International Journal on Emerging Technologies **15**(2): 62-67(2024)

ISSN No. (Print): 0975-8364
ISSN No. (Online): 2249-3255

Role of Different Components in Artificial Diet of Silkworm, *Bombyx mori* L.

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(Received 02 July 2024, Revised 06 August 2024, Accepted 29 August 2024)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Silkworm, *Bombyx mori* being a monophagous insect feeds chiefly on mulberry leaves. The presence of chemo-factors *viz.,* morin and β-sitosterol in mulberry leaves act as stimulating and attracting factors for feeding silkworms. With the advancement in research, more emphasis is laid on the development of artificial diet to continue silkworm rearing when mulberry leaves are insufficient or unavailable. However the development of proper artificial diet for silkworm is a high-tech procedure and requires complete knowledge of all the essential nutrients for preparation of diet. Artificial diet requires mulberry leaf powder, proteins, fatty acids, lipids, sterols, cellulose, minerals etc in a recommended amount. These nutrients play an essential role in the growth and development of silkworm larvae. The artificial diet is supplied once with gap of three to four days to young instar larvae of silkworm. The artificial diet is influenced by many factors *viz.*, physical factors, microbial agents and palatability of mulberry leaf. The preparation and feeding of artificial diet in a strategical manner can lower the dependence of sericulture on fresh mulberry leaves with significant decline in labour and production costs. This review aimed to summarize the composition and other important ingredients influencing the properties of artificial diet of silkworm *Bombyx mori*.

Keywords: anti-microbial, β-sitosterol, proteins, silkworm, artificial diet.

INTRODUCTION

Man made food or non-natural food for insects may be referred to as artificial diet (Grenier, 2012). There are many benefits of preparing artificial diet for insects which includes rearing of beneficial insects throughout the year independent of availability of natural food sources or favorable season for growing natural food. The other benefits include rearing of parasitoids and predator for biological control of pests, ease of transport, reduced storage space, handling of measurable dietary ingredients and low cost production (Morales-Ramos *et al.,* 2012). The artificial diet for insects was first of all developed by E. Bogdanow in 1908 for rearing of blue bottle fly, *Calliphora vonitoria.* The artificial diet for blue bottle fly was prepared from meat extract, peptones, starch and mineral salts (Northrop, 1917; Piper, 2017). Silkworm (*Bombyx mori* L.) being monophagous insect, occupies a special place within insect species suitable for several scientific studies (Gautam *et al.,* 2022). Mulberry leaves (*Morus* sp.) are the natural sole food source for silkworms (Li *et al.*, 2023). These leaves are palatable due to presence of volatile compounds (Chang *et al.,* 2021). The performance of silkworm and its productivity depends on the nutritional constituents of mulberry leaves. The nutritional quality of mulberry leaves is superior in spring season than in autumn (Rahmathulla, 2012). The other factors which influence the quality of nutritional quality of mulberry leaves are mulberry cultivar, maturity level of mulberry leaves and harvesting time of leaves (Yu *et al.,* 2018; Nayab *et al.*, 2020). The rearing of silkworms on natural food is sometimes impracticable because of unavailability of abundant mulberry leaf, rearing house, transportation facility and intensive labor requirement. The silkworm rearing over artificial diet can solve many grassroot problems faced in sericulture. The labour costs can be saved as the artificial diet is provided once in 3-4 days. Since the land prices are considerably higher and unavailabity of skilled labour, the artificial diet can be more a good approach for sustainable development (Bhattacharyya *et al.,* 2016).

Mulberry leaves have remarkable nutritional status consisting of proteins, sugars, vitamins, carbohydrates, lipids, and microminerals (Zhou *et al.,* 2015; Yu *et al.*, 2018). Mulberry leaves are efficient to sequester carbon and constitutes active biocompounds *i.e.,* flavonoids, polysaccharides, and alkaloids. These compounds perform many biological functions *viz.,* due to their innate properties of hypoglycaemic, immunoregulatory, antioxidant, and anticoagulan (Wen *et al.,* 2020; Zhang *et al.,* 2022; Hăbeanu *et al.,* 2023). Artificial diet possesses some remarkable property in terms of quality

