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Growth studies of popular silkworm (*Bombyx Mori* L) breeds influenced by dietary supplementaion of synthetic and phyto -ascorbate

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Abstract
Three popular silkworm breeds CSR2, CSR4 and PM were fed with three different sources of ascorbate *viz*., synthetic, crude quantified plant extract and ascorbate purified from plant. Supplementation was carried out with the already identified potential ascorbate doses during large-scale bioassay. Bivoltine breeds (CSR2 and CSR4) were fed with 0.05% ascorbate in crude Amla fruit extract (C) and 0.5% dose of synthetic (S) and purified ascorbate (P) in water. In case of multivoltine breed (Pure Mysore), 0.30% dose of "C", 3.00% of "S" and "P"were prepared in water. Daily larval and gonadal growth of three popular silkworm breeds CSR2, CSR4 and PM were recorded. In multivoltine breed, PM, no significant change was observed in the given traits with any of the three treatments. However, bivoltine breeds, CSR2 and CSR4, larval weight from 0 day of 5th instar to the 5th day was increased from 4.52 to 4.92 times over their controls (4.26 & 4.47 times). Improvement was more in 5th instar gonad weights than in larval weight. The 5th instar gonad weights were improved to the extent of 10.83 to 15.87% and 14.29 to 15.71% respectively vis-à-vis larval weight which increased only to the tone of 5.92-10.09% in bivoltine breeds (CSR2 and CSR4). There was a proportionate change in the tissue somatic index of gonad of treated and control larvae. The study gives an impression that ascorbate exerts its impact mainly on gonad than whole larval body. Further, in multivoltine silkworm breed, PM, gonad- tissue -somatic index was relatively higher than all the breeds studied. This appears likely that the gonads in the PM breed grow at relatively faster rate than rest of the silkworm breeds used.

Keywords: silkworm gonads, larval weight, tissue somatic index.

Introduction
In silkworm, *Bombyx mori* L, optimum gonadal growth is imperative for the production of viable gametes. Gonadal weights in females determine the quantum of fecundity and fertility which have direct correlation with silk production. In simple non-technical terms it can be stated that "a healthy gonad for higher fecundity and fertility". Nutrition is an important growth regulating factor in silkworm. Generally vitamins present in the mulberry leaves satisfy minimum needs of silkworm but the amount of vitamins present in mulberry leaves varies on the basis of environmental conditions, usage of fertilizers in field and mulberry varieties and other field practices (Ito, 1978) [12]. Ascorbic acid or vitamin C has always been regarded as indispensable for the growth and development of *Bombyx mori*. Most of the insects including silkworm are unable to synthesize ascorbate (Ito and Arai 1965, Bresci, 1951 and Lombardi, 1964) [13, 14, 2]. Ascorbic acid shows a particular behaviour as it is very susceptible to degradation, especially when in solution, or when exposed to light, oxygen, and free radicals.
Umpteen researchers have observed relationship of ascorbate supplementation and growth of silkworm. Supplementation of mulberry leaves with ascorbate has been studied more than any other vitamin (Etebari *et al.*, 2004). Several research demonstrated phagostimulatory (Ito, 1978; Dobzhenok, 1974) [12, 7] gustatory (Ito, 1961a) [10] and growth stimulating activity (Ito, 1961b & Tantray *et al.*, 2017) [11] of this nutrient. Effect of vitamin C on economic parameters of silkworm has been studied in detail (Tantray and Trivedy, 2008, Tantray *et al.*, 2011 & 2015) [20, 22]. The present work was attempted to determine the effects of vitamin C on popular breeds of the silkworm, *Bombyx mori* L.

Materials and Methods
The silkworm rearing was conducted by following standard rearing techniques (Rajan and Hemanthraju, 2005) [16]. Three silkworm breeds CSR2, CSR4 and PM were used for the

