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Gonadotrophic effect of synthetic and phytoascorbate on the silkworm, *Bombyx mori* L

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Abstract
Daily gonad weight increase and gonad tissue somatic indices of two commercial silkworm hybrids viz., CSR2 x CSR4 and PM x CSR2 were studied after dietary supplementation of botanical based and synthetic ascorbic acid. It was observed that both the sources of ascorbic acid exerted significant improvement on gonad weight of 5th instar larva. In general, the gonad weight was more in females (16.44 to 17.22%) than males (13.58 to 16.32%) over their respective controls though male gonad appeared bigger visually in all the sources of ascorbic acid. But the opposite trend was observed in case of tissue somatic index. Male had more tissue somatic index than the females.

Keywords: Gonad weight, tissue somatic indices, *Bombyx mori*

1. Introduction
Enrichment of mulberry leaf with a multitude of exogenous factors, such as vitamins, minerals, antibiotics, hormones and study their impact on silk production, has become the order of traditional research in sericulture (Sannapa *et al*., 2002; Etebari *et al*., 2004; Bhattacharya *et al*., 2004 & 2005 and Chakrabarty and Kaliwal, 2011) [21, 11, 4, 5, 7]. One important nutrient that attracted the attention of researchers in this field is vitamin C or L-ascorbic acid. This vitamin has been identified as a powerful antioxidant, potential phago-stimulant, efficient growth promoter and booster of silk production in the silkworm (Ito, 1961; Javed and Gondal, 2002; Hussain and Javed, 2002; Prasad, 2004; Etebari *et al*., 2004) [14, 16, 13, 19, 11]. The silkworm, *B. mori* has been classified among the insects which are unable to synthesize ascorbic acid or vitamin C (abbreviated as VC here) in their body and depend on exogenous supply to fulfill the requirement (Ito and Arai 1965) [15]. There is very close relationship reported between the addition of VC in diet and silkworm's growth. The growth of 1 gram fresh weight of larva needs about 0.668 mg (0.067%) VC. Although the function of VC to silkworm physiological activities has not been clear, it is known to promote silkworm food ingestion (Junliang *et al*., 1994) [17]. A scan through the information supplied by the earlier investigators with regard to synthetic VC supplementation, reveal that most of them have studied various aspects of the *B. mori* larval development and other productive aspects but no attempt has been made to determine its effect on the development of gonads in terms of weight gain and tissue somatic index and hence the present study was undertaken..

2. Materials and methods
The silkworm rearing was conducted as per the standard method (Rajan and Hemmantraju, 2005) [20]. Two commercial silkworm hybrids viz., CSR2 x CSR4 and PM x CSR2 were used for the daily gonad weight gain and gonad tissue somatic index study. Only the doses which were identified as the best doses during the large-scale bioassays of crude and synthetic vitamin C (VC) were used (Tantray *et al*., 2011 and Tantray and Trivedy, 2011) [1, 2]. Accordingly, 0.05% of crude Amla-based VC (C), 0.50% of purified (P) and synthetic VC (S) were prepared in water and used for CSR2, CSR4 and CSR2 x CSR4 hybrid. Similarly, for PM x CSR2 hybrid, 0.30% VC in crude extract (C), 3.00% of synthetic (S) and purified (P) were used. In case of purified VC, the dose equivalent to synthetic VC was 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 (Tantray *et al*., 2011) [1]. 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 daily gonad weight gain of silkworm hybrids as influenced by treatment of VC-quantified crude plant extract (C), purified VC (P) and synthetic VC (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 two gonads was recorded. The procedure was continued till the onset of spinning activity. Apart from recording the growth rate pattern of gonads, the silk gland tissue somatic index (SGTSI) was determined in respect of each treatment and control using the following formula (Reddy and Benchamin, 1989).

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

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).