feed which is germ-free and non-season specific. Hence silkworms can be reared in any season on artificial diet (Saviane *et al.,* 2014). Since silkworms are most attractive towards fresh mulberry leaves, but the artificial diet is considered as pathogen-free when silkworms are particularly reared as biological model for bioreactor to produce recombinant proteins and for many diseases, genetic or silk biomaterial studies (Panthee *et al.,* 2017; Paudel *et al.,* 2020). The rearing of the silkworm, *Bombyx mori,* on artificial diets was first achieved in 1960 (Horie, 1995). The practical application of artificial diets for silkworm rearing has saved a greater proportion of labour (Bhattacharyya *et al.,* 2016). The silkworm as a biological model in research is more accessible due to short gestation period, low-cost breeding, and does not require ethical approval (Panthee *et al.,* 2017; Aznar-Cervante *et al.,* 2021). This review is aimed to summarize the literature information about the composition and different factors influencing the artificial diet for silkworm *Bombyx mori.*

Composition of artificial diet for silkworms: The mulberry chawki gardens were promulgated to meet all the nutritional requirements of young instar worms. It requires a lot of space, time, labour and maintenance of garden to achieve successful chawki rearing. Artificial diet was developed to overcome the issues pertaining to successful chawki rearing (Bhattacharya *et al.,* 2016). However feeding behavior of silkworms plays a great role in rearing of silkworms on artificial diet (Cappellozza *et al.*, 2005; Dong *et al.,* 2017). The feeding behavior of silkworms depends mainly on three factors which include attracting factor, biting factor and swallowing factor (Song *et al.,* 2021). The combinations of these factors have been found important for positive acceptance of food and factors have identified chemically. Alpha-hexanol, beta-hexanol, citral, linalyl acetate, linalol and terpenyactate have been identified as attractants. Biting factors identified are beta-sitosterol, isoquercitrin and morin. The swallowing factors have been identified as cellulose, sugar, inositol, silica and potassium phosphate (Hamamura, 1959). The first successful rearing of silkworm B. mori on artificial diet from hatching to spinning was carried out by Fukuda *et al.* (1960). The composition of artificial diet for mulberry silkworm is given in Table 1. The addition of fresh leaf alcohol to fresh leaf aldehyde attracted *Bombyx mori* silkworm (Watanabe, 1958). Terpenes and esters were found to show the same action (Hirao and Ishikawa 1964). The chlorogenic acid found in water soluble part of mulberry leaves (Yamada and Kato 1966). 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 (Ito, 1978). It is possible to eliminate ascorbic acid from the formulation of artificial diet, at least for fifth instar stage without affecting the production or larval (Cappellozza *et al*., 2005). The possibility of utilization of ammonia by the silkworm, *Bombyx mori,* is of interest in connection with the biological role of silk formation because the removal of silk glands from 3rd- or 4th-instar larvae elicited aminoacidaemia 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 (Hirayama *et al.,* 1995).

Table 1: Composition of artificial diet for silkworm *Bombyx mori* (Roychodhury *et al.,* 1994; Roychodhury, 2003).

Sr. No.	Ingredient	Quantity (g)
1.	Mulberry leaf powder	400
2.	Defatted soyabean powder	200
3.	Phytosterol	3
4.	Sucrose	40
5.	Cellulose powder	115
6.	Corn starch	56
7.	Citric acid	37
8.	Vitamin C	10
9.	Salt mixture	40
10.	Sorbic acid potassium	2.40
11.	Vitamin B mixture*	3.82
12.	Agar powder	75
13.	Soybean powder	13
14.	Chloromycetin	0.15
15.	Propionic acid	6 .00 ml
16.	Distilled water	2.55 litre
	Total	995.37

Note: *Vitamin B mixture:- thiamine, riboflavin, biotin, folic acid, nicotinic acid, pantothenate, pyridoxine-Hcl.