comparative study. In this bioassay only the doses which were identified as the best doses during the large-scale bioassays of crude and synthetic vitamin C (VC) were used. In case of purified ascorbate, the dose equivalent to synthetic ascorbate was prepared. 0.05% of crude Amla-based ascorbate (C), 0.50% of purified (P) and synthetic ascorbate (S) were prepared in water and used for CSR2 and CSR4. On the other hand, for traditional multivoltine breed (Pure Mysore), 0.30% ascorbate in crude extract (C), 3.00% of synthetic (S) and purified (P) were prepared. All the treatments were given only once at 0h of 5th instar since this time of application resulted in significant improvement in economic traits of silkworm during large-scale bioassay. The treatments were sprayed to mulberry leaves @ 60 ml/ 200g for 100 larvae and the latter were kept under shade for 15 minutes to remove the excess moisture and fed *ad libitum* to 5th instar larvae. Treatments were preferably given with night feeding among the three feedings per day to ensure the proper ingestion of the leaves. The larval weight was recorded on maximum growth on last day of the 5th instar. When the larvae were ready for spinning, they were picked individually and mounted on plastic collapsible mountage. When about 75-80% worms were mounted, the remaining worms were also mounted at once and on 6th day of mounting, the cocoons were harvested.

The growth rate pattern of silkworm breeds as influenced by the treatment of the three sources of vitamin C (C, P &S) at effective dose and application time was studied. Five male and five female larvae were weighed daily. Larvae were sex wise dissected and gonads separated. Water was drained out using tissue paper and the weight of paired gonads was recorded. The procedure was continued till the onset of spinning activity. Apart from recording the growth rate pattern of larvae and gonads, the gonad tissue somatic index (SGTSI) were determined in respect of each treatment and control using the following formula (Reddy and Benchamin, 1989) [17].

$$\text{GTSI (\%)} = \frac{\text{Weight of gonads (g)}}{\text{Weight of larva (g)}} \times 100$$

Data with regard to daily growth, and gonadal growth *visa viz*., gonad tissue somatic index was recorded. The experiments were repeated thrice and the data statistically analyzed by ANOVA through Statistical Package for Social Science, SPSS 7.5 for Windows (Berkowitz and Allaway, 1998).

Results and Discussion

Daily larval weight gain

Effect of vitamin C-quantified crude plant extract (C), purified vitamin C (P) and synthetic vitamin C (S) treatment on daily increase in 5th instar larval weight of different silkworm breeds is summarized in the tables 1 to 3. In multivoltine breed, PM, no significant change was observed in the given trait with any of the three treatments (Table 1). However, bivoltine breeds, CSR2 and CSR4, responded well to the treatment. In CSR2 breed, larval weight was increased by 4.57, 4.52 and 4.52 times from 0 day of 5th instar to 5th day as compared to that of the control (4.26times) with the improvement of 7.18, 5.92 and 5.93% in the batches treated with C, P and S (Table 2). In CSR4 larvae, trend of body weight increase from 0 day of 5th instar to the 5th day (maximum body weight) was almost same as that of CSR2, by 4.87, 4.92 and 4.91 times in treatment C, P and S respectively compared to that of the control where the increase was 4.47 times. The improvement induced by the same treatments over the control was 9.08, 10.09 and 9.98% (Table 3).

Table 1: Daily record of larval weight (g) of silkworm breed, PM, treated with vitamin C-quantified crude plant extract, purified vitamin C and synthetic vitamin C

	5th instar daily larval weight (mg/larva)									
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Treat. C ♂♂	0.327	0.464	0.578	0.802	0.920	1.004	1.226	1.633	1.943	1.732
Treat. P ♂♂	0.329	0.468	0.579	0.800	0.939	1.005	1.237	1.646	1.978	1.712
Treat. S ♂♂	0.331	0.472	0.580	0.799	0.941	1.002	1.235	1.646	1.973	1.713
Control ♂♂	0.328	0.472	0.573	0.795	0.934	1.000	1.230	1.646	1.973	1.715
Treat. C ♀♀	0.471	0.891	1.278	1.557	1.724	1.964	2.202	2.521	2.795	2.567
Treat. P ♀♀	0.474	0.892	1.270	1.561	1.723	1.947	2.204	2.519	2.792	2.568
Treat. S ♀♀	0.472	0.889	1.270	1.536	1.731	1.958	2.200	2.524	2.870	2.579
Control ♀♀	0.473	0.892	1.272	1.564	1.722	1.957	2.198	2.522	2.866	2.573
Avg. treat. C	0.399	0.678	0.928	1.180	1.322	1.484	1.714	2.077	2.369	2.150
Avg. treat. P	0.402	0.680	0.924	1.181	1.331	1.476	1.720	2.082	2.385	2.140
Avg. treat. S	0.402	0.681	0.925	1.167	1.336	1.480	1.718	2.085	2.422	2.146
Avg. Control	0.401	0.682	0.922	1.179	1.328	1.478	1.714	2.084	2.420	2.144
SE±	0.001	0.002	0.003	0.002	0.002	0.003	0.002	0.001	0.002	0.002
CD 5%	0.003	0.005	0.007	0.007	0.007	0.007	0.007	0.003	0.006	0.007

C: crude vitamin C, P: purified vitamin C and S: synthetic vitamin C.

