Punjab University Journal of Zoology

OPEN ACCESS

37(1): 07-17 (2022)
https://dx.doi.org/10.17582/journal.pujz/2022.37.1.07.17

Review Article

An Overview of Sericulture and Enhanced Silk Production in *Bombyx mori* L. (Lepidoptera: Bombycidae) Through Artificial Diet Supplementation

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Article History
Received: April 13, 2020
Revised: December 25, 2021
Accepted: January 10, 2022
Published: March 17, 2022

Authors' Contributions
NS, HM and SA conceived the idea and prepared figures and/or tables. NS, MH, HMT and Azizullah wrote the manuscript. HMT, SYK and AK edited and approved the manuscript.

Keywords
Silk, Silk gland, Natural food, Food supplementation

Abstract | Sericulture is a labour-intensive, welfare-oriented and rural cottage industry being capable to engage amateurish family manpower for income generation via indoor activities. It has potential to provide a livelihood for the poor rural families and can play a crucial role to uplift the economy of our country. Unfortunately, this industry has not been on the list of priorities of our Government. Currently, Pakistan is importing silk from other countries to meet the demand of local market. Nutritional background of larval stage (1st, 2nd and 3rd instars etc.) significantly influences the status of the resulting larva, pupae, adult and fiber. For this purpose, sericulture productivity has been impressively modulated by fertilizing the silkworm's diet with natural food supplements or exogenous nutrients like Amway protein, honey, bovine milk, sericin, probiotics (*Bacillus cereus, B. subtilis, B. amyloliquefaciens, B. licheniformis, Lactobacillus casei, Saccharomyces cerevisiae* and Spirulina), vitamins (C and E), royal jelly, ascorbic acid, cowpea seed powder, AgNPs, secondary metabolites (phenols flavonoids, phenolic amino acids and proline) and white hen's egg at different larval instars. Economic parameters (pupal weight, shell ratio (%), cocoon weight, filament length, shell weight, denier, fibroin and sericin contents) and biological traits (fresh weight of each larva, silk gland, pupa and moth) of silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) have been enhanced with natural food supplements. The current review highlights details about the overview of sericulture, constrains of sericulture industry being faced by local farmers in Pakistan and impact of natural food supplements on silkworms growth and cocoon yield.

Novelty Statement | In this review article current activities related to sericulture and methods to improve economic and biological traits through diet supplementation have been discussed.

To cite this article: Shahzadi, N., Tahir, H.M., Ali, S., Bhatti, M.F., Azizullah, Khan, S.Y. and Khaliq, A., 2022. An overview of sericulture and enhanced silk production in *Bombyx mori* L. (Lepidoptera: Bombycidae) through artificial diet supplementation. *Punjab Univ. J. Zool.*, **37**(1): 07-17. https://dx.doi.org/10.17582/journal.pujz/2022.37.1.07.17

Introduction

The silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) is a domesticated, monophagous and economically important insect. Its primary food source is fresh mulberry leaves (Soumya *et al*., 2017; Zhang *et al*., 2019) enriched with carbohydrates, lipids, proteins, moisture and inorganic matter (Konala *et al*., 2013; Zhou *et al*., 2015). Moreover, silkworm is well-known to humans for thousands of years due to fabrication of natural silk as well as for its significant role in rural agro-industry of

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subtropical and tropical regions of the world (Ruiz and Almanza, 2018; Xu *et al*., 2018). Silk (a natural fiber) is produced by different arthropods including scorpions, silkworms, bees, spiders and mites with variations and similarities in properties and compositions (Altman *et al*., 2003; Wang *et al*., 2006). Silk mainly consists of two proteins (sericin and fibroin) which are synthesized by the silkworms in their foremost silk gland (Qi *et al*., 2017) and ejected by spinnerets during final stage of the larval development (5th instar) (Hossain *et al*., 2015; Sekar *et al*., 2016). Enormously nutritious mulberry leaves make the silkworms more resistant to ailments as well as enabled them to elevate silk productivity (Kaur *et al*., 2013). Optimum environmental conditions like moderate relative humidity (RH) (60-75%) and temperature (25-28°C) are necessary for the larval growth and development, adult emergence and mass production of silk. On the other hand, unfavorable conditions like high temperature and RH affect the mechanical (strength, rigidity) and morphological (coloration) properties of the cocoons which ultimately altered the spinning behavior and sericin curative properties (Offord *et al*., 2016; Ramachandra *et al*., 2001). In addition, these two environmental factors also have a reflective impact on protein and carbohydrate profile of the insects, egg hatch ability, larval mortality, pupation and overall performance of the silkworm lines (Hussain *et al*., 2011; Kumar *et al*., 2012).

