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Original Research Article

Impact of Cholecalciferol (D_3) supplementation on biology and cocoon yield of silkworm, *Bombyx mori* L.

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Abstract

The impact of different levels of the Cholecalciferol (Vitamin D_3) supplementation on silkworm larvae was evaluated by rearing under controlled conditions of temperature, relative humidity and photoperiod (25 ± 1 °C, 75 ± 5 % and 16:08 h of light to darkness ratio, respectively) at Sericulture Research Laboratory, Lahore, Pakistan. Larvae were fed on mulberry leaves of "Chinese Husung" variety supplemented with vitamin 'D_3'@ 0.5, 1, 1.5 and 2 % solutions. The significant increment in larval length (2.81, 4.17 and 5.91 cm in $D_{3\ (2\%)}$ as compared to 2.69, 3.79 and 5.56 cm in control) were recorded in 3rd, 4th and 5th instars, respectively. The results also delineated significant variations in larval weight (0.39, 1.0 and 2.81 g in $D_{3\ (2\%)}$ as compared to 0.28, 0.78 and 2.54 g in control, during 3rd, 4th and 5th instars, respectively). Larval food consumption recorded during 3rd, 4th and 5th instars showed significantly higher mean values (2.63, 3.23 and 4.43 g in $D_{3\ (2\%)}$ as compared to control (2.26, 2.60 and 2.74 g), respectively. The dietary supplementation also affected cocoon weight (1.39 g) and Shell Weight (0.33 g) and consequently Cocoon Shell percentage (23.74 %) recorded in $D_{3\ (2\%)}$ in comparison with 1.25 g, 0.24 g and 19.2 % in control, respectively. The results indicated improvement in better food consumption, larval weight and length and cocoon production when mulberry leaves supplemented with D_3. The study highlighted the significance of dietary supplementation with vitamin D_3 of "Chinese Husung" mulberry variety for rearing of silkworm.

Keywords: Larval Length, Cocoon Weight, D_3, Mulberry, Sericulture

Introduction

Sericulture has served the humanity by providing natural animal silk for centuries. The history of cottage industry as a source of income for folks dates back to 4500BC. Sericulture industry has not been able to flourish in most part of the world mainly due to its great dependence on mulberry as sole food, low nutritional level of mulberry leaves, environmental concerns, silkworm diseases and lack of training facilities for the silkworm rearers, etc. The success of this industry is based on availability of high yielding mulberry varieties, rearing of silkworm larvae for cocoon production under prevailing favorable environment and marketing facilities (Hussain et al., 2011). Mulberry leaves provide proteins, vitamins and other nutrients from which silk proteins are synthesized. Quality and quantity of mulberry leaves along with environmental factors affect production of raw silk spun by larvae before pupation in the form of cocoons. Several studies have been conducted on food supplementation and developing artificial diet for rearing of silkworms. The perusal of literature indicated food supplementation of silkworm larvae

have resulted in the improvement of commercial and biological aspects of sericulture whereas artificial diet had little success in commercial rearing. Supplementation of mulberry with nitrogen (Khan and Muslim, 1999; Hussain and Javed, 2002; Haq and Saleem, 1985) resulted in better growth of larvae and improved quantity and quality of cocoons. The commercial traits of silkworm cocoons were enhanced when mulberry leaves were supplemented with vitamin C (El-Karaksy and Idriss, 1990; Babu et al., 1992; Chauhan and Shing, 1992; Hussain and Javed, 2002; Etebari et al., 2004a; Chang and Li, 2004). Supplementation of mulberry leaves with Vitamin B enhanced resistance in silkworm larvae against conditions of environmental stress which resulted in body weight increment as compared to control (Das and Medda, 1998; Rahmathulla et al., 2002; Raman et al., 2007). VitaminD_3 having significant role in cell metabolism associated with glucose, may improve physiology of silkworm contributing in enhanced performance for biological and economic traits (Ewer, 2005). In recent years, many attempts have been made to improve the quality and quantity of silk through supplementing the leaves with nutrients, spraying with antibiotics, vitamins, hormones and hormone analogues, plant products or using extracts of plants (Kanafi et al., 2007; Ahsan et al., 2013). The proposed research work was undertaken after perusal of literature where little work was found on D_3 supplementation with reference to silkworm growth and development and its impact on commercial traits of cocoons. Thus, the study was conducted to evaluate the impact of vitamin D_3 on the performance of silkworm growth and cocoon production.