Table 2: Daily record of larval weight (g) of silkworm breed, CSR2, treated with vitamin C-quantified crude plant extract, purified vitamin C and synthetic vitamin C

	5th instar daily larval weight (mg/larva)						
	0	**1**	**2**	**3**	**4**	**5**	**6**
Treat. C ♂♂	0.876	1.030*	1.491*	1.685*	2.778*	3.158*	3.015*
Treat. P ♂♂	0.876	1.029*	1.497*	1.694*	2.783*	3.158*	3.024*
Treat. S ♂♂	0.877	1.031*	1.495*	1.688*	2.782*	3.159*	3.035*
Control ♂♂	0.876	1.012	1.376	1.569	2.653	3.044	2.786
Treat. C ♀♀	0.883	1.682*	2.250*	2.933*	3.114*	4.879*	4.587*
Treat. P ♀♀	0.885	1.682*	2.256*	2.854*	3.107*	4.795*	4.652*
Treat. S ♀♀	0.896	1.683*	2.254*	2.905*	3.106*	4.849*	4.632*

Control ♀♀	0.884	1.576	2.234	2.660	2.886	4.529	4.308
Avg. treat. C	0.879	1.356*	1.871*	2.309*	2.946*	4.018*	3.801*
Avg. treat. P	0.880	1.356*	1.877*	2.274*	2.945*	3.977*	3.838*
Avg. treat. S	0.886	1.357*	1.875*	2.297*	2.944*	4.004*	3.833*
Avg. Control	0.880	1.294	1.805	2.114	2.770	3.787	3.547
SE±	0.0014	0.0015	0.0019	0.0016	0.0017	0.0015	0.0015
CD 5%	0.0042	0.0044	0.0056	0.0048	0.0051	0.0045	0.0044

C: crude vitamin C, P: purified vitamin C and S: synthetic vitamin C.

Table 3: Daily record of larval weight (g) of silkworm breed, CSR4, treated with vitamin C-quantified crude plant extract, purified vitamin C and synthetic vitamin C

5th instar daily larval weight (mg/larva)							
	0	**1**	**2**	**3**	**4**	**5**	**6**
Treat. C ♂♂	0.608	1.030*	1.459*	1.720*	2.578*	3.044*	2.635*
Treat. P ♂♂	0.609	1.050*	1.480*	1.712*	2.612*	3.103*	2.654*
Treat. S ♂♂	0.599	1.012*	1.470*	1.714*	2.589*	3.049*	2.614*
Control ♂♂	0.608	0.894	1.369	1.671	2.306	2.817	2.531
Treat. C ♀♀	0.923	1.581*	2.006*	2.826*	3.780*	4.414*	4.293*
Treat. P ♀♀	0.929	1.589*	2.011*	2.851*	3.830*	4.459*	4.338*
Treat. S ♀♀	0.931	1.590*	2.013*	2.855*	3.880*	4.464*	4.336*
Control ♀♀	0.923	1.434	1.879	2.430	3.478	4.020	3.942
Avg. treat. C	0.766	1.306*	1.732*	2.273*	3.179*	3.729*	3.464*
Avg. treat. P	0.769	1.319*	1.745*	2.282*	3.221*	3.781*	3.496*
Avg. treat. S	0.765	1.301*	1.742*	2.284*	3.234*	3.756*	3.475*
Avg. Control	0.766	1.164	1.624	2.051	2.892	3.419	3.236
SE±	0.0014	0.0015	0.0019	0.0016	0.0017	0.0015	0.0015
CD 5%	0.0042	0.0044	0.0056	0.0048	0.0051	0.0045	0.0044

C: crude vitamin C, P: purified vitamin C and S: synthetic vitamin C.