In the last few years, significant and novel innovations have been made in sericulture through enriching the mulberry leaves with exclusive food components such as horse gram flour, ascorbic acid, probiotics, bovine milk, white hen's egg, honey and Amway protein (Quraiza *et al*., 2008; Rani *et al*., 2011; Hossain *et al*., 2015; Thulasi *et al*, 2015; Masthan *et al*., 2017; Helaly, 2018) for better silk quantity and quality along with enhanced silkworm's growth. Keeping this information in view, we are urged to explore the excellent, easily available and cost less dietary supplements to enhance the silk production and quality by the silkworm.

Sericulture

Sericulture is the great invention of the ancient Chinese (Liu *et al*., 2010; Yilmaz *et al*., 2015) which entails cultivation of mulberry plants, development of silkworm, collecting, reeling and weaving of silk (Kawade *et al*., 2014; Rahmathulla, 2012) as demonstrated in Figure 1. It is a short gestated (about 40 days), least resource intensive and commercially alluring financial activity (very low investment leads to high returns), additionally does not longer require high education and thought to be a promising strategy to alleviate the poverty in the rural areas (Goswami and Bhattacharya, 2013; Mubin *et al*., 2013; Rahmathulla, 2012). It is being practiced at monetary scale in more than 25 countries all over the world and provides employment for 30 million households of distinctive international locations like China, Korea, India, Thailand, Vietnam, Brazil and Bulgaria. In Pakistan, sericulture had been brought underneath the Forest Department since 1975 in specifically province Punjab (Mubin *et al*., 2013). Several studies have indicated that sericulture industry in Pakistan was once a remunerative occupation but due to several reasons and with the passage of time, this industry is near to demise. The main reasons behind the deterioration of this industry include lack of interest and attention of state, less and old productive silk seeds, unavailability of standardized conditions and infrastructure, inappropriate rearing sheds and scarcity of advancement in research, policies and technology (Rahim and Hyder, 2017). Besides the fundamental products (silk cocoon and natural silk fiber which are the two most important assets for the income of serial culturists) of the sericulture, a number of secondary products have also exceptional commercial value (Kim *et al*., 2010). Accordingly, effective utilization of the bye merchandise of sericulture like moths, pupae, sericin, silk fiber waste, silkworm excreta, fruits, roots and mulberry leaves, for value addition is must for putting sericulture on sound footing (Buhroo *et al*., 2018). Furthermore, there are innovative commercial products that have been obtained from the sericulture waste with widespread fascinating destination for cosmetics (skin and hair products), pharmaceutical (antibacterial, anti-viral, anti-diabetic, hypotensive, antivirus and hypoglycemic products), foodstuffs (juice, vinegar, marmalade, wine, oil, fruit distillate, natural coloring and dried fruit powder), zootechnic (fodder for swine, goats, rabbits, poultry, fur animals and sheep) and ecological (landscape and phytoremediation) importance (Buhroo *et al*., 2018; Dong *et al*., 2017; Soumya *et al*., 2017).

Silkworm (Bombyx mori) silk

Silk has long been known as 'Queen of Textiles' owing to its elegance, luxurious grandeur and comfort properties (Basu, 2015). *B. mori* silkworm belongs to the family Bombycidae, also known as mulberry silkworm, is well characterized, renowned and employed silk; Conversely, Saturniidae (*Antheraea mylitta*) fabricates the non-mulberry silk. Although spider silk is no longer usually employed on account of dearth of commercially established supply chains due to wilder nature and smaller yield in contrast to silk of *B.mori* (Hardy and Scheibel, 2009). Chinese have been attributed for invention of the silk with a record which could be traced back to more than 380 million years (Shera *et al*., 2019). A well-known raw silk is being produced and exported by Brazil, Italy and China whilst imported by Italy, Japan and India. Bulgaria is the pinnacle producer of raw silk and fresh cocoons in Europe. Largest fresh cocoons producer around the world is China and engender about 84 % of the global raw silk. However, India is categorized 2nd, contributing about 15% to the world raw silk production (Popescu, 2013).

