic treated cocoon was $15.97 \pm 0.82mg, 16.95 \pm 0.75$ mg and 15.35 ± 0.85 mg respectively. The calculated shell ratio for vitamin treated cocoons was 16.84 ± 0.75 mg, $14.92 \pm 0.60\ mg$ and $14.07 \pm 0.65\ mg$ at 2, 4 and 6% concentrations respectively.

Table 1: Protein content *B.mori* larvae fed with Probiotic and Vitamin

Treatment		Silk gland (mg/gm)	Haemolymph(mg/gm)	Muscles (mg/gm)
Control		16.10 ± 0.45	18.10 ± 0.80	20.40 ± 0.70
	2	17.30 ± 0.58 **	19.25 ± 0.64	22.05 ± 0.82 **
	4	19.10 ± 0.65**	19.90 ± 0.48*	22.45 ± 0.75 **
	6	17.40 ± 0.92*	18.55 ± 0.74	21.10 ± 0.52
	2	18.30 ± 0.40**	19.54 ± 0.74	21.40 ± 0.65 *
	4	16.00 ± 0.62	19.22 ± 0.62	19.30 ± 0.38 *
	6	14.75 ± 0.82 *	17.64 ± 0.45 *	16.32 ± 0.40 **

Mean ± S.D, *Significant **Highly Significant at P−<0.05, All other deviations are not significant.

Table 2: Carbohydrate content of *B. mori* larvae fed with Probiotic and Vitamin.

Treatment		Silk gland (mg/gm)	Haemolymph(mg/gm)	Muscles (mg/gm)
Control		12.70 ± 0.75	9.90 ± 0.50	6.10 ± 0.35
	2	12.60 ± 0.60	10.30 ± 0.48	7.20 ± 0.28 **
	4	15.20 ± 0.54**	10.00 ± 0.36	7.10 ± 0.42 **
	6	14.50 ± 0.30 **	9.58 ± 0.72	6.50 ± 0.70
	2	14.35 ± 0.52**	12.05 ± 0.72 **	8.15 ± 0.38 **
	4	11.80 ± 0.40 *	8.60 ± 0.46 *	6.40 ± 0.65
	6	9.20 ± 0.48 **	8.20 ± 0.64**	6.05 ± 0.40

Mean ± S.D, *Significant **Highly Significant at P−<0.05, All other deviations are not significant.

Table 3: Lipid content of *B. mori* larvae fed with Probiotic and Vitamin

Treatment		Silk gland (mg/gm)	Haemolymph(mg/gm)	Muscles (mg/gm)
Control		1.10 ± 0.04	1.92 ± 0.12	2.60 ± 0.56
	2	1.05 ± 0.03	1.84 ± 0.09	3.15 ± 0.32
	4	1.34 ± 0.04**	2.15 ± 0.17*	3.00 ± 0.38
	6	1.10 ± 0.06	1.81 ± 0.09	2.10 ± 0.49
	2	1.56 ± 0.07**	2.00 ± 0.18	2.54 ± 0.62
	4	1.01 ± 0.05 *	2.32 ± 0.31 *	2.64 ± 0.91
	6	0.94 ± 0.06 **	1.90 ± 0.15	2.42 ± 0.45

Mean ± S.D, *Significant **Highly Significant at P−<0.05, All other deviations are not significant.

Table 4: Economic characters of *B. mori* larvae fed with Probiotic and vitamin

Parameters	Control	Probiotic%			Vitamin %		
		2	4	6	2	4	6
Larval Weight(mg)	1540±35.50	1600±56.00	1680±62.20**	1590±50.60	1740±48.75**	1560±82.50	1510±75.10
Cocoon weight(mg)	1350±48.40	1365±46.00	1445±58.54*	1400±50.60	1520±50.40**	1340±6.75	1350±60.40
Pupal weight(mg)	1141±28.90	1148±32.65	1200±32.15*	1184±25.84*	1264±30.54**	1140±2.80	1160±.54
Shell weight(mg)	208±22.90	218±18.40	245±28,54.*	215±24.50	256±3010*	200±42.00	190±35.20
Shell ratio (%)	15.40±0.80	15.97±0.82	16.95±.0.75*	15.35±0.85	16.84±0.75**	14.92±0.60	14.07±0.65*
Filament lenghth (m)	830.47±19.87	867.83±25.11*	886.35±35.36 *	835.47±17.87	900.3±22.13**	792.74±18.54*	758.24±18.91**

Mean ± S.D, *Significant **Highly Significant at P−<0.05, All other deviations are not significant