Materials and Methods

Preparation of stock culture for larval rearing

The larvae were reared in room disinfected with 4 % formalin prior to shift the larvae and by keeping rearing room air tight for 24 h. The incubation of eggs was performed in hatching plates measuring 15 x 30 cm kept at recommended temperature (25 ± 1 °C) and RH conditions (75±5% RH) following Krishnaswami, (1978).

Incubation and Hatching

The silkworm eggs were obtained, provided with acid treatments and spread over a sheet in single layer at Sericulture Research Laboratory, Lahore, Pakistan. The eggs were subjected to incubation (25 ± 1 °C and 75 ± 5 % RH with 16 h light: 8 h of darkness) as followed by Hussain et al., (2011).

Larval Rearing

Early larval instars (1^{st} - 3^{rd}) were reared at 27 ± 1 °C temperature and RH conditions of 85 – 90 % whereas 4^{th} - 5^{th} larval instars were reared at 25 ± 1 °C temperature and 70 – 80 % RH (Rahmathulla, 2012). There were 100 larvae in each replication during 1^{st} - 3^{rd} instar. Newly hatched larvae were transferred to rearing trays and fed with fresh tender chopped leaves of mulberry variety 'Chinese Husung'. The feeding schedule for larval rearing comprised of 02, 03 and 04 times a day, during 1^{st}, 2^{nd}, 3^{rd} and 4^{th} - 5^{th} instars, respectively (Hussain et al., 2011).

Experimental Design

In present work, Vitamin D_3 supplementations were given to silkworm larvae by dipping mulberry leaves in the Vitamin D_3 solution to evaluate its effects on different parameters of cocoon and larval growth of silkworm (Table 1). The research work was carried out in Complete Randomized Design (CRD) and replicated 04 times to minimize the error. In the beginning of 3^{rd} instar, the larvae were divided into 20 groups with each replicate containing 100 larvae.

Table – 1: Different treatments used in the study to evaluate the impact of vitamin D_3 on silkworm larvae of Chinese race fed on Mulberry variety Chinese Husung

Treatments	Description
$D_{3\ (0\%)}$	Control: Mulberry leaves without any supplementation
$D_{3\ (0.5\%)}$	Mulberry leaves dipped in 0.5% D_3 Solution
$D_{3\ (1\%)}$	Mulberry leaves dipped in 1% D_3 Solution
$D_{3\ (1.5\%)}$	Mulberry leaves dipped in 1.5% D_3 Solution
$D_{3\ (2\%)}$	Mulberry leaves dipped in 2.0 % D_3 Solution

Mounting and Cocoon Spinning

The 5^{th} instar mature larvae stopped feeding and searching for place to pupate were handpicked and transferred for cocoon spinning under controlled conditions (25 ± 1°C and 75 ± 5% RH). After the completion of cocoon construction by pupating larvae,

fresh cocoons were harvested on 8th day of spinning to allow uniform cocoon crop (Hussain et al., 2011).

Data Collection and Analysis

The data was subjected to ANOVA to determine significance and treatment means were compared by Tukey's Test.

Larval length in each instar was recorded with the help of measuring tape whereas larval weight was documented with the help of Electronic Balance (Hussain et al., 2011). Data recording on larval length and weight were accomplished on the completion of 3rd, 4th and 5th instars before molting.

Food Consumption

The data pertaining to larval food consumption in each instar (3rd to 5th) were obtained by using following method:

Food Consumption (g) =
dry weight of offered leaves(g) - dry weight of residual leaves (g)

Cocoon Characteristics

The weight of freshly harvested cocoons without removing pupae was calculated for cocoon weight whereas after removing pupae shell weight was determined by Electronic Balance by following method (Rao et al., 2006; Hussain et al., 2011).

$$\textbf{Cocoon Weight (g)} = \frac{\text{Average weight of 5 female cocoons} + \text{Average weight 5 male Cocoons}}{10}$$

Shell Weight (g) =
Cocoon weight with pupa(g) − Cocoon weight without pupa(g)

$$\textbf{Shell Ratio } (\%) = \frac{\text{Shell Weight (g)}}{\text{Cocoon Weight (g)}} X100$$

Results and Discussion

The impact of vitamin D_3 on growth of silkworm larvae and cocoon was investigated in the present study. The data on larval length, weight and food consumption were recorded during 3rd - 5th instars whereas cocoon characteristics were also determined after the completion of cocoon spinning.