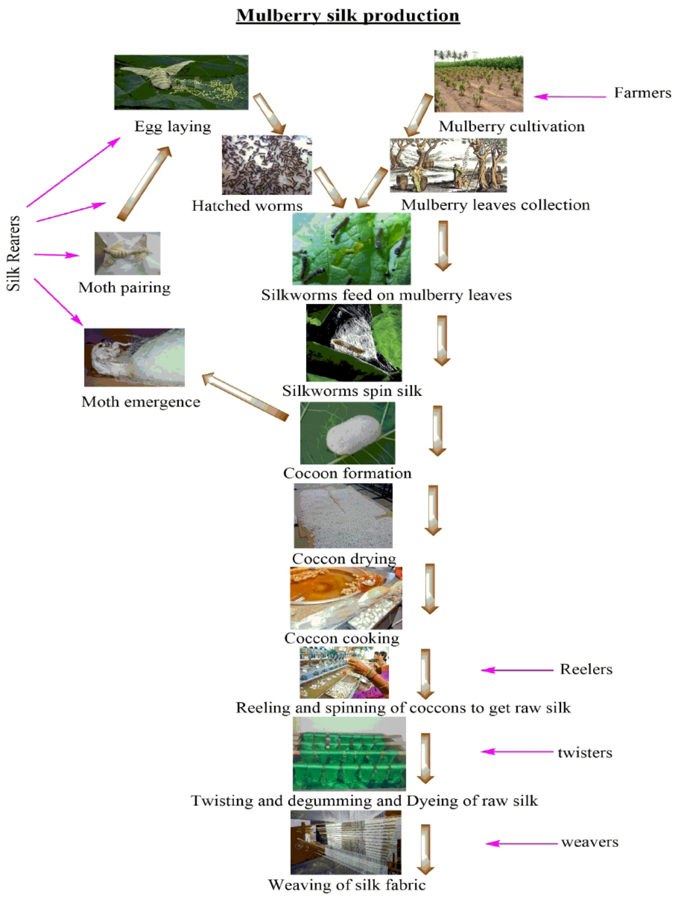

Figure 1: Phases of mulberry silk production and processing.

Composition of silk

The silk cocoons of *B. mori* are comprised of two major proteins, namely fibroin and sericin, intimately linked but unique in terms of properties and structure (Altman *et al*., 2003; Cao and Zhang, 2016; Inoue *et al*., 2000; Khyade and Yamanka, 2018; Yusoff *et al*., 2019). Generally, the proportion of silk sericin is about 15–35% while silk fibroin ranges from 65% to 85% (Altman *et al*., 2003; Cao and Zhang, 2016; Inoue *et al*., 2000). Moreover, a non-sericin like part made of mineral, salts, wax, sugars, other impurities and pigments is additionally present (Cao and Zhang, 2016). The central structural protein of the silk fiber is silk fibroin (Yusoff *et al*., 2019) and being biocompatible protein has appropriate characteristics for pharmaceuticals ceasing and remarkable drug release properties (Ageitos *et al*., 2019). Furthermore, silk fibroin has also been extensively used as a biomaterial for regeneration and tissue engineering purposes (Ju *et al*., 2016; Kim *et al*., 2016, 2017; Su *et al*., 2019). Exclusively, silk fibroin supported the adhesion, proliferation and differentiation of a number of cell types, including osteoblasts, endothelial cells, epithelial cells, glial cells, keratinocyte and fibroblast (Martínez-Mora *et al*., 2012; Yamada *et al*., 2004). Sericin is typically adhesive protein, wrapped around the fibroin to keeps the integrity of silk fibers and being separated by using thermo-chemical treatment (degumming) (Jiang *et al*., 2006; Koh *et al*., 2015; Porter and Vollrath, 2009).

