ISSN 1313 - 8820 (print)
ISSN 1314 - 412X (online)

Volume 11, Number 1
March 2019

AGRICULTURAL SCIENCE AND TECHNOLOGY

2019

An International Journal Published by Faculty of Agriculture,
Trakia University, Stara Zagora, Bulgaria

AGRICULTURAL SCIENCE AND TECHNOLOGY, VOL. 11, No 1, pp 8 - 12, 2019
DOI: 10.15547/ast.2019.01.002

Correlations between some economic larval traits of silkworm

M. Gündüz*, Ü. Şahan

Department of Animal Science, Faculty of Agriculture, Uludag University, 16059 Gorukle, Bursa, Turkey

(Manuscript received 25 August 2018; accepted for publication 18 December 2018)

Abstract. *Silkworm, Bombyx mori L. is an economic insect that has great importance on cocoon and silk production. The development of sericultural industry depends on quality and yield in terms of qualitative and quantitative silkworm characters. Mature larval weight and silk gland mass are important factors in silkworm breeding. Silk gland mass has significant impact on the synthesis of silk protein in fifth larval stage. The investigation was conducted to evaluate the correlations between silk gland mass and mature larva weight of M and ZF (China), and N and ZB (Japan) lines and their hybrids MxN, ZFxZB. In this research, silkworm lines and hybrids were reared at the laboratory with 600 silkworm larvae for each four lines and their hybrids to determine these silkworm traits. Analysis of variance indicated that the mean values in Chinese lines and hybrids were found to be significant (P<0.01) for mature larva weight. On the other hand, mean value of silk gland mass was determined as important for Chinese, Japanese lines and hybrids (P<0.01). Both mature larva weight and silk gland mass have positive correlation in Chinese lines (r=0.659) and hybrids (r=0.643) (P<0.01). Otherwise, the correlation between these characters were not found to be significant for Japanese lines. As a result, it is necessary to search the correlation between different characters and to protect the genetic diversity in silkworm lines.*

Keywords: sericulture, lines, hybrids, silk gland mass, mature larva weight

Introduction

It is well known that silk gland is a larval specific tissue for the synthesis of silk protein in silkworm and begins to degenerate shortly before pupation (Zhang et al., 2006; Bashir, 2013). The growth of silk gland in silkworm is of paramount importance to the sericultural industry as it is responsible for the synthesis of silk proteins, the basic raw material of the silk cocoon (Shimizu, 2000; Sutherland et al., 2010). Silk glands, present in the larval stage of the silkworm, produce threads of silky material to form the cocoon and are mainly composed of three parts: the anterior, the middle, and the posterior silk glands, each playing different roles in silk secretion. In the body of the silkworm, after the alimentary canal, the silk gland is considered the second largest gland, consisting of three distinct regions posterior (15cm long), middle (7cm long) and anterior (2cm long), (Prudhomme et al., 1985; Inoue et al., 2000).

The development of silk glands has great importance to the sericultural industry because silk glands are responsible for the synthesis of silk protein. For years, structural and functional aspects of the silk gland of silkworm *Bombyx mori* have thoroughly been investigated (Sasaki and Noda, 1973; Tashiro et al, 1976; Sailaja and Sivaprasad, 2010).

Silkworm larvae contain a silk gland that is a larval specific tissue used for the synthesis of silk protein in Lepidopteran insects and that starts generating soon before pupation. (Zhang et al, 2006; Bashir, 2013). Most emphasis in the silkworm industry is given to the silk gland because it is the only gland that is accountable for the production of protein and acts as a raw material for the cocoon (Shimizu, 2000; Sutherland et al., 2010). During the larval period silkworm larvae consume only mulberry leaves, for the production of luxuriant silk thread in the form of cocoon (Goldsmith, 2009; Banno et al., 2010; Rahmathulla, 2012).