Role of different ingredients in preparation of artificial diet

1. Mulberry leaf. The artificial diets prepared by different workers for their experiment constitutes mulberry leaf as chief ingredient. The mulberry leaves may be used in powder form or paste form. The artificial diet was prepared by using 25% dried and pulverized mulberry leaf (Cappellozza *et al.,* 2005). The mulberry leaf was added with soya flour, corn flour, horse gram flour and complete artificial diet (Serinutrid) were used to improve the silkworm production (Pallavi *et al.,* 2011). "Serinutrid" manufactured by theM/s. Sericare, Aschem Agencies Private Limited, Bangalore constitutes 28% of mulberry powder, 25% of soya powder, 4% of salt mixture, 0.3% of sterol, 3% of sugar, 8% of cellulose powder, 7% of gelling agent and 3 % of preservative as artificial diet to feed silkworms (Rajaram *et al.,* 2012). These ingredients are important to enhance mulberry leaf quality and are considered as essential ingredients I synthetic artificial diet.

2. Protein. Silkworms derive proteins directly from mulberry leaf and convert to silk proteins. Silkworm converts 72-86 % of the mulberry leaf proteins into silk proteins, among which 30% is derived from tissue & blood and 60% of the absorbed amino acids are used for silk production (Qadir *et al.,* 2022; Sudan *et al.,* 2023). Protein acts as essential ingredients for silkworm growth and silk production. The presence of amino acids in the mulberry leaves protein directly influence the silkworm larval growth (Islam *et al.,* 2023). Defatted soya meal is main protein source for preparation of artificial diet. Nearly thirteen essential amino acids and five non-essential amino acids are

required for silkworm growth and development. The fortification of mulberry leaves with nutrient supplements *viz.,* carbohydrates, proteins, vitamins, amino acids, sterols, antibiotics and hormones etc., enhance the crop production and improve the fibre quality (Qadir *et al.,* 2022; Islam *et al.,* 2023). The nutritional effects of mulberry leaf powder proteins and dietary soybean in the semi-artificial diet were studied (Mahmoud, 2013). The low-molecular weight peptides or small peptides had an optimistic effect on silkworm growth and silk production (Jha *et al.,* 2014; 2015).

3. Vitamin B and C. Silkworm larvae require nine vitamins of which ascorbic acid is most important phagostimulant for silkworms (Etebari *et al.,* 2004). Ascorbic acid is present in large amounts in mulberry leaves and profoundly influences the growth and development of *Bombyx mori* (Aneesha and Kumar 2022). The larval development can be improved by vitamin B and C as coenzymes in amino-acid metabolism and antioxidant agents. These vitamins may increase amino acid concentrations in larval tissues which directly leads to improvements in productivity (Zannoon *et al.,* 2008). The improvement of cocoon and silk characters in the experiment may be attributed conversion efficiency of dietary nitrogen into the cocoon shell. The increase in the cocoon and filament characters might be due to increased protein conversion efficiency of the silk glands which is due to increased availability of vitamins. The supplementation of mulberry leaves with ascorbic acid (1%) and vitamin B complex (0.5%) improved cocoon yield and silk filament quality (Sarker *et al.,* 1995). Folic acid or folate is one of the vitamin B which is essential for the normal development of larvae‟s spine, brain and skull. It is also reported to be necessary for nucleic acid biosynthesis in insects (Rahmathulla *et al.,* 2007).

4. Sterols and lipids. Insects require dietary sterols because there are unable to synthesize itself (Nagata and Nagasawa 2011). Sterols are important for insect growth, survival and development. Cholesterol is utilized as constituents of cell membranes and precursors of molting hormones, ecdysteroids (Bhattacharyya *et al.,* 2016). β-sitosterol is present in the mulberry leaves and its conversion to cholesterol is important biological process for *Bombyx mori* larvae (Nagata *et al.,* 2006). The β-sitosterol appears to contribute for activation of biting and dietary selection process in artificial diet. Therefore, sterol requirement of silkworm provide information link between nutritional quality and feeding behavior (Horie, 1995).

Fatty acids in combination with sterols accelerate silkworm larval growth and development. The fatty acids present in mulberry leaves are linolenic, palmilic, linoleic, lauric, stearic and oleic acid are required for silkworm growth (Nakasone and Ito 1967).