A **B**

Plate 1: Dissected out gonads, **A:** Female and **B:** Male

Table 1. Daily record of gonad weight (mg) of silkworm hybrid, CSR2 x 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 ♂♂	2.247	4.720*	6.131*	8.175*	9.736*	12.855*	15.612*
Treat. P ♂♂	2.249	4.730*	6.013*	7.937*	9.736*	13.861*	15.507*
Treat. S ♂♂	2.249	4.807*	6.213*	7.974*	9.747*	12.859*	15.592*
Control ♂♂	2.250	4.138	4.699	5.930	8.646	10.975	13.169
Treat. C ♀♀	2.309	6.055*	8.079*	8.749*	10.982*	16.223*	20.032*
Treat. P ♀♀	2.309	6.052*	8.128*	8.769*	11.064*	16.222*	20.112*
Treat. S ♀♀	2.309	6.044*	8.128*	8.765*	10.664*	16.221*	20.538*
Control ♀♀	2.283	5.600	6.977	7.800	8.711	14.570	17.658
SE±	***0.109***	***0.135***	***0.126***	***0.145***	***0.138***	***0.140***	***0.132***
CD 5%	***0.326***	***0.404***	***0.369***	***0.423***	***0.414***	***0.420***	***0.395***

C: crude vitamin C, **P**: purified vitamin C, **S**: synthetic vitamin C and ***** significant at 5%

Table2. Daily record of gonad weight (mg) of silkworm hybrid, PM x 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 ♂♂	2.229	4.061*	6.046*	8.578*	11.401*	16.967*	19.601*
Treat. P ♂♂	2.240	4.093*	6.079*	8.549*	13.225*	17.535*	19.320*
Treat. S ♂♂	2.241	4.124*	6.155*	8.565*	13.155*	16.979*	19.445*
Control ♂♂	2.231	3.561	5.053	6.346	10.971	13.882	16.639
Treat. C ♀♀	2.312	5.517*	7.501*	9.275*	11.539*	18.073*	20.737*
Treat. P ♀♀	2.312	5.507*	7.268*	9.365*	11.691*	18.022*	20.782*
Treat. S ♀♀	2.312	5.470*	7.259*	9.283*	11.437*	18.072*	20.991*
Control ♀♀	2.299	4.987	5.988	7.868	9.846	14.690	18.129
SE±	***0.109***	***0.132***	***0.131***	***0.131***	***0.131***	***0.127***	***0.127***
CD 5%	***0.319***	***0.385***	***0.383***	***0.383***	***0.383***	***0.372***	***0.382***

C: crude vitamin C, **P**: purified vitamin C, **S**: synthetic vitamin C and ***** significant at 5%

Table 3. Daily record of gonad tissue somatic index (%) of silkworm hybrid, CSR2 x CSR4, treated with vitamin C-quantified crude plant extract, purified vitamin C and synthetic vitamin C

5th instar daily gonad tissue somatic index (%)							
	0	***1***	***2***	***3***	***4***	***5***	***6***
Treat. C ♂♂	0.187	0.204	0.235*	0.271*	0.351*	0.401*	0.503*
Treat. P ♂♂	0.187	0.203	0.230*	0.265*	0.344*	0.395	0.506*
Treat. S ♂♂	0.187	0.206*	0.237*	0.264*	0.335*	0.401*	0.518*
Control ♂♂	0.187	0.190	0.201	0.211	0.288	0.389	0.456
Treat. C ♀♀	0.172	0.183	0.199*	0.198	0.212*	0.250	0.306*
Treat. P ♀♀	0.172	0.181	0.194*	0.196	0.212*	0.270*	0.309*
Treat. S ♀♀	0.172	0.181	0.197*	0.197	0.212*	0.252*	0.310*
Control ♀♀	0.172	0.175	0.176	0.190	0.201	0.244	0.272
SE±	***0.0082***	***0.0052***	***0.0046***	***0.0040***	***0.0038***	***0.0030***	***0.0031***
CD 5%	***0.0245***	***0.0155***	***0.0138***	***0.0112***	***0.0101***	***0.0080***	***0.0092***

C: crude vitamin C, **P**: purified vitamin C, **S**: synthetic vitamin C and ***** significant at 5%

Table 4. Daily record of gonad tissue somatic index (%) of silkworm hybrid, PM x CSR2, treated with vitamin C-quantified crude plant extract, purified vitamin C and synthetic vitamin C