The silk gland is the principal site for manufacturing the two silk proteins; as fibroin and sericin and also other 90 proteins (Jin et al., 2004; Zhang et al., 2006). In the silk gland, the proteins are utilized for the manufacturing of silk proteins, as fibroin and sericin. Fibroin is the structural centre of the silk, and sericin is the sticky material encompassing it. Its main structure consists mostly of the repetitive amino acid sequence. Sericin is a kind of protein synthesized by *Bombyx mori* silkworms in the production of silk (Vollrath, 2000; Kancevicha and Lukyanchikovs, 2012).

Physiological and biochemical properties are important to study for evaluating the weight of silk glands, as they play an essential role in the production of cocoon. The weight of the silk gland varies in different breeds and is a characterstic feature among breeds (Reddy et al., 2015). On the other hand, silk productivity and seed production is being affected by the weight of mature larvae in different breeds of silkworms, therefore this trait is supposed to be most important. That indicates the importance of larval weight for gaining the higher cocoon weight and ultimately the more silk yield. Moreover, larval weight is one of the major factors that determines the health of the larvae, and thereby the quality of cocoon spun. Due to the higher metabolism and development of silk glands in the fıfth instar, it is during this period when larvae reach their maximum size and weight (Ueda et al., 1971; Nguku et al., 2007).

Environment plays a great role in the development of silk gland and synthesis of silk. Silk gland grows during the fourth and fifth larval instars, the rate of which is modulated by environmental factors such as mulberry leaves quality and quantity, temperature and relative humidity (Shimizu, 2000).

*e-mail: mervegunduz@uludag.edu.tr

The purpose of the present study was to investigate the correlations between silk gland mass and mature larva weight of *Bombyx mori* L. lines (M and ZF - China, N and ZB - Japan) and their hybrids (MxN and ZFxZB).

Material and methods

Object of study

This experiment was conducted at Kozabirlik (silkworm egg production unit). For the purpose of the study, four silkworm *Bombyx mori* L. lines M, ZF (Chinese) and N, ZB (Japanese) and their hybrids (MxN, ZFxZB) were used. To prevent diseases and to maintain good sanitation, the rearing room and other rearing appliances were disinfected with a 5% formaldehyde solution following the procedure suggested by Ullah and Narasimhanna (1978). The eggs were incubated at 25±1°C temperature and 75-80% relative humidity for about 10-12 days until hatching (Krishnaswami, 1978).

Animals and experiment design

Silkworm lines and hybrids were reared in the laboratory with standard rearing conditions of 75-80% relative air humidity (RH) and photoperiod of 16L:8D (Lim et al., 1990). Temperature and relative humidity inside the laboratory were measured (EagleTech HTC-2 Digital CD Temperature Humidity Meter Clock Hygrometer Thermometer) every day for the duration of the experiment. Totally, 600 silkworm larvae were reared for each pure line and their hybrid combinations to determine silkworm larval characters. In the end of the fifth larval instar on the 8^{th} day, at the peak of growth of the fifth instar 10 larvae were randomly selected from each of the four lines and two hybrids to measure mature larva weight and silk gland mass. Firstly, 10 mature larvae were measured individually from each line and hybrid. After that, silk gland was extracted with optical tweezers from the abdomen of the larvae to weigh silk gland mass with a (±0.01g) precision scale and expressed in gram (Precisa Gravimetrics AG, Switzerland). Also, the removed silk gland was cleaned and gently soaked with filter paper to remove the adhered water on it.

Statistics

Data processing was performed in one-way ANOVA using the general linear model as summing equal variances and statistical analysis was performed using the program Minitab software (Minitab, 2017). The Pearson correlation was used to determine the correlation of different parameters of mature larvae weight and silk gland mass.