5. Sugar and Minerals. Sugar or glucose is not only energy source but also a feeding inducer required for preparation of artificial diet. Calcium, Magnesium, Manganese, Iron, Zinc, Potassium, Phosphorus are essential minerals required for growth and development of silkworm (Reddy, 2010; Urbanek-Krajnc *et al.,* 2022; Mahanta *et al.,* 2023).

Factors influencing formulation of artificial diet

1. Physical factors. Physical factors are important in the maintenance of good quality of diets (Ito, 1980; Parra, 2012). The main factor in diet is water content, which constitutes 75% approximately. However, the proper water content must be pre-determined for each diet as some dietary ingredients, such as agar, sugar, cellulose powder directly influences the hardness or softness of diets (Ito, 1980). Physical property is modified by the inclusion of cellulose powder or by the increase of its level in the diet. The food consumption rate is accelerated by the increase in dietary cellulose powder, and growth of larvae is inversely accelerated by the less nutritive diet (Ito, 1980; Horie, 1995 Bhattacharya *et al.,* 2016). Silkworm cannot digest cellulose but optimal amount of cellulose powder in diet is considered to contribute to improve the physical property of diet. Dietary agar also plays some important role in enhancing the physical property of artificial diet (Ito, 1980). Autoclaving time in diet preparation is also reported to influence the hardness of the diet. The pH value of the diet also affects the physical property of diet (Cappellozza *et al.,* 2005).

2. Antimicrobial Agents. Artificial diets decompose very quickly. The addition of antimicrobial agent is necessary to prevent microbial contamination (Pisochi *et al.,* 2018). Some kinds of antimicrobial substances are found to be harmful for the silkworm. It is necessary to keep the dietary concentrations of those antimicrobial agents at the safe level (Ito, 1980). Although, it is still impossible to prevent the decomposition of diet completely under the conventional rearing conditions. In the artificial diets, sorbic acid, propionic acid and chloramphenicol are added at their optimal concentrations. Under the aseptic condition, the bacterial contamination can be kept away, but this method is not practical in a large scale rearing in sericulture (Ito, 1980).

2. Palatability of diet. The most of the nutritive compounds have their own specific taste and act as either feeding stimulants or inhibitors (Ito, 1980). Some sugars enhance the feeding of the silkworm greatly and some amino acids suppress the feeding considerably (Song *et al.,* 2021). For silkworms, taste is more preferred than odour of the diet (Ishikawa *et al.,* 1969). The main ingredient in diet is Soybean meal which contains water-soluble deterrent substance. It can be easily extracted by washing with a 90% ethanol (Ito *et al.,* 1975). Mulberry leaf powder is the main constitutent and enhances both feeding efficiency and growth of silkworm (Cai *et al.,* 2019). The leaf powder quantity can be lowered to zero at final stage according to requirement of larvae and growth phase, however it did not influence feeding efficiency (Tao *et al.,* 2022). Fresh mulberry leaves are dried to leaf powder at lower temperature, high temperature is not recommended. Larval growth is highly reduced on diet consisting of leaf powder which dried at high temperature (Ito, 1980; Ma *et al.,* 2018).

CONCLUSION

Nutrition plays an essential role in growth and development of silkworm. Since nature provides all the nutrients and additional governing factors for enhancing quality of mulberry leaf as a potential feed for silkworm, *B. mori*. More emphasis should be laid on development of artificial diet homogenous to mulberry leaves for silkworm, *B. mori*. The artificial diet should contain mulberry dry leaf powder, carbohydrates, protein, amino acids, fatty acids, minerals, vitamins in required amounts in addition to attracting and biting factors to support the luxuriant growth of silkworm. The physical factors, antimicrobial agents, pH and mulberry leaf palatability which greatly influences the properties of artificial diet should be taken in proper consideration while formulation of diet. Although, many researchers are still working on the efficacy of artificial diet at commercial level in sericulture. However, artificial diet is quite implementable while working on the research models of silkworm to explore specific mechanisms.

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How to cite this article: Jasmeena Qadir and Tajamul Islam (2024). Role of Different Components in Artificial Diet of Silkworm, *Bombyx mori* L. *International Journal on Emerging Technologies, 15*(2): 62–67.