Silk sericin

Noteworthy silk sericin protein is especially hydrophilic component of silk with molecular weight ranging from 20 to 400 kDa and incorporates 18 different amino acids with predominantly polar amino acid inclusive of threonine (6%), glutamic acid (5 %), serine (25 %) and aspartic acid (17 %) (Aramwit *et al*., 2009; Gimenes *et al*., 2014; Padamwar and Pawar, 2004; Wu *et al*., 2007). Polar groups (amino, hydroxyl and carboxyl) of the side chains of amino acids, their structural organization, organic composition and solubility make viable co-polymerization, cross-linking and combinations of sericin with other polymers. All collectively express unique properties of the sericin as an antioxidant, moisturizing, healing, antibacterial and antitumor/anticancer as well as provides defense against ultraviolet radiation (Takechi *et al*., 2014). Beside these, sericin solution (0.05 wt. %) absorbs UV radiations ranging from 223 to 300 nm, resist to free radicals, stimulate cell proliferation, inhibit tyrosinase activity and retain water capacity as well as being used as a vital ingredient for tissue engineering and skin repair (Su *et al*., 2019). Sericin protein can be utilized in food industry because of its beneficial properties to the development of variety of new products owing to its metallic ion-chelating and antioxidant properties (Sasaki *et al*., 2000; Wu *et al*., 2008). It is predictable that 50,000 tons of unused sericin is thrown away each year in the degumming waste water worldwide. Lately, there has been an eminent attention for reuse and retrieval of sericin which in turns minimizes the environmental issues and has significant commercial and scientific value (Jin *et al*., 2004). Sericin has also been investigated for the biological effects and also has therapeutic consequences in diabetes (Yang *et al*., 2002). A number of researchers have found that because of anti-elastase and mitogenic properties, sericin has various potential applications in biomedical field (Lamboni *et al*., 2015) (Figure 2).

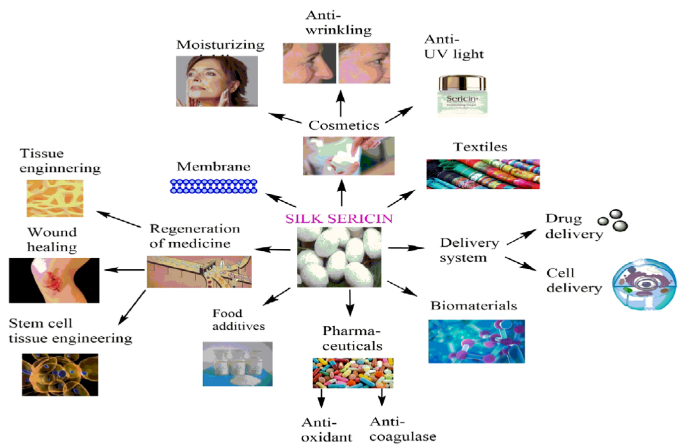

Figure 2: Application of silk sericin.

Silk fibroin

The fibroin fiber is enveloped by sericin with subsequent gummy layers which ensures the uniformity of cocoon via cementing the bunch of silk threads, all collectively for the aim of defending the developing silkworm (Jena *et al*., 2018). Among the most explored silk-based materials for the tissue engineering is silk fibroin. It has potential to release and transport the insulin in sustained manner, which is a powerful strategy for the cure of corneal injury (Cubayachi *et al*., 2019). Consequently, the requirement for water vapor permeability, biodegradability, elevated mechanical resistance and biocompatible materials displays the rising interest of silk fibroin in biomedical field (Altman *et al*., 2003; Fuchs *et al*., 2006; Gupta *et al*., 2007; Servoli *et al*., 2005; Wenk *et al*., 2011). Silk fibroin supports cell proliferation and cell attachment for different kinds of cell types (Jin *et al*., 2004). The biomaterials based totally on silk have determined to be appropriated for diverse applications, such as skin wound dressing, bone tissue scaffold, vascular tissue regeneration and drug delivery (Zhao *et al*., 2015) (Figure 3).

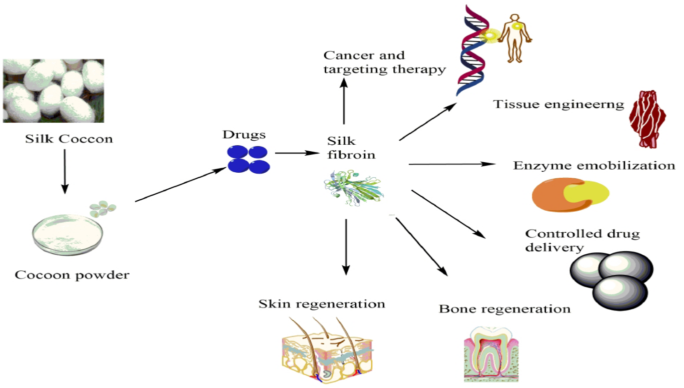

Figure 3: Applications of silk fibroin.