Results and discussion

The results of mean values of mature larva weight and silk gland mass were evaluated for different silkworm lines and their hybrids are given in Table 1. Analysis of variance indicated that the mean value in Japanese lines and hybrids were found significant (P<0.01) for mature larva weight. The mean values of mature larval weight in M line and ZF line ranged from 38.5±0.20g to 34.6±0.20g in Chinese lines, also from 41.5±0.34g (ZFxZB) to 38.9±0.34g (MxN) in hybrids, respectively. These results were in accordance with Talebi et al. (2010) who reported differences in mean value for larval weight in line C_{108} (3.40g) and NB_4D_2 (3.45g). Besides, Rayar (2010) explained the same mean performance on multivoltine x bivoltine silkworm hybrids in terms of mature larva weight. In another research, the average larval weight was found among 4.21g and 3.8g for reared silkworms (Balanay, 2002).

Table 1. The mean values of mature larvae weight and silk gland mass in different silkworm lines and their hybrids

Lines and Hybrids	Characters ($\bar{X} \pm S_{\bar{x}}$), n=10	
	Mature larvae weight (g)	Silk gland mass (g)
Lines		
Chinese		
M	38.5±0.20^{a}	4.38±0.08^{a}
ZF	34.6±0.20^{b}	3.95±0.08^{b}
Japanese		
N	38.0±0.17	4.26±0.08^{b}
ZB	37.7±0.17	5.02±0.08^{a}
Hybrids		
M x N	38.9±0.34^{b}	5.13±0.08^{b}
ZF x ZB	41.5±0.34^{a}	6.02±0.08^{a}

*a, b means in the same row with different superscripts are significantly different (P<0.01)
(n= 10 in each replicate)

It is necessary to focus on larval weight that may have been influenced by the ability of different silkworm strains to absorb consumed food in different percentages. During the whole larval period, almost 25-30g of fresh mulberry leaves are consumed by the silkworm larva, *Bombyx mori* L. of the dry material digested, 54% in the young silkworm and 60% in the grown silkworm is retained in the body, 25% of the food ingested is converted to silk protein. The female of the grown silkworm utilizes about 10% of the food digested for the formation of the eggs (Şahan, 2011). In the last larval instar the maximum weight gained by newly hatched larvae increases to 10 000 times and also the silk gland grows almost 160 000 during the fifth larval instar when differentiated with the newly hatched silkworm larvae (Krishnaswami, 1973). It was found that the silkworm larvae reared under favourable environmental conditions with good quality of mulberry leaves and adequate quantity, they will exceed their minimal weight and reach optimal weight (Saha et al., 2009).

The mean values of silk gland mass were found significant for Japanese lines and hybrids and are given in Table 1. Moreover, mean value was recorded significantly higher for silk gland in ZFxZB (6.02±0.08g) hybrid followed by MxN (5.13±0.08g) hybrid when compared with ZF (3.95±0.08g). The efficiency and benefit of the sericulture industry rely on the quantity of silk proteins produced in the silk gland and the quality of silk extracted from the cocoons. In the commercial productivity of silk, the silk shell weight instantly influences the silk content of the cocoon, through which the estimation of the productivity is regulated (Mendonça et al., 2010; Kavitha, 2012). The weight of the silk gland of *Bombyx mori* L. was extensively affected because of the synthesis of protein. The development of silk gland is activated all of a sudden toward the start of the fifth instar, the weight of silk gland elevates quickly because of growth of individual cells (Tashiro, 1968).

Dhawan and Gopinathan (2003), additionally reported that the size and weight of the silk gland increment with each larval instar. Typically, the silk gland of *B. mori* develops gradually during the early phase, quickly elevates in weight and size during the fifth larval instar, and once the cocoon has been formed, starts to decline (Sehnal et al., 1990). Hugar and Kaliwal (1998) noticed that in the fifth instar larvae were the most active and during this activity the larvae develop immense amount of food reserves which are gathered for cocoon spinning, metamorphosis and reproduction. Essentially more prominent growth and development of silkworm larvae and silk gland are acquired under the optimum conditions of 25°C and 70% RH; this may add to the development of sericulture industry (Rahmathulla and Suresh, 2013).

