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ARTIFICIAL DIET AS AN ALTERNATIVE IN SILKWORM (*Bombyx mori* L.) FEEDING - A REVIEW

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Abstract

Silkworm (Bombyx mori L.) is an important monophagous insect in the sericulture industry. The mulberry leaves (Morus alba L.) are known as valuable plants rich in nutrients and nutraceuticals due to the presence of chemo-factors (morin, β-sitosterol) and antioxidants (flavonoids, anthocyanin and alkaloids), the traditional feed in the rearing of silkworm larvae. The mulberry is also used in pharmaceutical, food, beverage and healthcare industries, being considered a suitable plant for sustainable development. Over time, research on using the artificial diet in silkworms has been focused on different topics: i) rearing silkworms in the season when mulberry leaves are insufficient or not available and ii) as an organism model for human diseases (i.e., tumour, degenerative and metabolic diseases) due to the low breeding cost, short generation time, genetic background, large progeny size and numerous genes homologous to humans. This review aimed to summarize the literature information about the impact of nutrition as a key factor in silkworm rearing and the effectiveness of using an artificial diet based on different ingredients in silkworm productivity and health.

Key words*: artificial diet, nutrients, mulberry, silkworm (Bombyx mori L.)*

INTRODUCTION

Silkworm (*Bombyx mori* L.) is a remarkable monophagous, lepidopterous insect that occupies a special place within insect species suitable for several scientific studies. Their traditional feed consists of mulberry (*Morus* sp.) leaves (Saviane et al., 2014) that are more palatable compared to other vegetable leaves, selecting it as a preference due to the volatile compounds contained (Paudel et al., 2020). The rearing performance of silkworms and productivity depends on the nutritional composition and quality of mulberry leaves which usually are higher in the spring season than in autumn (Manjula et al., 2011; Lee and Choi, 2012; Zou et al., 2012; Elangovan et al., 2013; Chen et al., 2020; Qin et al., 2020). The other factors influencing mulberry leaves' nutritional and functional contents are cultivar, harvesting time, and the leaves maturity degree (Iqbal et al., 2012; Lee & Choi, 2012; Zou et al., 2012). However, Hu et al. (2013) noticed that the distribution of the leaves in the mulberry tree also affects the nutritional quality (the top leaves had higher nutritional contents than the mature leaves). Mulberry leaves have an excellent nutrient profile consisting of proteins, carbohydrates, sugars, lipids, vitamins, and microminerals (Zhou et al., 2015; Yu et al., 2018). Moreover, mulberry leaves are more capable of sequestering carbon (Hăbeanu et al., 2023). The bioactive compounds, i.e., flavonoids, polysaccharides, and alkaloids contained by mulberry leaves have hypoglycaemic, immunoregulatory, antioxidant, and anticoagulant properties (Wang et al., 2010; Devi et al., 2013; Wen et al., 2020; Zhang et al., 2022).

Conversely, the artificial diet quality did not change by season to mulberry leaves and may be used in germ-free rearing systems (Saviane et al., 2014). Although mulberry leaves are more attractive for *B. mori*, due to the fact that an artificial diet is pathogens-free, it is required when the silkworms are used as a biological model for various human diseases or as a bioreactor to obtain recombinant proteins and biomaterials based on silk (Kaito & Sekimizu, 2007; Kato et al., 2010; Tatemastu et al., 2012; Panthee et al., 2017; Paudel et al., 2020). Using invertebrates such as the silkworm as a biological model is more accessible due to the short generation time, implies low-cost breeding, and does not require ethical approval (Panthee et al., 2017; Aznar-Cervante et al., 2021). This review aimed to summarize the literature information about the impact of nutrition as a key factor in silkworm rearing and the effectiveness of using an artificial diet based on different ingredients in silkworm productivity and health.

MATERIALS AND METHODS

For this review study, the relevant scientific articles with full-text available in English were searched in databases such as Web of Science, PubMed, ScienceDirect, Google Scholar, MDPI, Springer, and Elsevier by using the following keywords: "Artificial diet", "*Bombyx mori* L.", "*Morus alba* L.", "Mulberry leaf", "Nutrients", "Silkworm". We reviewed the relevant articles published over the last 20 years. References of included studies were also consulted as additional bibliographic sources.

RESULTS AND DISCUSSIONS

Nutrient requirements of silkworm

The chemical elements in the food necessary for the proper metabolism and growth of insects are known as their nutrient requirements (House, 1962). The correct balance of nutrients is crucial for most insects that have been studied (House, 1965). The nutrient requirements, i.e., carbohydrates, proteins, amino acids, fats, vitamins, minerals and as well as water, are commonly found in the mulberry leaves consumed by silkworms. The feeding strategy of silkworm larvae determines their subsequent development, cocoons and egg production (Shamsuddin, 2009).