Fortification of the mulberry leaves with supplementing/ exogenous nutrients

Earlier studies has confirmed that sustenance of silkworm is a merely factor which augments quantity and high-quality of silk (Vijila, 2018). The consequences of distinctive dietary supplements on the silkworm have been extensively studied (Tantray, 2016). Better volume and high-quality of the cocoon and silk production can be executed through the fortification of mulberry leaves with additional nutrients (Thangapandiyan *et al*., 2019). Secondary metabolites can be recommended as leaf nutrient for increasing the farmer's earnings (Sivakumari *et al*., 2019). A complete detail about the impact of different food additives/exogenous nutrients on silkworm's growth and economic parameters of sericulture is described in Table 1.

Probiotics

Gururaj *et al*. (1999) reported that microbial infections due to pathogens on silkworm prompt a shift in the metabolic profile and the activities of distinct enzymes including invertase, amylase, protease and trehalose. Enrichment of mulberry leaves with the probiotic microorganisms like *Saccharomyces cerevisiae* Meyen ex E.C. Hansen (Saccharomycetales: Saccharomycetaceae) has influenced the enzymatic profiles (elevation of amylase and invertase activity which are essential for better food utilization with increased larval growth) and prompt disease resistance with improved quantitative economic parameters of the silkworm (Esaivani *et al*., 2014). Subsequently, probiotics elevate the manufacturing of nutritional vitamins and improve the host resistance as well as enable the silkworms to compete with pathogens (bacteria) by synthesizing the organic and antibiotic substances (Singh *et al*., 2005). Another study has evaluated that the supplementation of *Bacillus licheniformis* (Weigmann) Chester (Bacillales: Bacillaceae) resulted in maximum larval weight, effective rearing rate, cocoon mass, weight of shell and pupae, shell ratio, silk productivity and filament length. It additionally reduces the larval mortality by disease prevalence and fiber denier as well as being used for the commercial rearing of the silkworms (Vijila, 2018). It was also found that *S. cerevisiae* (Yeast) serves as an immune modulating agent. Consequently, energy budget, level of protein content and the commercial characteristics of silkworm were enhanced at a higher rate (Amala and Ranjith, 2011). Spirulina (blue-green micro algae) has attracted more attention recently due to its interesting composition including 18 amino acids and different vitamins including the tocopherol, vitamin B12, thiamine, biotin, folic acid riboflavin, beta-carotene, niacin, pyrodozoic acid, carbohydrates (mucopolysaccharides, glycogen and rhamnose etc.), 50 vital minerals, trace elements, chlorophyll and GLA omega-3 fatty acid (Venkatesh *et al*., 2009). Mulberry leaves supplemented with Spirulina (blue green algae) as a feed to silkworm proved to be very effective in revamping the larval and cocoon characteristics (Venkatesh *et al*., 2009; Venkataramana *et al*., 2003). Previous studies confirmed that blue green algae (Spirulina) and *S. cerevisiae* (yeast) yielded better fibroin content indicating the good high-quality silk when compared to *Lactobacillus acidophilus* (Moro) Hansen and Mocquot and *L. sporogenes* Horowitz-Wlassowa and Nowotelnow (Lactobacillales: Lactobacillaceae) (Masthan *et al*., 2017). In addition, a list of commonly used probiotics for improved silk production in silkworm is given in Figure 4.

Amway nutrilite

Amway nutrilite is a fat-free and protein rich commercial nutrient, widely used as a supplemental diet. The enhanced larval growth and silk production could be augmented with the supplementation of nutrilite (Raman *et al*., 2007). Notably, it becomes enriched in soya protein (80%) (Chief ingredient of nutrilite) and performs a crucial role in the growth and development of the silkworm as well as improves the economic traits of the sericulture. It also contains 9 crucial amino acids (lysine (544 mg), isoluecine (408 mg), leucine (696 mg), tryptophan (104mg), threonine (320mg), histidine (216 mg) and valine (432 mg), phenylalanine and tyrosine (722mg),

Table 1: Different Food supplements /Food additives and their economic and biological effects on silk and silkworm.