In addition to this, some application has been performed on silk gland mass. For instance, Malik and Reddy (2010) showed higher silk gland weight and somatic index rate when silkworm was treated with linoleic acid.

The aim of silkworm breeding is the genetic progress of characters to enhance profitability of the sericulture industry (Mubasher et al., 2010; Seidavi, 2010). The relationship between different characters was clearly obtained by methodical examinations which enabled breeders to practice for different traits (Darmand et al., 2011). Estimating the correlation of effective factors is useful for determining the components of complex traits and also correlation between some silkworm characters may have a fundamental significance for breeding programs. It is important to know the correlation between the specific traits of quantitative characters in the silkworm. A positive correlation between the characters indicates the genetic progress of the breeding methods.

The study was undertaken to analyze correlation between mature larvae weight and silk gland mass. Correlation between mature larval weight and silk gland mass of silkworm, *Bombyx mori* L. is presented in Table 2.

Table 2. Correlation coefficient between mature larval weight and silk gland mass of silkworm, *Bombyx mori* L.

Lines and Hybrids		Silk gland mass (g)
Chinese	Mature larva weight (g)	0.659** (0.002)
Japanese	Mature larva weight (g)	-0.250 (0.288)
Hybrids	Mature larva weight (g)	0.643** (0.002)

*Means in the same line with no common superscript are significantly different at P< 0.01; Data in parenthesis are 'p' values.

It seems, there is a high and positive correlation between Chinese lines (M and ZF) (r=0.659) and hybrids (MxN and ZFxZB) (r= 0.643) for these larval characters (P<0.01). On the contrary, the correlation was not found significant for Japanise lines in terms of mature larva weight and silkgland mass (r= - 0.250) (P>0.05) in the study.

To evaluate the quantitative traits in silkworm, silk gland is considered an important character for its determination. Many studies showed the correlation between silk gland and other characters. Devaiah et al. (1985) showed a positive association between silk gland and larval weight. Emilia and Dezmirean (2012) have investigated correlation between silk gland mass and mature larval weight (r= 0.3077). The high and positive correlation between these traits has been seen from the results of Bashir (2013). Most of the genetic characters in silkworm are under polygenic control, affected by environmental components and nutrition (Yokoyama, 1979).

To understand the relationship between different commercial characters is one of the vital parameters in breeding programmes. Many researches have been done on determining correlation among economic traits of silkworm to enhance the production through selection systems (Seidavi et al., 2008; Singh et al., 2011). Hence, the data produced from studies might be utilized during the breeding procedures in reproducing programs for new breeds of silkworm with preferable economic characters. Duration of larval growth is counter related with increments of cocoon production, silk quality and fecundity (Bharathi and Govindappa, 1987; Mahesha, 2013).

It is important to know the relationship between characters for increasing some quantitative characteristics in silkworm breeding. Mostly correlation is directly related to the cocoon character because of cocoon weight, shell weight, shell percentage in silkworm breeding. Recently, most studies have been focused on silk gland mass, silk gland length and larval characters. The correlation between silk gland weight and larval weight, and also between silk gland length and larval weight was found by Murugesh et al. (2013). It has been shown that silk gland weight has a positive correlation with larval weight, likewise with cocoon weight, shell weight in various researches (Bashir, 2013). Additionally to these characters, the relationship between larval silk gland and shell weight was revealed by Kasmaei and Mahesha (2012).

Moreover, a positive relationship was found between the fifth instar larvae weight and growth of silk gland (Ueda et al., 1971; Miranda et al., 2002). The larval weight showed a positive correlation among few characters such as cocoon weight, filament length, and denier other than silk gland weight (Mahesha, 2013). As per a report by Kamili (1994), silk gland weight is directly correlated to silk production and various silkworm strains have distinctive silk gland weight affecting their silk generation ability.