Carbohydrates are a major energy source for silkworms (Ito, 1967) that can be produced from lipids and amino acids (Nation, 2001). Although some chemicals were used more quickly than others, silkworm larvae didn't show any particular requirements for carbohydrates, similar to other insect species. Pentoses often needed to be more utilized. By the hexoses examined, glucose, fructose, and mannose were efficiently used. Disaccharides were all of good quality, notably sucrose, cellobiose, and maltose. Melezitose and raffinose, two trisaccharides, had a high value (Ito, 1967). Sugar provides energy and carbon to silkworm larvae and stimulates their feeding. Among sugar alcohols, it was noticed that D-sorbitol promotes silkworm larva feeding (Ito, 1960 a, b). Sasaki et al. (2013) also reported improved silkworm larva feeding by adding myo-inositol and sucrose.

Proteins are fundamental substances in the body that plays a vital role in cell molecular structure and survival. Its ability to combine with other active substances, such as enzymes and hormones, allows it to regulate metabolism and physiological functions in silkworms. Around 70% of the silk proteins synthesized by silkworms are obtained directly from the protein present in mulberry leaves (Bhattacharyya et al., 2016). Adequate protein intake is essential for the development of ovaries and eggs in adult female silkworms, as it is necessary for the secretion of juvenile hormones. In contrast, male silkworms typically do not require protein to mature their sperm upon adulthood. Optimal proteins nutritional requirements vary by age, sex, physiological status and stress (Nation, 2001; Borah & Boro, 2020).

Amino acids (AA) are considered the most important components of silkworm nutrition. The silkworm larvae rely on the amino acids from mulberry leaves for their growth, development, and cocoon formation. The twelve essential AA for the growth and development of the silkworm larvae includes arginine, lysine, methionine, histidine, leucine, isoleucine, phenylalanine, threonine, tryptophan, valine, aspartic and glutamic acids (Borah & Boro, 2020). Besides this 12 essential AA, the silkworm must have semi-essential AA, proline. Meanwhile, alanine, cystine, glycine, serine, and tyrosine, known as non-essential AA, also play a significant role in promoting the growth of the silkworm. The addition of these non-essentials is necessary for optimal growth. Even when acidic AA were previously present in the diet, the nutritional effect of these non-essentials was still evident. The silk fibre fibroin includes alanine, serine, glycine, and tyrosine derived from the silkworm feed. Alanine, in particular, is crucial in metabolizing glucose, tryptophan, and organic acid (Borah & Boro, 2020).

Lipids are a group of compounds that include fatty acids, alcohols of varying chain lengths, steroids and their esters, phospholipids, and other similar substances. Silkworms have the ability to transform carbohydrates into lipids and store them in their body tissues (Nation, 2001). Fatty acids, phospholipids, and sterols are essential components of cell walls and perform various specialized functions. For normal growth and development, silkworm larvae require polyunsaturated fatty acids, particularly linoleic and linolenic acids (Genc et al., 2002). Mouths that lack these fatty acids experience wing formation defects, and their scales stick to the pupal case upon emergence. Even a small amount of lipids or sterols in their diet can positively influence the growth and development of silkworms.

Silkworms need sterols in their diet because they cannot synthesize enough to meet their physiological requirements. Sterols are present in all cellular membranes and serve as a precursor to the silkworm moulting hormone (Yuan et al., 2020). Therefore, a deficiency of sterols in their diet can result in an inability to moult, and silkworms typically die in their early stages (Nation, 2001; Genc et al., 2002). The addition of β-sitosterol is beneficial for the growth and development of silkworm larvae (Nagata et al., 2006).

Vitamins are organic compounds that predominantly serve as enzyme co-factors and catalysts. They regulate cellular metabolism and physiological functions (Shamsuddin, 2009). The growth and development of silkworms primarily depend on vitamin B complex and vitamin C intake. The main functions of these vitamins and their effect on silkworms are presented in Table 1 (Ito, 1978; Borah & Boro, 2020).

Table 1. Vitamins requirements for silkworms

Items	Role[1]	Minimum req. mg/g of dry diet[2]	Mulberry leaves content mg/g dry matter[2]	Effect on silkworm[1]
Vitamin C (ascorbic acid)	- antioxidant; - protection against oxidative damage to DNA, membrane lipids and proteins.	-	-	- addition of 1-2% vit. C increases the weight of larvae and survival rate; - absence of vit. C in the instars I and II delayed the growth.
Vitamin B1 (thiamine)	- energy metabolism.	0.5	6.7	- no effect on larvae weight and silk gland; - increase larval duration, cocoon and shell weights, and fecundity.
Vitamin B2 (riboflavin)	- energy released from carbohydrates, fats, and proteins.	5	13-21	- increase certain economic traits and improve silk production.
Vitamin B3 (niacin)	- cell respiration; - release of energy and -metabolism of carbohydrates, lipids and proteins.	20	69-99	-higher vit. B3 dose affects feed intake, growth, increased mortality in the moulting phase, and incomplete moulting.
Vitamin B5 (pantothenic acid)	- cofactor for enzymes; - precursor of coenzyme A; - metabolism of carbohydrates; - synthesis and degradation of fats; - synthesis of sterols and steroid hormones.	5	16-35	
Vitamin B6 (pyridoxin)		20	43-50	- lower vit. B6 dose increases the larval body weight, pupal weight, silk gland weight, cocoon and shell weight. - higher vit. B6 dose affects the optimum growth and development of the silkworm.
Vitamin B8 (biotin)	- carbohydrate and fat metabolism, - synthesis of fatty acids.	1	0.2-0.8	- minimal optimal level of vit. B8 for growth and survival of the silkworm is much lower than those of other vitamins.
Choline and Inositol	- production of cell membranes.	750 1000	930-1550 4000	- need a higher level for optimal growth.