Enhancement of mulberry leaves with vitamin E not has denoting out come on food expending in silkworm larvae (Mosallanejad *et al.*, 2002). Shafique, (1993) has describe that dry matter gobble by silkworm was corresponding to nitrogen amount of the leaves. Mahmood, (1989) has reported that nitrogen rise the body weight of larvae and gave finer cocoon production. He culminate that leaves plunge in 0.2% N solution bring out the larvae with topmost weight as cillated to the other doses Rehman,(1997) has be over that ideal doses of minerals in copious amalgamation, when used intensify silk production and silkworm growth to a better compass than control. During the intact larval life, mean nourishment was transmute into body matter was 74.55%. The A*loe vera* tonic at 2.0% concentration ensue better larval growth and raise the weight of cocoon (Manimuthu and Isaiarasu, 2010). The amino acid plays a pivotal role in glucose, tryptophan and organic acid metabolism. Little research has been supervised on amino acids addition; their results upgrade the silk building (Etebari and Matindoost, 2005). Suprakash & Pal (2002) amplify fresh mulberry leaves with 3 levels of vitamin B complex by dunk them in 0.5, 1.0 and 1.5% solution, and nourish the dried leaves to larvae of several *Bombyx mori* contest: the 0.5% level expand the weights of larvae and cocoons, and the shell ratio. Silk production basically depends on the *Bombyx mori* larval protein metabolism which in turn needs more energy create hap, spinning requires more muscular activity and silk is being produced by the silk gland. The quality of the leaves has a deep primacy of silk produced by the *B. mori* (Priyadharshini *et al*., 2008). Nutritional additive include vitamins, amino acids, protiens and probiotics when added to larval feed tend to increase nutritional efficiency and economic feature of silkworm (Etebari and Matindoost, 2005; Amalarani *et al.*, 2011; Singh *et al.*, 2005). From the forgoing literature, it is apparant that the impact of L-Serine on feed potency of *B. mori* is fragmentary. Therefore, it has been map out in the present study to know the feed efficacy and larval growth rate to be famed in *B. mori* in relation to silk production.

Similar trend was observed by Udupa, (1986) and Tayade, (1987), food ingestion and swallowable and growth in the larval phases are associated and the rate of digestion in silkworm escalate with the push on of instar, which is towering, about 65% in the fifth instar (Ueda, 1982).

Rath (2010) divulge that 95 - 96% of the gross food inlet of different larval instars was put away during the last two instars, which corroborate the result of the present detection. Probiotics make vitamins and crash the chewable compounds that steer to the nutritional upgrade and bracing appetite Irianto and Austin (2002). Gibson and Robert (1995) highlight that probiotics with probiotic encourage bacteria in the intestinal tract, that better the host intestinal balance. Digestive enzymes such as amylase, protease and lipase are building due to oral administration of dietary pre and probiotics in rabbit fish upgrade the concentration of intestinal enzymes (Lee and Lee, 1990 and El - Dakar *et al*., 2007) and foster digestion. Also set up that vitamin B complex notably better growth and development, with valuable effects on the economic attribute of the cocoon (Etebari and Fazilati, 2003). The upgrade of cocoon and silk characters in this experiment may be ascribing to upgrade of the output of turning of dietary nitrogen into the cocoon shell. Increase in silk protein showed appreciable in silk percentage when larvae of *B. mori* fed by ascorbic acid. The soar protein or nitrogen in the silkworm diet causes the surge of total protein in the larval haemolymph (Islam *et al*., 2004). Countless studies have outlined a range of compounds for better the economic characters of the larvae, such as body weight and cocoon weight (Bentea *et al*., 2011). The result of divergent vitamins on the dietary embellishment of mulberry leaves and it was found that all the vitamins exhibit a fringe benefit on *B. mori* growth and development (Kanafi *et al*., 2007). Upgrade the silkworm diet (mulberry leaves) with external nutrients such as proteins, carbohydrates, amino acids, vitamins, minerals, hormones, antibiotics and gauge their bang on larval growth, metabolism and silk production has become the order of traditional analysis in sericulture. Ascorbic acid notably increased the weight of *B. mori* larvae and pupae. Various authors propose that the boost in larval weight was allied to phagostimulation of ascorbic acid (Bhattacharya and Kaliwal, 2004). In higher doses of vitamin B or C in silkworm diet, the larval weight was significantly decreased (El-Karaksy and Idriss, 1999) bespeak that cater of elevated concentrations of ascorbic acid decreased the silkworm larval weight owing to hyper vitaminosis (Etebari *et al*., 2004). Low concentrations of thiamine notably lesson the developmental periods of silkworm (Khan and Saha, 1999). The larval length, width and weight have been refining when the larvae fed with the augmentation of vitamin C treated mulberry leaves (Ramesh *et al*., 2007). This monitoring procure support from the research findings of that nourish of silk worm on mulberry leaves enhance with multi vitamins from fourth instar escalate female cocoon shell weight at 2.5% concentration, when pupal weight got raised at 1% concentration.

Nithya *et al.* (2008) have also describe that climb in silk protein manifest distinct swap in silk percentage when larvae of *B. mori* nourished by ascorbic acid. The increase protein or nitrogen in the silkworm diet ideal the climb of total protein in the larval haemolymph. Supplementation of silkworm diet with various compounds can impact the amount of protein (Etebari and Fazilati, 2003). In order to study the impact of pre and probiotics on the protein level of silkworm larvae *B. mori*, three different tissues *viz*., silk gland, body tissue and haemolymph were selected in the current study. According to Bai (2013), the protein at ease of the body tissue of *B. mori* ranged from 11.21±0.25 (1% bifilac) to 13.58±0.25mg/dl (5% immunorm) among the treated larvae. Krishnan *et al*. (1995) also detailed, that the level of storage proteins in the haemolymph was increased by the surge concentrations of hydrolyzed soy protein enhancement. Thus it is deduce that silkworm larvae cater for with mercantile Probiotics and Vitamin profitable effect the cocoon building.

Conclusion

Silkworm hold on at most on a mulberry leaves. Nutritional grade of mulberry leaves escalated by rampart with live micro-organisms but these micro-organisms need indigestible matters to carry on its hold on for a long time to carry out its role unreservedly.

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