Conclusion

Considering the results of this study, correlation between silk gland mass and mature larva weight has importance for Chinese lines (M, ZF) and hybrids (MxN, ZFx ZB). In conclusion, Chinese lines and hybrids can be suggested for selection programs in terms of economical silkworm characters as mature larvae weight and silk gland mass in silkworm breeding.

References

Basavaraja HK, 1996. Studies on the synthesis of productive bivoltine strains of *Bombyx mori* L. through hybridization, selection and genetic evaluation. Thesis for PhD, University of Mysore, Mysore, India (Ind).

Bashir M, 2013. Physiological and biochemical changes in silkglands of *Bombyx mori* L. in response to seasons. Thesis for PhD, The Faculty of Postgraduate Studies Sher-e-Kashmir University of Agricultural Sciences, Kashmir, India (Ind).

Balanay PG, 2002. Performance evaluation of newly F1 silkworm hybrids under laboratory condition. PTRI TC, Kalingagan, Phillipines.

Banno Y, Shimada T, Kajiura Z and Sezutsu H, 2010. The silkworm - an attractive BioResource supplied by Japan. Experimental Animals, 59, 139-146.

Bharathi D and Govindappa S, 1987. Effect of prostaglandin F2 on growth pattern of silkworm larvae, *Bombyx mori* L. Sericologia, 27, 355-358.

Darmand S, Lavvaf A, Seidavi AR, Eila N, Nematollahian S and Farahvash T, 2011. Estimation of genetic parameters in three commercial silkworm lines of Iran. African Journal of Biotechnology, 10, 13324-13330.

Dhawan S and Gopinathan KP, 2003. Cell cycle events during the development of the silk glands in the mulbery silkworm *Bombyx mori*. Development Genes and Evolution, 213, 435-44.

Devaiah MC, Rajashekhargouda R, Yelashetty S and Govindan R, 1985. Growth and silk production in Samia cynthia ricini Boisduval as fed on four different host plants. Indian Journal of Sericulture, 24, 33-35.

Emilia F and Dezmirean DS, 2012. Correlation between larvae mass and silk glands mass of silkworm bred in Transylvania. Bulletin UASVM Animal Science and Biotechnologies, 69, 1-2.

Goldsmith MR, 2009. Recent progress in silkworm genetics and genomics. In: Molecular Biology and Genetics of the Lepidoptera (eds. M.R. Goldsmith and F. Marec), CRC Press. New York, ABD, pp. 25-47.

Hugar II and Kaliwal BB, 1998. Effect of Benzyl-6- aminopurine and indole –3-acetic acid on the biochemical changes in the fat body and haemolymph of the bivoltine silkworm, *Bombyx mori* L. Bulletin of the Sericultural Experiment Station, 9, 63-67.

Inoue S, Tanaka K, Arisaka F, Kimura S, Ohtomo K and Mizuno S, 2000. Silk fibroin of *Bombyx mori* is secreted, assembling a high molecular mass elementary unit consisting of H-chain, L-chain and P25, with a 6:6:1 molar ratio. Journal of Biological Chemistry, 275, 40517-40528.

Jin H, Chen J, Karageorgiou V and Altman G, 2004. Human bone marrow stromal cell responses on electrospun silk fibroin mats. Biomaterials, 25, 1039-1047.

Kamili AS, 1994. Detection and estimation of components of genetic variation in bivoltine silkworm (*Bombyx mori* L.). Thesis for PhD, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Kashmir, India (Ind).

Kancevicha V and Lukyanchikovs A, 2012. Silk fibroin bombyx mori yarns for bio-resistant woven aortic prostheses. Material Science, Textile and Clothing Technology, 7, 13-16.

Kasmaei FG and Mahesha HB, 2012. Analysis of correlation between haemolymph and midgut tissue amylase with commercial characters of silkworm *Bombyx mori* L. Annals of Biological Research, 3, 3518-3532.

