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Artificial Diet Based Silkworm Rearing System-A Review

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ABSTRACT

There are about 1000 varieties of silkworm, Bombyx mori L., among them bivoltine and multivoltine races are particularly used for rearing in India. Silkworm is a monophagous insect which has special significance in sericulture industry. It totally depends on the mulberry leaves at their larval stage. Mulberry leaves are a traditional food for silkworm larvae due to presence of morin. Human beings have benefited by the silkworm in various ways and scientists have been continuously trying to improve the techniques of silkworm rearing. The physiology of Bombyx mori (L.) has been studied comprehensively due to the economically valuable silk production.

Mulberry belongs to the genus Morus of the family Moraceae. Three types of mulberry are found, white (Morus alba L.), red mulberry (M. rubra) and black mulberry (M. nigra). Among them, only white mulberry is recognized as the food source for silkworm. Now a days, sericulture research developed number of supplement nutrients with mulberry leaves for silkworm rearing. There are number of foods used as an ingredient for artificial diet of silkworm. Artificial diet encourages the small landless farmers to take up sericulture and it also helps to reduce labour cost for mulberry cultivation. In this review, one attempt was made to discuss on artificial diet based silkworm rearing and different ingredients used in artificial diet preparation.

Key words: *Artificial diet, silkworm, mulberry, protein, sterol.*

INTRODUCTION

India is the second largest producer and also the largest consumer of silk in the world due to development of innovative technologies in mulberry cultivation as well as in silkworm rearing. The best silk quality is obtained from the cocoons of the larvae of mulberry silkworm *Bombyx mori* L. reared in captivity (sericulture). Sericulture is an art and science of technology fundamentally a village based and welfare oriented industry that plays an important role in our national economy. Sericulture depends on rearing of silkworm on mulberry leaves; and it can be stated that silk productions are directly correlated with larval growth and development on mulberry. The production of mulberry leaves both in terms of quality and quantity change due to different climatic factors and field practices.

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One of the alternatives for improving larval feeding is enrichment of mulberry leaves with different supplementary nutrients such as vitamins[30]. There are about 1000 varieties of silkworm[4]; among them, bivoltine and multivoltine races are particularly used for rearing in India. Among them, *Bombyx mori* L. is mostly domesticated and is believed to have derived from the original mandarina silkworm *Bombyx mandarina* Moore[33]. Silkworm *(Bombyx mori* L.*)* is a monophagous insect which has special significance in sericulture industry.

The growth and development of silkworm larvae and subsequently cocoon productions are greatly influenced by mulberry leaves quality. Nearly 70 per cent of the silk produced is directly derived from mulberry leaf proteins. Hence, silkworm should be fed abundantly with good quality mulberry leaves for the successful cocoon production and high yield of silk. Although the Mulberry (*Morus sp.*) leaf is considered as the traditional food for silkworm, but now a day's many attempts has been made to establish artificial diet. First time, artificial diets were applied in sericulture in Japan in 1977 for the rearing of young larvae of the silkworm. The practical application of artificial diets in sericulture which has enabled to save a enormous deal of labour for the rearing and the rearing young healthy larvae has rapidly expanded. Though many artificial diets have developed after extensive research, but it was observed that silkworm exhibits better growth rate and cocoon production when fed by mulberry leaves only. The mulberry used must be fresh enough to meet the preference of silkworm; it must be fed 3 or 4 times a day. But such kind of rearing system has faced some problems:

1. High land-cost for mulberry fields
2. Rearing houses and farmer's habitation
3. Good transportation facility
4. Intensive labour

So system of rearing silkworm with artificial diet may solve the above problems. Farmers who rear silkworms can purchase the artificial diet instead of cultivating. The labour of rearing can be avoided because the artificial diet given to silkworm is once in every 3 or 4 days. In addition, the system could be extremely useful in recreation cultivation, education, and biological research. As land-price is high and labour is lacking so application of artificial diet becomes more practical for sericulture development. Prof. Hou of National Chung-Hsin University at Taiwan and his co-workers have done good work with diet rearing system[34]. It was observed from their results that among the three most popular mulberry varieties in Taiwan the most suitable mulberry leaf powder for the artificial diet are leaves on upper shoots of the variety Taisong-2 and Taisong-3. However there are local mulberry varieties, as a constituent of the diet which supports the silkworm development comparable to that of fresh leaf. For making leaf powder a rolling-tank-type oven was found to be better than common vertical-multiple-shelf oven. As it allows rapid drying of larger amount of mulberry leaves despite of its smaller drying chamber, leaf drying process is operated rapidly and are of good quality. He also found that mulberry shoots themselves are good source of cellulose. To protect it from microbial contamination, dry component of the diet per 100g are mixed with antibiotics like 10mg of chloramphenicol, 5mg of dihydrostreptomycin, 1500 mg of propionic acid, and 200 mg sorbic acid, which support whole developmental stages of silkworm. This new system could rear silkworms up to 4th instar through inexpensive method and high quality cocoons are produced when applied with mulberry.