Reference	**(Quraiza et al., 2008)**	**(Thangapandiyan et al., 2019).**	**(Nguku et al., 2007)**
Consequences	Elevate economic parameters and protein content of silk gland, fat body and muscle	Improve the economic traits and the nutrient efficacy in silkworm	Augment the larval, cocoon and pupal weights in silkworm
Duration		4 times per day till the formation of cocoon	
Dose	1% and 2%	300ppm AgNPs	0.5gms-dried powder per 20ml-distilled water
Larval stages in *Bombyx mori*	1st instar	3rd instar	fourth instar till spinning
Food supplements	Ascorbic acid	AgNPs and spirulina	Royal jelly
Reference	**(Sivakumari et al., 2019).**	**(Brahma et al., 2018)**	**(Thulasi and Sivaprasad, 2013)**
Consequences	Responsible for higher shell ratio, cocoon and shell weight along with improved renditta and reel-ability.	Vitamin C yield better result in concentration of silk gland protein in comparison to vitamin E. Furthermore, vitamins C facilitate the metabolism in the silkworms and also influences on its larval growth and development throughout metamorphosis.	Ascorbic acid is responsible for better growth and economic parameters of silkworm. Lemon juice enhances the shell weight, shell protein and denier.
Duration	4 times a day	7th days till the formation of cocoon	Once in a day
Dose		1%	1%
Larval stages in *Bombyx mori*	All larval instars	5th instar	4th and 5th instars
Food supplements	Secondary metabolites (phenols flavonoids, phenols total amino acids and proline)	Vitamin C and E	Ascorbic acid + Lemon juice
Reference	**(Lattala et al., 2014)**	**(Khyade and Gosavi, 2016; Manjula et al., 2011)**	
Consequences	Spermidine raise better growth and silk production in silkworm	Enhance silk length, silk weight as well as improve the digestion of food and elevate the quantity of silk	
Duration	3 times in a day	5 times in a day	
Dose	25µM-75µM	7.5%	
Larval stages in *Bombyx mori*	5th instar	3rd to 5th instars	
Food supplements	Spermidine	Cowpea seeds powder	

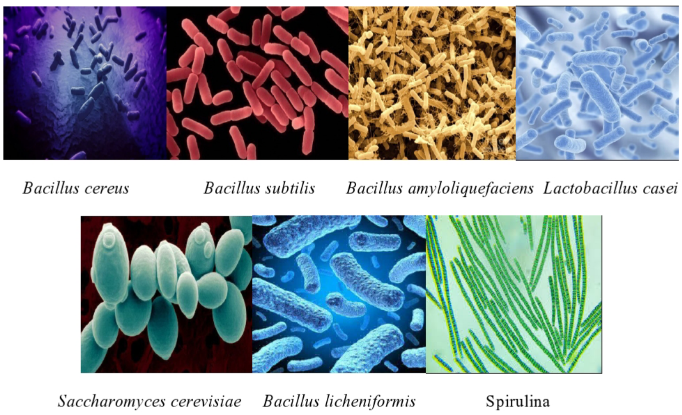

Figure 4: Common probiotics used commonly as food additives for improved silk production in silkworm.

responsible to modulate the silk coding gene expression and silk protein synthesis in silkworm. Profitable gains in the different economic traits of sericulture, under the influence of Amway nutrilite (1% in distilled water to the fourth larval instar till it begin to spinning), are executed through its significant growth stimulating effects on silkworm, more predominantly with reference to the reinforcing silk protein synthesis in silk gland. Simultaneously, the nutrilite could possibly retard the synthesis of floss in sericulture (Thulasi *et al*., 2015). In addition, Amway nutrilite (10%), protein enrichment with the mulberry leaves had been traced and found significant enhancement on the energy budget (Relative Growth Rate (RGR), weight gain, Relative Consumption Rate (RCR), Efficiency of Conversion of Digested Food (ECD), Approximate Digestibility (AD), Efficiency of Conversion of Ingested Food (ECI) and economic characters (cocoon and shell mass, larval weight, shell ratio, filament length, filament width, denier, filament width sericin and fibroin content) of the silkworm (Rani *et al*., 2011). Even though, the cause for growth stimulating effect of Amway nutrilite has not been evaluated so far (Quraiza *et al*., 2008; Thulasi and Sivaparasad, 2013, 2015).