Larval Body Length (3rd Instar)

The larval lengths recorded at the end of 3rd instar showed significant differences (F = 10.105, P = 0.000) in means i.e. 2.65, 2.69, 2.74, 2.78, and 2.81cm in control, $D_{3\ (0.5\%)}$, $D_{3\ (1\%)}$, $D_{3\ (1.5\%)}$, and $D_{3\ (2\%)}$, respectively (Table 2).

Larval Body Length (4th Instar)

Larval length recorded at the end of 4th instar indicated significant variations (F = 5.016, P = 0.001) in larval length i.e. 3.79, 3.88, 4.08, 4.13 and 4.17 cm exhibited in different treatments control, $D_{3\ (0.5\%)}$, $D_{3\ (1\%)}$, $D_{3\ (1.5\%)}$, and $D_{3\ (2\%)}$, respectively (Table 2).

Larval Body Length (5th Instar)

Data recorded at the end of 5th instar showed significant differences (F = 6.023, P = 0.000) in larval length i.e. 5.56, 5.68, 5.80, 5.86 and 5.91 cm in control, $D_{3\ (0.5\%)}$, $D_{3\ (1\%)}$, $D_{3\ (1.5\%)}$, and $D_{3\ (2\%)}$, respectively (Table 2).

Larval Body Weight (3rd Instar)

Larval weight (g) recorded at the end of 3rd instar yielded significant differences (F= 4.338, P = 0.002) in larval weight (g) i.e. 0.28, 0.29, 0.33, 0.37, and 0.39 g in control, $D_{3\ (0.5\%)}$, $D_{3\ (1\%)}$, $D_{3\ (1.5\%)}$, and $D_{3\ (2\%)}$, respectively (Table 2).

Larval Body Weight (4th Instar)

Larval weight (g) recorded at the end of 4th instar showed significant variations (F = 5.645, P = 0.000) in in larval weight (g) i.e. 0.78, 0.84, 0.91, 0.97 and 1.0 g in control, $D_{3\ (0.5\%)}$, $D_{3\ (1\%)}$, $D_{3\ (1.5\%)}$, and $D_{3\ (2\%)}$, respectively (Table 2).

Larval Body Weight (5th Instar)

Larval weight (g) were recorded at the end of 5th instar gave significant differences F = 1.398, P = 0.0236 in mean larval weight i.e. 2.54, 2.59, 2.67, 2.76 and 2.81 g in control, $D_{3\ (0.5\%)}$, $D_{3\ (1\%)}$, $D_{3\ (1.5\%)}$, and $D_{3\ (2\%)}$, respectively (Table 2).

Table – 2: Effect of Vitamin D_3 on larval growth, larval weight and food consumption during 3rd, 4th and 5th larval instars of silkworm reared at controlled conditions of Temperature and RH

Treatments	Body Length (cm)			Body Weight (g)			Food Consumption (g)		
	3rd Instar	4th Instar	5th Instar	3rd Instar	4th Instar	5th Instar	3rd Instar	4th Instar	5th Instar
Control	2.65 a	3.79 a	5.56 a	0.28 a	0.78 a	2.54 a	2.26 a	2.60 a	3.74 a
$D_{3\,(0.5\%)}$	2.69 b	3.88 b	5.68 b	0.29 a	0.84 b	2.59 b	2.35 b	2.97 b	3.84 b
$D_{3\,(1\%)}$	2.74 c	4.08 c	5.80 c	0.33 b	0.91 c	2.67 bc	2.44 c	3.12 c	4.29 c
$D_{3\,(1.5\%)}$	2.78 c	4.13 d	5.86 c	0.37 c	0.97 d	2.76 c	2.52 d	3.19 d	4.35 d
$D_{3\,(2\%)}$	2.81 d	4.17 de	5.91 d	0.39 c	1.00 d	2.81 d	2.63 e	3.23 e	4.43 e

a, b, c means with different letters in a column differ significantly at $p < 0.05$ Tukey's Test

Table – 3: Effect of different levels of Vitamin 'D_3' on Cocoon Weight (g), Shell Weight (g) and Cocoon Shell Ratio (%) of silkworm reared at controlled conditions of Temperature and RH