Daily gonad weight gain

Data regarding the effect of vitamin C-quantified crude plant extract (C), purified vitamin C (P) and synthetic vitamin C (S) on daily gonad weight increase of different silkworm breeds are presented in tables, 4-6. Gonad weight was at parity between treated and control larval groups of multivoltine breed, PM (Table 4). However, in CSR2 male larval groups treated with C, P and S, increase in daily gonad weight was recorded by 9.70, 9.61 and 9.61 times respectively as compared to that of the control where 8.40 times increase was recorded from 0 day till the last day of 5th instar and the improvement of 15.43, 14.39 and 14.31% was recorded in the last day compared to control. Female larvae with the same treatments had 9.96, 9.99 and 10.06 times increase in gonad weight over that of the control (8.69 times). Improvement in gonad weight in the last day of 5th instar was recorded as 14.67, 15.06 and 15.87% (Table 5). In CSR4 male larvae treated with C, P and S, body weight increase from 0 day to the 5th day of 5th instar was 10.11, 10.10 and 10.11 times compared to control which had 9.11 times increase, giving the improvement of 10.89, 10.83 and 10.89% over the control. In female larvae same treatments induced the increase of 10.68, 10.67 and 10.68 times compared to that of the control (9.55 times) with the improvement of 11.80, 11.81 and 11.80% (Table 6).

In the present study it was interesting to note that three different forms of ascorbate *viz.*, ascorbate-quantified crude plant extract, (C), purified ascorbate, (P) and synthetic ascorbate (S) in their respective doses were equally effective thereby exerting almost similar influence on larval and gonad weights. It was observed that the treatments induced prominent change in 5th instar gonad weights than in larval weight. The 5th instar larval weight increased only to the tone of 5.92-10.09 % in bivoltine breeds, CSR2 and CSR4 (Table 2 & 3) as compared to 10.83 -15.87% increase in gonad weight (Table 5 & 6). The study gives an impression that ascorbate exerts its impact mainly on gonad than whole larval body. The ascorbate is believed to have played its role on the activation of protein synthesis (Walingo, 2005) in silk gland. There is no report available with regard to the impact of ascorbate on silkworm gonads. However, a lot of work has been done in higher animals. Sodek *et al.*, (1982) reported that ascorbate stimulated the protein synthesis and improved their hydroxylation in adult mouse periodontal tissues. Supplementation of mulberry leaves with vitamin B_{12} which is not present in the leaves could increase the synthesis of nucleic acid and proteins in the silk gland of silkworm, *B. mori* (Das and Medda, 1988) [6]. Dabrowski and Ciereszko (1996) [5] demonstrated in wild fish, *Perca flavescens* that diet enrichment with ascorbate resulted in a dramatic increase of ascorbate in ovary and testis. According to them incorporation of ascorbate may take place during the late vitellogenesis. The results of the present study indicated that the ascorbate treatment induces more improvement in female gonad than in male in both the bivoltine breeds studied. The present results are comparable with the results obtained by Dabrowski (1991) [4] who reported the higher concentrations of ascorbate in ovary than testis of the fish, *Salvelinus alpinus.* He also suggested that the endogenous stores of ascorbate cannot fulfill the requirement of gonads and therefore should be supplemented exogenously. These results indicated that gonads are in some manner concerned with the utilization of ascorbate. Higher concentrations of ascorbate in gonads give an impression of more requirement of the vitamin in these organs. In current study it is plausible to speculate that the increase in gonad weight is due to additional supply of ascorbate in mulberry leaves. Cao and Schteingart (1942) [3] reported the appreciable increase in gonadal size of rats when fed with ascorbate supplemented diet along with gonadotropin. Ascorbate has been reported by many researchers (Lutwak-Mann, 1958 and Hershberger *et al.*, 1965) [15, 9] as an important nutrient for natural functioning of gonads in animals.

Table 4: Daily record of gonad weight (mg) of silkworm breed, PM, treated with vitamin C-quantified crude plant extract, purified vitamin C and synthetic vitamin C