Kavitha S, Sivaprasad S, Saidulla B and Yellamma K, 2012. Effect of zinc chloride and zinc sulphate on the silkworm, *Bombyx Mori* growth tissue proteins and economic parameters of sericulture. The Bioscan An International Quarterly Journal of Life Sciences, 7, 189-195.

Krishnaswami S, Narasimhanna MN, Suryanarayan SK and Kumararaj S, 1973. Manual of Sericulture. Second Edition, Silkworm Rearing, FAO, Rome, Italy.

Krishnaswami S, 1978. New technology of silkworm rearing. Bull. 3, CSR and TI, Mysore, India.

Lim SH, Kim YT, Lee SP, Rhee IJ, Lim JS and Lim BH, 1990. Sericulture training manual, Food and Agriculture Organization of the United Nations, FAO Agricultural Services Bulletin, Rome, Italy.

Mahesha HB, Ghasemi KF and Thejaswini PH, 2013. Analysis of correlation between commercial characters of silkworm *Bombyx mori*. International Journal of Biology Pharmarcy and Allied Sciences, 2, 1071-1082.

Malik FA and Reddy YSR, 2010. Effect of foliar application of linoleic acid on nutritive and economic characters of the mulberry silkworm *Bombyx mori* L. Indian Journal of Entomology, 72, 42-47.

Mendonça GA, Marchini LC and Macedo LPM, 2010. Cocoon production of the silkworm, *Bombyx Mori* L. (Lepidoptera: Bombycidae), fed on leaves of mulberry hybrids. Revista Caatinga, 23, 118-122.

Miranda JE, de Bortoli SA and Takahashi R, 2002. Development and silk production by silkworm larvae after topical ap-