Essential components of food required for silkworm rearing

i. Protein as essential nutrients for larval growth:

Almost 70% silk proteins produced by silkworm are directly derived from the mulberry leaf protein[41]. Some workers clearly described that protein acts as essential ingredients in silkworm diet for silkworm growth and silk production. Arai and Ito[2] established the presence of amino acid in the mulberry leaves protein can influence the larval growth. Elevation of dietary protein to an optional level[21] and supplementation of low nutritive proteins with their limiting amino acids[25] have found to accelerate the growth of the silkworm. Ito[27] stated that rich sources of dietary proteins like soya protein are known to promote growth and improve the economic characters of the silkworm. Horie and Watanabe[20] found soybean meal as a protein source in silkworm diet that can increase the weight of silkworm larvae and fresh silk glands. Krishnan *et al*[32]. showed that the hydrolyzed soya-protein (P-soyatase) supplementation decreased the larval duration, increased the accumulation of haemolymph protein (SP-1: female specific protein and SP-2; an arlyphorin), larval weight and cocoon characters. Moustafa *et al*[37]. studied the nutritional effect of tested level of mulberry leaf powder and dietary soybean in the semi-artificial diet. El-Sayed[11] and El-Hattab[10] found the effects of dietary protein on larval growth and silk production. Low molecular weight peptides or small peptides had an optimistic effect on silkworm growth and silk production[28,29] .

ii. *Vitamin C and Vitamin B as Nutrition for Silkworm*

For the growth and development of *Bombyx mori* L-ascorbic acid (vitamin C) has always been regarded crucial. In fact, in mulberry leaves ascorbic acid is present in large amounts[5,35], stimulatory effect of ascorbic acid was seen on silkworm voluntary feeding during the first instar stage[24]. The larval development can enhance by vitamin C and B as coenzymes in amino-acid metabolism and antioxidant agents, which may increase amino-acid concentrations in larval tissues, leading to improvements in productivity. This hypothesis coincides with that of Babu *et al*[3]. who observed that leaf consumption was greater in ascorbic-acid-fortified leaves as compared to control; practically vitamin C content of tissues and body fluids are highly dependent upon the amounts ingested with food. Suprakash & Pal[48] also found that vitamin B complex significantly improved growth and development, with beneficial effects on the economic characteristics of the cocoon. The improvement of cocoon and silk characters in the experiment may be attributed to improvement of the efficiency of conversion of dietary nitrogen into the cocoon shell. In other words, the increase in the cocoon and filament characters might be due to increased protein conversion efficiency of the silk glands as a result of increased availability of vitamins and the positive antibacterial effect of honey on the growth of many kinds of bacteria. This is supported by Sarker *et al*[47]. who reported that supplementation of mulberry leaves offered to silkworm with ascorbic acid (1%) and vitamin B complex (0.5%) improved cocoon yield and silk filament quality. Bee-honey is also highly beneficial, improve rearing and the quality of the silk filament. Supplementation of mulberry leaves with vitamin B increased the resistance against poor environmental conditions and increased body weight in silkworm and Vitamin B12 could increase the synthesis of nucleic acids and protein in the silk gland of silkworm[9]. Silk production is increased by Riboflavin which also reduces the choline and uric acid excretion and at end its derivatives are sprayed on mulberry leaf, thus helps silkworm to increase the fiber yield[26]. Folic acid or folate is one of the vitamin B which is essential for the normal development of larvae"s spine, brain and skull and also reported that it is necessary for nucleic acid biosynthesis in insects. It was also reported that dietary supplementation of folic acid to silkworm larvae did not significantly increase the glycogen content of the body but haemolymph trehalose content increases significantly[44]. Etebari[12] showed that hyper vitaminosis does not only cause enhancement of larval and cocoon weight but it also has many negative effects too. Enrichment of mulberry leaves with vitamin E did not have significant effect on food consumption in silkworm larvae[36].