Honey

Honey being multifactorial nutrient comprises of metals (Co, Cr, Cu, Mn, Zn and Fe), enzymes (Diastase, invertase, catalase, glucose oxidase etc.), a variety of sugars carbohydrates (82%), proteins, minerals and vitamins (B and C). It is a natural sweetener, produced with the aid of honey bees from nectar of the flora (Council, 2002) along with fascinating characteristics and chemical composition which vary relying on its environmental, botanical and geographical origin (Ball, 2007; Falco *et al*., 2003; Garcia *et al*., 2005). Moreover, honey has been exploited as an ingredient of veterinary and human medicines owing to its significant healing, prophylactic and nourishing properties (Cooper *et al*., 2002; Iglesias *et al*., 2004; Taormina *et al*., 2001). Honey is a profitable supplementary diet. An earlier study has reported that mulberry leaves treated with 2% aqueous honey were offered to fifth instar larvae, which in turn modulated larval growth and metabolism, reduced floss output (sericulture waste) and elevated silk production and quality (Bhatti *et al*., 2019; Thulasi and Sivaparasad, 2015). Accordingly, honey being cost effective and easily accessible food additive not solely stimulates the silk protein synthesis in silk gland but also mobilizes the protein reserves from body and improves the economic parameter of sericulture (Bhatti *et al*., 2019; Sivaprasad and Thulasi, 2014).

Milk

Milk is a multifaceted liquid that simultaneously offers bioactive compounds in the form of minerals, proteins, fatty acids, carbohydrates and other nutrients that facilitate crucial growth and development of silkworms. It additionally contains many polyamines, enzymes and peptides which plays a pivotal role to modulate the various regulatory processes (Haug *et al*., 2007). Likewise, transforming growth factor (TGFβ, a bioactive compound) has been identified in bovine milk and human, which assist differentiation, growth, and immune response (Donnet-Hughes *et al*., 2000) and influenced tremendous impacts on body and cocoon weights of silkworm (Konala *et al*., 2013). Subsequently, few researchers has reported that mulberry leave enriched with bovine milk were consumed by the fifth larval instar up to the spinning of cocoon, prompted the highest lipid in the haemolymph of larvae (Helaly, 2018) which improved growth rate and silk production (Helaly, 2018; Konala *et al*., 2013).

Egg white

Beside other food supplements, egg white is an excellent source of selenium and riboflavin as well as affluent in fundamental vitamins and amino acids which are requisite for optimal growth of an insect (Szalay, 2015). Regarding the biochemical traits, white hen's eggs provide the highest total protein content in the haemolymph of the 5th larval instar. Helaly (2018) studied that rearing of silkworm on the mulberry leaves enriched with the white hen's eggs, given at odd days during fifth instar until the cocoon formation, induced noteworthy increase in the weight of silk gland, full-grown larva, female pupa, cocooning percentage, cocoon shell, weights of fresh cocoon, silk filament length, silk size and silk weight.

Conclusion and future perspective

We have outlined in this assessment compelling evidence regarding the impact of natural food supplements (bovine milk, Amway protein, honey, white hen's egg, probiotics and sericin) on growth, survival, economical parameters (larval weight, cocoon weight, pupal weight, cocoon weight, filament length, shell weight, cocoon width, denier, fibroin and sericin content) and biological characters (fresh weights of each of larva, silk gland, pupa and moth) of silkworms. We are urged to explore the remarkable and economical food additives that will help us to improve the silk quality and quantity in silkworms which in turn improve the economy of our country. We strongly recommended the proper utilization of natural food supplements and maintenance of optimum conditions for the rearing of silkworms to boost up the silk industry.

Acknowledgements

We are really thankful to Punjab Higher Education Commission (PHEC) to provide funds for our project to perform experiments to enhance silkworm's economic and biological traits through diet supplementation.

Conflict of interest

The authors have declared no conflict of interest.

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