Treatments	Cocoon Weight (g)	Shell Weight (g)	Cocoon Shell (%)
Control	1.25 a	0.24 a	19.20 a
$D_{3\,(0.5\%)}$	1.30 b	0.27 b	20.76 b
$D_{3\,(1\%)}$	1.32 bc	0.29 c	21.96 c
$D_{3\,(1.5\%)}$	1.35 c	0.31 c	22.96 d
$D_{3\,(2\%)}$	1.39 d	0.33 d	23.74 e

a, b, c means with different letters in a column differ significantly at $p < 0.05$ Tukey's Test

Larval Food Consumption (3rd Instar)

The comparison of means revealed significant differences in food consumption at various level of D_3 in comparison with the control ($F = 12.674$, $P = 0.000$). Larval food consumption (g) recorded at the end of 3rd instar showed significant variations i.e. 2.26, 2.35, 2.44, 2.52 and 2.63 g in control, $D_{3\,(0.5\%)}$, $D_{3\,(1\%)}$, $D_{3\,(1.5\%)}$, and $D_{3\,(2\%)}$, respectively (Table 2).

Larval Food Consumption (4th Instar)

Data recorded at the end of 4th instar showed significant differences ($F= 17.701$, $P = 0.000$) in mean food consumption (g) i.e. 2.60, 2.97, 3.12, 3.19 and 3.23 g in control, $D_{3\,(0.5\%)}$, $D_{3\,(1\%)}$, $D_{3\,(1.5\%)}$, and $D_{3\,(2\%)}$, respectively (Table 2).

Food Consumption (5th Instar)

Data recorded on food consumption at the end of 5th instar yielded significant differences ($F = 5.895$, $P = 0.000$) in mean food consumption i.e. 3.84, 4.29, 4.35 and 4.43 g in control, $D_{3\,(0.5\%)}$, $D_{3\,(1\%)}$, $D_{3\,(1.5\%)}$, and $D_{3\,(2\%)}$, respectively (Table 2).

Cocoon Weight (g)

Cocoon weight (g) recorded at the end of spinning after 5th instar gave significant variations ($F = 2.675$, $P = 0.0466$) in cocoon weight i.e. 1.25, 1.30, 1.28, 1.25 and 1.33g in control, $D_{3\,(0.5\%)}$, $D_{3\,(1\%)}$, $D_{3\,(1.5\%)}$, and $D_{3\,(2\%)}$, respectively (Table 3).

Cocoon Shell Weight (g)

Cocoon shell weight (g) calculated after removing pupa showed significant differences (F = 1.437, P = 0.0466) in mean Shell weight i.e. 0.24, 0.26, 0.25, 0.25 and 0.27g in control, $D_{3\,(0.5\%)}$, $D_{3\,(1\%)}$, $D_{3\,(1.5\%)}$, and $D_{3\,(2\%)}$, respectively (Table 3).

Cocoon Shell (%)

Cocoon Shell (%) calculated presented differences (F = 1.715, P = 0.0466) at different treatments in comparison with the control i.e. 21.25, 20.16, 19.69, 20.82 and 21.28 % in control, $D_{3\,(0.5\%)}$, $D_{3\,(1\%)}$, $D_{3\,(1.5\%)}$, and $D_{3\,(2\%)}$, respectively (Table 3).

Discussion

The study was undertaken to evaluate the impact of Vitamin D_3 on larval performance for biological characteristics of larvae and commercial traits of cocoon spun by the silkworm larvae under laboratory conditions. The results indicated significant differences in larval growth i.e. larval weight, larval length and food consumption when mulberry leaves were supplemented with D_3. Our results are in accordance with the results of earlier researchers who reported that the monophagous habit of silkworm has led to search for alternative food sources including artificial diet and food supplementation of mulberry leaves during larval feeding to boost cocoon yield. Amino acids, Vitamins, proteins, sugars and minerals affect silkworm growth and development (Khan and Saha, 1997; Faruki, 1998; Mora et al., 2008; Castillo et al., 2016). Researchers have reported positive impact of vitamin supplemented food on the reproduction of silkworm females (Khan and Saha, 1997). Some of the works have illustrated no effect of vitamins supplemented with mulberry leaves on silkworm and cocoon characteristics. Such results showing no effect of vitamin E on larval food consumption was reported (Mora et al., 2008; Castillo et al., 2016).