iii. *Effect of different Salt on silkworm growth and development:*

Salt may represent a limiting factor for the growth of insects, principally true for all types of diet composition[53]. The salt significantly improved the growth of the developmental stages, increased the cocoon characters, elicited early cocoon production and increased the reproductive potential of the silkworms. Nutritional supplementation of nickel chloride, potassium iodide and copper sulphate increased the economic parameters of the silkworm. It is reported that nickel chloride considerably increased the growth of silkworm larvae, pupae, adults and subsequently cocoon production but higher salt concentrations produced terminal effects on these parameters[22]. The cocoon weight was increased after feeding silkworm larvae with nickel and zinc fortified mulberry leaves[7].

iv. *Requirement of sterol for silkworm development:*

Insects require dietary sterols because there are unable to synthesize sterols *de novo*[31,38]. Sterols are crucial to insects for survival and development, since cholesterol is utilized not only as constituents of cell membranes, but also as precursors of molting hormones, ecdysteroids[8,14]. β-sitosterol present in the mulberry leaves and conversion of β-sitosterol to cholesterol is crucial for *Bombyx* larvae[39]. In addition to nutritional regulation of sterol requirements, β-sitosterol appears to contribute for activation of biting[16] and dietary selection in this. Therefore, sterol requirement of *B. mori* should provide important information on the relationship between nutritional regulation and feeding behaviour.

To address the molecular conversion of β-sitosterol in *Bombyx* larvae, we analyzed the composition of sterols in an artificial diet and in *Bombyx* larvae. Substantial sterol components of the midgut, epidermis, silk gland, and hemolymph of *Bombyx* larvae fed with mulberry leaves was reported much earlier on the basis of analytical records of preparative gas chromatography[42].

Artificial Diet: an alternative food for silkworm

An complete rearing of the silkworm on artificial diets was first achieved in 1960[13,23]. At that time, on artificial diet based rearing, the larval growth was poor, mortality was high, development was delayed, and cocoons were small. At present there is little difficulty in rearing the silkworm on artificial diets and in obtaining cocoons of good quality in a laboratory scale.

However**,** different authors tried to establish artificial diet with different composition (both in terms of quality and quantity) for silkworm rearing. They used different essential food as ingredients of artificial diet.

i. Composition of Artificial Diet:

Special mulberry gardens are maintained to meet the specific nutritional requirement of young instar silkworm, so artificial diet was developed in order to avoid such preconditions. Contrary to the initial belief that the popular and productive silkworm hybrids commercially exploited in India and their parental strains would accept the artificial diet for young instar rearing, they did not accept the diet and the FR (Feeding Response) was low. To overcome this bottleneck, it was realized that the only option available was to modify the selection response of silkworm for the feeding behaviour. Thus, commercial breeds were planned to change gradually to feed on artificial diet through selection. This would ultimately facilitate to develop a common artificial diet for all the evolved hybrids. When Japan faced a similar problem with silkworm strain, *Sawa J* regarding their abnormal feeding behaviour, efforts was made to evolve exclusive silkworm strains for rearing only on artificial diet. By exploiting the polyphagous nature of *Sawa J*, special strains and commercial hybrids were further developed for rearing on artificial diet[49]. Different artificial diet composition made up by different author was reported in their articles. After 24 years of continued research, Hamamura[15] reported the substances involved in 3 step feeding behaviour of *Bombyx mori* silkworm found in mulberry leaves as attracting factor (Citral), biting factor (β-sitosterol, morin or isoquercitrin) and swallowing factor (cellulose powder) and supplementary factors (potassium diphosphate, sucrose, inositol and silicasol) in agar agar jelly pure form were prepared. Watanabe[52] reported that in addition to the fresh leaf alcohol, the fresh leaf aldehyde also attracted *Bombyx mori* silkworm. Several terpenes and esters having the same action were reported by Hirao and Ishikawa[17].

Yamada and Kato[54] worked on chlorogeninc acid, found in water soluble part of mulberry leaves and Hamamura *et al*[16]. worked on the role of polyphenolic acid in growth of silkworm. According to Arai and Ito[2], amino acid present in the mulberry leaves protein was analyzed and mixture of the same was included in the artificial diet in place of soybean powder.

L-ascorbic acid (vitamin C) has always been regarded indispensable for the growth and development of *Bombyx mori*. In fact, ascorbic acid is present in large amounts in mulberry leaves[5,35], the exclusive food for the silkworm, but they are incapable of synthesizing it. Ascorbic acid is usually added to silkworm food (enrichment) in a quantity generally varying from 1–2% of the dry weight of the artificial diet, which is considered as optimum content of this vitamin[26]. On the basis of worked done by Cappellozza *et al*[6]. it seems possible to eliminate ascorbic acid from the formulation of artificial diet, at least for fifth instar stage without affecting the production or larval mortality and the length of the larval cycle. Further studies will elucidate the mechanism of ascorbic acid requirements in different silkworm strains. The possibility of utilization of ammonia by the silkworm, *Bombyx mori*, is of interest in connection with the biological role of silk formation.