5th instar daily gonad weight (mg/larva)										
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Treat. C ♂♂	3.125	6.701*	9.035*	14.203	16.492	18.703	19.765	28.326	36.251	42.811
Treat. P ♂♂	3.174	6.425	9.362*	14.123	16.470	18.751	19.875	28.428	36.437	42.831
Treat. S ♂♂	3.200	6.373	9.311*	14.231	16.480	18.750	19.741	28.327	36.378	42.831
Control ♂♂	3.130	6.250	9.232	12.174	16.496	18.747	19.728	28.426	36.366	42.792
Treat. C ♀♀	4.516	6.512	8.907	23.137*	23.699	28.000*	32.031	40.421	45.456	50.651
Treat. P ♀♀	4.513	6.575	9.188	22.242*	24.069*	27.960*	32.672*	40.451	47.692	51.247
Treat. S ♀♀	4.651	6.707	8.979	21.327	24.682*	28.131*	32.635*	40.501	46.847	51.173
Control ♀♀	4.520	6.606	8.762	21.000	23.967	27.480	32.117	40.461	46.465	51.205
SE±	0.132	0.163	0.163	0.171	0.163	0.167	0.171	0.163	0.163	0.171
CD 5%	0.395	0.488	0.488	0.513	0.488	0.501	0.513	0.488	0.488	0.513

Table 5: Daily record of gonad weight (mg) of silkworm breed, CSR2, treated with vitamin C-quantified crude plant extract, purified vitamin C and synthetic vitamin C

5th instar daily gonad weight (mg/larva)							
	0	**1**	**2**	**3**	**4**	**5**	6
Treat. C ♂♂	2.210	3.403	5.961*	8.982*	12.096*	19.922*	21.443*
Treat. P ♂♂	2.231	3.456*	6.145*	9.162*	12.318*	19.823*	21.453*
Treat. S ♂♂	2.232	3.441*	6.097*	9.156*	12.210*	19.922*	21.443*
Control ♂♂	2.213	2.984	5.407	6.761	10.981	14.810	18.599
Treat. C ♀♀	2.315	4.979*	6.923*	9.800*	13.066	21.079*	23.059*
Treat. P ♀♀	2.315	4.962*	6.408*	9.960*	16.713*	21.208*	23.133*
Treat. S ♀♀	2.315	4.896*	6.389*	9.801*	16.562*	21.098*	23.297*
Control ♀♀	2.315	4.374	5.000	7.936	13.296	16.788	20.110
SE±	0.135	0.150	0.148	0.150	0.150	0.150	0.150
CD 5%	0.404	0.451	0.431	0.451	0.451	0.451	0.448

C: crude vitamin C, P: purified vitamin C and S: synthetic vitamin C.

Table 6: Daily record of gonad weight (mg) of silkworm breed, CSR4, treated with vitamin C-quantified crude plant extract, purified vitamin C and synthetic vitamin C

5th instar daily gonad weight (mg/larva)							
	0	**1**	**2**	**3**	**4**	**5**	6
Treat. C ♂♂	2.136	3.533*	8.727*	10.712*	18.975*	19.922*	21.585*
Treat. P ♂♂	2.136	3.503*	8.723*	10.721*	18.907*	19.981*	21.575*
Treat. S ♂♂	2.136	3.533*	8.741*	10.712*	18.971*	19.894*	21.585*
Control ♂♂	2.136	3.443	6.760	8.955	14.561	18.970	19.465
Treat. C ♀♀	2.172	4.265*	9.019*	13.120*	19.955*	20.020*	23.200*
Treat. P ♀♀	2.172	4.265*	8.949*	13.100*	19.955*	20.020*	23.203*
Treat. S ♀♀	2.172	4.275*	8.982*	13.119*	19.955*	20.030*	23.203*
Control ♀♀	2.324	3.924	7.064	10.120	15.831	18.752	22.200
SE±	0.136	0.167	0.159	0.153	0.166	0.165	0.161
CD 5%	0.407	0.501	0.475	0.459	0.498	0.496	0.483

C: crude vitamin C, P: purified vitamin C and S: synthetic vitamin C.

Besides gonad weights, daily gonad tissue somatic index was calculated in order to have a clear idea of the present supplementation. As discussed above, the gonad weight was more in females than males though male gonad appeared bigger visually in all the three sources of ascorbate (C, P and S). But the opposite trend was observed after calculating relative tissue somatic index of all the breeds. Male had more tissue somatic index than the females in all the breeds. Further, in multivoltine silkworm breed, PM, gonad- tissue - somatic index was relatively higher than all the breeds studied, though no significant improvement was observed between its treated and control groups. This appears likely that the gonads in the PM breed grow at relatively faster rate than rest of the silkworm breeds used. There is no report available regarding the effect of ascorbate on gonad-tissue somatic index.

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