plication of methoprene. Journal of the Science of Food and Agriculture, 59, 585-588.
Minitab Inc, 2013. MINITAB release 17.1.0 statistical software for Windows. Minitab Inc, USA.
Mubasher H, Shakil AK and Muhammad A, 2010. Evaluation of genetic potential of inbred pure line of silkworm for breeding and cocoon production in Pakistan. African Journal of Food Science, 4, 300-302.
Murugesh KA, Mahalingam CA and Mohankumar S, 2013. Silk gland tissue somatic ındex and silk quality traits of bivoltine and multivoltine races of silkworm, *Bombyx mori* L. The Madras Agricultural Journal, 100, 524-527.
Nguku EK, Muli EM and Raina SK, 2007. Larvae, cocoon and post-cocoon characteristics of *Bombyx mori* L. (Lepidoptera: bombyxidae) fed on mulberry leaves fortified with Kenyan royal jelly. Journal of Applied Sciences and Environmental Management, 11, 85-89.
Prudhomme JC, Couble P, Garel JP and Daillie J, 1985. Silk synthesis. In: Comprehensive insect physiology, biochemistry and pharmacology (eds. G.A. Kerkut and L.I. Gilbert), 13, Pergamon, New York, USA.
Rahmathulla VK, 2012. Management of climatic factors for successful silkworm (*Bombyx mori* L.) crop and higher silk production: A Review. Hindawi Publishing Corporation Psyche, 12.
Rahmathulla VK and Suresh HM, 2013. Influence of temperature and humidity on growth and development of silk gland of a bivoltine silkworm hybrid. Iranian Journal of Entomology, 3, 24-29.
Rayar SG, 2010. Evaluation of bivoltine silkworm hybrids suitable to nontraditional sericulture region of Karnataka. Karnataka Journal of Agricultural Sciences, 23, 798-799.
Reddy BV, Divya P and Anitha M, 2015. Quantitative profile Analysis of Mulberry Silkworm *Bombyx mori*. L (CSR2XCSR4). International Letters of Natural Sciences, 7, 34-41.
Saha AK, Chaudhuri AB, Moorthy SM and Roy S, 2009. Studies on Determination of Larval Critical Weight in Silkworm, *Bombyx mori* L. - An Index of Quality. International Journal of Industrial Entomology, 19, 243-248.
Sailaja B and Sivaprasad S, 2010. Photoperiodic modulation of circadian protein rhythm in the silk gland of *Bombyx mori* during fourth instar development. The Bioscan, 5, 177-183.
Sasaki T **and Noda H**, 1973. Studies on silk fibroin of *Bombyx mori* directly extracted from the silk gland. 1, Molecular weight determination in guanidine hydrochloride or urea solutions. Biochimica et Biophysica Acta, 310, 76-90.
Sehnal F and Akai H, 1990. Insect silk glands: their types, development and function, and effects of environmental factors and morphogenetic hormones on them. International Journal of Insect Morphology and Embryology, 19, 79-132.
Seidavi AR, Bizhannia AR, Ghanipoor M and Qotbi AAA, 2008. Investigation on improvement possibility of resistance, production and reproduction traits in 3p, 2p and p generations in three Japanese pure lines of silkworm *Bombyx mori* L., using individual selection in 3p generation. Asian Journal of Animal and Veterinary Advances, 3, 443-447.
Seidavi AR, 2010. Estimation of genetic parameters and selection effect on genetic and phenotype trends in silkworm commercial pure line. Asian Journal of Animal and Veterinary Advances, 5, 1-12.
Shimizu M, 2000. Structural basis of silk fibre; in Structure of silk yarn: biological and physical aspects, (ed. Nobumasa Hojo). Taylor & Francis Inc. New Delhi, India.
Singh T, Bhat MM and Khan MA, 2011. Critical analysis of correlation and heritability phenomenon in the silkworm, *Bombyx mori* (Lepidoptera: bombycidae). Advances in Bioscience and Biotechnology, 2, 347-353.
Sutherland TD, Young JH, Weisman S, Hayashi CY and Merritt DJ, 2010. Insect silk: one name, many material. Annual Review of Entomology, 55, 171-188.
Şahan Ü, 2011. İpekböcekçiliği, İpekböceği Yetiştirme ve Islahı, Koza Üretimi, Ham İpek, Yumurta Üretimi, Hastalıklar ve Dut Yetiştirme, 1, 69, Dora Yayınları. Bursa, Turkey (Tr).
Talebi E and Subramanya G, 2010. An investigation on heterosis and inbreeding depression in the silkworm (*Bombyx mori* L.). ARPN Journal of Agricultural and Biological Science, 5, 52-55.
Tashiro Y, Morimoto T, Matsuura S and Nagata S, 1968. Studies on the posterior silk gland of the silkworm, *Bombyx mori*. I. Growth of posterior silk gland cells and biosynthesis of fibroin during the fifth larval instar. The Journal of Cell Biology, 38, 574-88.
Tashiro Y, Shimaadzu T and Matsura S, 1976. Lysosomes and related structures in the posterior silk gland cells of *Bombyx mori* L. In late larval Stadium. Cell strucure and Function, 1, 205-222.
Ueda S, Kimura R and Suzuki K, 1971. Studies on the growth of the silkworm, *Bombyx mori* L. III. Relative increase in body weight and silk gland weight in the 5th instar larvae. Bulletin of the Sericultural Experiment Station, 25, 1-20.
Ullah SR and Narasimahanna MN, 1978. Handbook of practical sericulture. Central silk Board.
Vollrath F, 2000. Strength and structure of spiders' silks. Journal of Biotechnology, 74, 67-83.
Yokoyama T, 1979. Silkworm selection and hybridization. Working paper on genetics in relation to Insect Management. Rockefeller Foundation, 71-83.
Zhang YQ, Ma Y, Xia YY, Shen WD, Mao JP and Xue RY, 2006. Silk sericin–insulin bioconjugates: Synthesis, characterization and biological activity. Journal of Controlled Release, 115, 307-315.