Akao[1] proposed that silk formation was a kind of excretion of excess amino acids by which the silkworm larvae could survive the danger of amino-acidaemia. This was proposed because the removal of silk glands from 3rd- or 4th-instar larvae elicited amino-acidaemia and most larvae died before pupation. Ammonium nitrogen is used by the silkworm, *Bombyx mori*, for silk production in the same manner as non-essential amino acids[18].

Recently, two kinds of artificial diets have been used to simplify the process for the manufacturing of the prepared diets from the dried diets. One is the "Pellet diet", is produced by using a twin-spindle extruder and can be fed to the silkworms by soaking with a suitable amount of water just before use. Another one is namely "Yuneri diet" can be fed to the silkworms by mixing with hot water at around 80o C without steaming[19].

ii. *Effect of Artificial diet on silkworm rearing system:*

The positive FR (Feeding Response) in the strains under the study on artificial diet feeding can be attributed to the presence of mulberry leaf powder in the diet composition which largely promotes the FR in silkworm. Nihmura[43] suggested that it was highly desirable to include mulberry leaf powder or mulberry leaf extracts as feeding stimulatory substance in the artificial diet in order to evoke uniform and prompt FR of newly hatched larvae. Many productive breeds having low response to artificial diet were made to adapt to the artificial diet over generations through selection. Trivedy *et al*[50,51]. evolved five multivoltine and six bivoltine strains. The improved strains were developed which accepted the diet and performed well with respect to their economic traits and almost at par with that of mulberry reared counterparts[50,51]. Nair *et al*[40]. created a larger pool of potential multivoltine silkworm strains for rearing exclusively on artificial diet during the young instar which will form breeding resource material for prospective hybrids.

iii. *Dependence of artificial diets on mulberry leaves:*

All artificial diets utilized by different worker for their experiment is composed by mulberry leaf as ingredients. The mulberry leaves may be used as powder form or fed after crushing as pest form. Likewise Cappellozza *et al*[6]. prepared artificial diet by using 25% dried and pulverized mulberry leaf out of the diet dry weight. Pallavi *et al*[45]. also used mulberry leaves with soya flour, corn flour, horse gram flour and complete artificial diet (Serinutrid) to improve the silkworm nutrition. Rajaram *et* *al*[46]. utilized "Serinutird" manufactured by the M/s. Sericare, Aschem Agencies Pvt. Ltd., Bangalore consist of the 28 % mulberry powder, 25 % soya powder, 4 % salt mixture, 0.3% sterol, sugar 3%, cellular power 8%, jelling agent 7%, and preservative 3 %. It is also necessary to improve mulberry leaf quality as they are essential for all kind of synthetic artificial diet.

CONCLUSION

It was generally considered that artificial diets could not be used for silkworm rearing throughout the whole instars for silk production, due to the cost of the diet. However, with the breeding of polyphagous silkworms and the development of low cost artificial diets this practice may become possible. Both theoretical and applied studies are currently in progress, including breeding of silkworms, improvement of artificial diets, development of rearing system and economical evaluation of the silkworm rearing system on artificial diets throughout the instars and the effective production of silkworm eggs. The new method of rearing of the silkworm on artificial diets is a renovated technique and its rapid expansion is expected in sericulture. To improve the quality of artificial diet, the quality of mulberry leaf must be improved first. Since, the primary purpose of mulberry cultivation in most of the Asian countries is to feed the silkworm with its leaf, the impact of the salt that accumulated in leaf of mulberry on silkworm"s growth and development need to be investigated in details. However, further studies such as genomic investigation, systematic biology-based researches and other extensive proteomic studies are necessary to elucidate more aspects in this area. More studies are needed to clarify the ability of different organic and inorganic components like ammonia assimilation and utilization by *Bombyx mori* in relation to silk formation. Further, knowledge on genetic basis of salinity tolerance in mulberry through identification of QTLs will facilitate utilization of molecular markers for identification desirable parents for cross hybridizations and screening of hybrids at early developmental stages. Emphasis should also be given to explore the natural genetic variations in salt tolerance among crop plants and their wild relatives. Regarding mulberry, recent developments in genetics, tissue culture, transgenesis, and linkage mapping have shown that research in the coming years will definitely change the salt tolerant capacity of mulberry. Similarly, emphasis has to be given for developing mechanisms by which mulberry plant can regulate excessive ion uptake and translocation in leaves.

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