Our findings illustrated that cocoon weight was enhanced with the supplementation of vitamin D_3 with increasing trend with increase in the quantity of Vitamin D_3. The results are in line with earlier works reporting impact of food supplementation with mulberry leaves adding in economic traits of cocoons (Rahmathulla et al., 2002; Mora et al., 2008). Our results were also supported by the earlier studies conducted on nutrient supplementation in which enhancement of growth and cocoon weight of silkworm owed to variations in nutritive value (Ganesh et al., 2012; Balasundaram et al., 2008). Larval body length and body weight was significantly affected by food supplementation with multivitamins (Balasundaram et al., 2013). Multivitamin and Mineral supplementation of mulberry leaves enhances food intake and conversion efficiency of silkworm (Prassad, 2004).

Feeding of silkworm larvae on mulberry leaves augmented with multi-vitamins from 4th instar onwards enhanced female cocoon shell weight (Etebari and Matindoost, 2005; Mora et al., 2008). Vitamins play significant role in the physiology and metabolism of organisms like silkworm and enhance their performance where direct supplementation of vitamin on larvae affect larval metabolism (Mora et al., 2008; Castillo et al., 2016). Evangelista et al., (1997) reported increase in larval and cocoon weight under multi-vitamin supplementation. Our findings were also in line with earlier researchers who reported growth indices were affected by doses of vitamin b and c supplemented up to certain limits by producing significant increment in larval body weight and length (Ahsan et al., 2013). The present study demonstrated that the performance silkworm races reared in Pakistan on Chinese Husung variety if supplemented with D_3 would enhance cocoon production significantly. The sericulture industry in Pakistan needs to be strengthened by introducing high yielding and highly nutritive mulberry varieties coupled with disease free silk seed resistant to environmental stress.

Conclusion

The impact of different levels of the Vitamin D_3 supplementation @ 0.5, 1, 1.5 and 2.0 % solutions was evaluated by rearing silkworm larvae under controlled conditions during last three larval instars. Larval body length, body weight and food consumption showed positive increment when larvae were fed on mulberry leaves supplemented with D_3 as compared to control. The results pertaining to Cocoon Weight, Cocoon Shell Weight and Cocoon Shell Percentage also showed positive trends when reared on supplemented food. The study emphasized on the use of D_3 supplementation to enrich the mulberry leaves with higher nutritive value to achieve significant economic benefit from Chinese races.

References

Ahsan KM, Khan AR and Ferdous T, 2013. Growth and development of the mulberry silkworm, *Bombyx mori* L. On vitamin b and c supplemented diet. Bangladesh J. Zool. 41(2): 199-206.

Babu M, Swamy MT, Rao PK and Rao MS, 1992. Effect of ascorbic acid enriched mulberry leaves on rearing of *Bombyx mori* L. Indian J. Seric. 31: 111-114.

Balasundaram D, Ganesh P, Selvisabhanayakam P, Mathivanan V and Ramesh V, 2013. Studies on the nutritional supplementation of vitamin C treated MR_2 mulberry leaves fed by 5^{th} instar larvae of silkworm, *Bombyx mori* (L.) (Lepidoptera: Bombycidae) in relation to feed efficacy and growth rate. Int. J. Biochem. Biotechnol. 3(1): 11-18.

Balasundaram D, Selvisabanyakam V and Mathivanan, 2008. Studies on comparative feed efficacy of mulberry leaves MR_2 and MR_2 treated with vitamin C on *Bombyx mori* L. (Lepidoptera: Bombycidae) in relation to larval parameters. J. Curr. Sci. 12(2): 31-35.

Castillo Y, Suzuki J, Watanabe K, Shimizu T and Watarai M, 2016. Effect of Vitamin A on Listeria monocytogenes Infection in a Silkworm Model. PloS one. 11(9): e0163747.

Chang CL and Li QX, 2004. Dosage effects between dietary niacin and other B vitamins on larval development of Ceratitiscapitata (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 97: 536-540.

Chauhan TPS and Singh K, 1992. Studies on the effect of ascorbic acid (vitamin C) on the fecundity in the mulberry silkworm (*Bombyx mori* L.). Sericologia. 32(4): 567-577.

Das S and Medda AK, 1998. Effect of cyanocobalamin on protein and nucleic acid contents of ovary of silkworm, *Bombyx mori* L. during larval, pupal and adult stages of development. Int. J. Trop. Insect. Sci. 9(5): 641-646.

El-Karaksy IA and Idriss M, 1990. Ascorbic acid enhances the silk yield of mulberry silkworm, *Bombyx mori* L. Indian J. Entomol. 109: 81-86.