5th instar daily gonad tissue somatic index (%)							
	0	***1***	***2***	***3***	***4***	***5***	***6***
Treat. C ♂♂	0.215	0.243*	0.295*	0.365*	0.391*	0.502*	0.593*
Treat. P ♂♂	0.216	0.243*	0.296*	0.365*	0.390*	0.496*	0.594*
Treat. S ♂♂	0.216	0.245*	0.299*	0.364*	0.383*	0.502*	0.600*
Control ♂♂	0.215	0.223	0.272	0.290	0.347	0.433	0.544
Treat. C ♀♀	0.208	0.221	0.238*	0.252*	0.296	0.339*	0.399*
Treat. P ♀♀	0.207	0.219	0.226*	0.256*	0.344*	0.353*	0.400*
Treat. S ♀♀	0.207	0.218	0.227*	0.252*	0.341*	0.341*	0.403*
Control ♀♀	0.208	0.209	0.193	0.233	0.305	0.308	0.364
SE±	***0.0103***	***0.0066***	***0.0053***	***0.0045***	***0.0037***	***0.0030***	***0.0030***
CD 5%	***0.0309***	***0.0197***	***0.0158***	***0.0136***	***0.0112***	***0.0091***	***0.0091***

C: crude vitamin C, **P**: purified vitamin C, **S**: synthetic vitamin C and ***** significant at 5%

3. Results and discussion

3.1 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 hybrids are presented in tables, 1 and 2. In CSR2 x CSR4, increase in male gonad weight of larvae treated with C, P and S was to the tone of 8.92, 8.94 and 9.13 times compared to that of the control (7.85 times) with the improvement of 13.58, 13.92and 16.32%. Respective treatment in females induced the increase in gonad weight by 6.76, 6.72 and 6.75 times over the control which had 5.77 time gonad weight increment. The improvement compared to control was 17.22, 16.44 and 17.08% (1). In PM x CSR2 males same treatments were capable of bringing out the increase in gonad weight to the extent of 9.31, 9.28 and 9.37 times compared to that of the control (8.12 times) with the improvement of 14.53, 14.18 and 15.30%. With same treatments in females, 8.48, 8.36 and 8.41 times increase in gonad was observed compared to that of the control (7.24 times) with the percentage improvement of 17.14, 15.47 and 16.22% (2).

3.2 Daily gonad tissue somatic index (GTSI)

GTSI of the treated and control silkworm has been summarized in Tables, 3 and 4. Generally in both the hybrids gonad weight was more in females than males although male gonad appeared visually bigger. GTSI had an opposite pattern, more in males than females. In CSR2 x CSR4 males, respective treatments were capable of bringing out the increase in GTSI to the extent of 10.33, 11.06 and 13.69% over the control, whereas, in females 12.38, 13.76 and 14.11% over the control (Table 3). PM x CSR2 hybrid treated with C, P and S had increase in male GTSI to the tone of 8.86, 9.12 and 10.19% and the females had 9.67, 9.94 and 10.74% improvement over the control in the last day of 5th instar (Table 4).

VC-quantified crude plant extract, (C), purified VC, (P) and synthetic VC (S) in their respective doses were equally effective thereby exerting almost similar influence on larval gonad weight. In general, the gonad weight was more in females than males though male gonad appeared bigger visually in all the three sources of VC (C, P and S). But the opposite trend was observed in case of tissue somatic index. Male had more tissue somatic index than the females. There is no report available regarding the effect of VC on gonad-tissue somatic index. The VC is believed to have played its role on the activation of protein synthesis (Walingo, 2005) [23] in silk gland. There is no report available with regard to the impact of VC on silkworm gonads. However, a lot of work has been done in higher animals. Sodek *et al*., (1982) [22] reported that VC 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) [8]. Dabrowski and Ciereszko

(1996) [10] demonstrated in wild fish, *Perca flavescens* that diet enrichment with VC resulted in a dramatic increase of VC in ovary and testis. According to them incorporation of VC may take place during the late vitellogenesis. The results of the present study indicated that the VC treatment induces more improvement in female gonad than in male in both the hybrids studied. The present results are comparable with the results obtained by Dabrowski (1991) [9] who reported the higher concentrations of VC in ovary than testis of the fish, *Salvelinus alpinus.* He also suggested that the endogenous stores of VC 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 VC. Higher concentrations of VC 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 VC in mulberry leaves. Cao and Schteingart (1942) [6] reported the appreciable increase in gonad size of rats when fed with VC supplemented diet along with gonadotropin. VC has been reported by many researchers (Lutwak-Mann, 1958 and Hershberger *et al*., 1965) [18, 12] as an important nutrient for natural functioning of gonads in animals.

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