Etebari K and Mantindoost L, 2005. Application of multi-vitamins as supplementary nutrients on biological and economical characteristics of silkworm, *Bombyx mori* L. J. Asia Pac. Entomol. 8(1): 107-112.

Etebari K, Ebadi R and Mantindoost, 2004a. Effects of vitamin C on biological, biochemical and economical characteristics of the silkworm, *Bombyx mori* L. Int. J. Indust. Entomol. 8: 81-87.

Evangelista A, Carvalho AD, Takahashi R and De-Carvalho AD, 1997. Performance of silkworm (*Bombyx mori* L.) fed with vitamin c and mineral supplement. Revista de Agric. Piracicaba. 72(2): 199-204.

Ewer J, 2005. How the ecdysozoan changed its coat. PLoS Biology. 3(10): 349.

Faruki SI, 1998. Nutritive effect of thianomin enriched mulberry leaves on the silkworm, Bombyx mori L. Univ. J. Zool. *Rajshahi* Univ. 17: 39-44.

Ganesh P, Selvisabhanayakam P, Balasundaram D, Pradhap M, Vivekananthan T and Mathivanan V, 2012. Effect of food supplementation with silver nanoparticles (AgNps) on feed efficacy of silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). Int. J. Res. Biol. Sci. 2: 60-67.

Haq M and Saleem M, 1985. Quantitative and qualitative feeding effect on silkworm, *Bombyx mori* L. Acad. J. Agri. Res. 23 (1): 45-46.

Hussain M, Khan SA, Naeem M and Nasir MF, 2011. Effect of rearing temperature and humidity on fecundity and fertility of silkworm, *Bombyx mori* L. Pak. J. Zool. 43(5): 979-985.

Hussain M and H Javed, 2002. Effect of 0.2% N with Various Combinations of Ascorbic Acid on Growth and Silk Production of Silkworm (*Bombyx mori* L.). Asian J. Plant Sci. 1(6):650-651.

Kanafi RR, Ebadi R, Mirhosseini SZ, Seidavi AR, Zolfaghari M, Etebari K, 2007. A review on nutritive effect of mulberry leaves enrichment with vitamins on economic traits and biological parameters of silkworm, *Bombyx mori* L. Invert. Surviv. J. 4: 86-91.

Khan AR and Saha BN, 1997. Nutrition of mulberry silkworm, *Bombyx mori* on feed supplemented with calcium lactate. J. Ecobiol. 9: 53-58.

Khan SA and Muslim MM, 1999. Effect on nitrogen feeding on the larval development and cocoon characters of silkworm (*Bombyx mori* L.). Acta Sci. 9(1): 51-56.

Krishnaswami S, 1978. New Technology of Silkworm Rearing. Bulletin No.2, Cental Sericultral Researchand Training Institute, Mysore, p.23

Mora JR, Iwata M, von Andrian UH, 2008. Vitamin effects on the immune system: vitamins A and D take centre stage. Nat. Rev. Inmunol. 8: 685-698.

Prassad PR, 2004. Effect of fortification of Ascorbic Acid through mulberry leaf on cocoon traits of Pure Mysore race of silkworm, *Bombyx mori* L. Indian J. Entomol. 66: 37-39.

Rahmathulla VK, 2012. Management of climatic factors for successful silkworm (Bombyx mori L.) crop and higher silk production: A Review. Psyche: J. Entomol. doi:10.1155/2012/121234

Rahmathulla VK, Suresh HM, Mathur VB and Geethadevi RG, 2002. Feed conversion efficiency of Elite bivoltine CSR hybrids silkworm, *Bombyx mori* L. reared under different environmental conditions. Sericologia. 42: 197-203.

Raman C, Manohar SL, Xavier N and Krishnan M, 2007. Expression of silk gene in response to p-soyatose (hydrolyzed soy bean protein) supplementation in the fifth instar male larvae of *Bombyx mori*. J. Cell Biol. Mol. Sci. 6(2): 163-174.

Rao, CGP, Seshagiri SV, Ramesh C, Basha K, Ibrahim H, Nagaraju J and Chandrashekaraiah M, 2006. Evaluation of genetic potential of the polyvoltine silkworm, *Bombyx mori* L. germplasm and identification of parents for breeding programme. J. Zhejiang Univ. Sci. 7: 215-220.