Sources: [1]Borah and Boro (2020); [2]Ito (1978).

Minerals act as a limiting factor for the growth of insects, which is particularly true for all types of dietary compositions (Ito, 1978). Salt significantly enhanced the growth of developmental stages, improved cocoon characteristics, triggered early cocoon production, and increased the reproductive potential of silkworms. Dietary supplementation with nickel chloride, potassium iodine, and copper sulphate elevated the economical parameters of silkworms. Nickel chloride boosted the growth of silkworm larvae, pupae, and adults, as well as cocoon production, but higher concentrations of salt had adverse effects on these parameters (Ito & Nirminura, 1966). Feeding silkworm larvae with mulberry leaves fortified with nickel and zinc increased cocoon weight (Wright, 1984).

Chemical composition of mulberry leaves

To cover the required nutrients of *B. mori* and produce artificial diets suitable for healthy and adequate growth, it is necessary to know the nutritional value starting with mulberry leaves, considered an excellent functional feed.

The chemical composition of mulberry leaves, fresh and meal, according to the literature database (Heuze et al., 2019 - www.feedipedia.org), is given in Table 2.

Crude protein, fat content and gross energy value were higher in fresh mulberry leaves than in meal, while crude fibre and its fractions, ash, and condensed tannins were lower in fresh mulberry leaves versus meal. Proteins are the most prevalent nutrient in leaves that larvae take over and are found in the pupae composition (Hăbeanu et al., 2023). Practically, protein and their AA from mulberry leaves are converted into silk.

Table 2. Proximate composition of mulberry leaves (*Morus alba*)[1]

Items	Mulberry leaves	
	fresh	meal
Dry matter (DM, % as fed)	30.2	90.5
Crude protein (% DM)	19.1	18.0
Crude fat (% DM)	5.6	3.5
Crude fibre (% DM)	13.5	13.7
Neutral detergent fibre (% DM)	30.9	37.0
Acid detergent fibre (% DM)	22.3	25.1
Ash (% DM)	12.3	12.8
Lignin (% DM)	5.4	6.1
Condensed tannins (% DM)	7.0	30.0
Gross energy (MJ/kg DM)	18.2	17.5

Source: [1]Heuze et al. (2019) - www.feedipedia.org

Fresh mulberry leaves have an excellent AA profile (Table 3) with a total of 80.6 g/16 g N, from which 38 g/16 g N essential AA and 42.6 g/16 g N non-essential AA. The essential/non-essential AA and essential/total AA ratios represent 0.89% and 0.47%, respectively (Heuze et al., 2019 - www.feedipedia.org). Olteanu et al. (2015), expressing the AA profile of mulberry leaves meal at % DM, reported 19.43% total AA, from which 19.15% essential AA and 10.28% non-essential AA. The essential/non-essential AA and essential/total AA ratios represent 0.85% and 0.47%. Amino acids are protein building blocks and function as signalling molecules that control feed intake, protein phosphorylation, gene expression, and intercellular communication (Yang et al., 2018). The primary AA in silk fibroin are glycine, serine, alanine, and tyrosine (Mondal et al., 2007). According to Qin et al. (2020), the decreased levels of glycine, serine, alanine, and tyrosine in an artificial diet could affect the biosynthesis of silk protein in silkworm larvae. Therefore, AA supplementation of artificial diet may enhance silkworm larvae feeding efficiency (Qin et al., 2020).

The functional characteristics of mulberry leaves are also given by their valuable fatty acids (FA) composition (Hăbeanu et al., 2023), such as myristic, palmitoleic, oleic, octadecatrienoic, octadecadienoic, hexadecenoic and octadecanoic acids (Horie et al., 1985). Olteanu et al. (2015) found that the mulberry leaves meal contains 36.68% of fat, saturated FA, 13.60% monounsaturated FA, and 44.11% polyunsaturated FA (PUFA), from which n-3 PUFA represents 29.27%, and n-6: n-3 ratio 0.51% (Table 4).

Table 3. Amino acids profile of mulberry leaves (*Morus alba*)

Items	Mulberry leaves	
	fresh[1] (g/16 g N)	meal[2] (% DM)
Lysine	4.2	1.68
Methionine	1.9	0.34
Threonine	3.9	1.53
Arginine	7.4	1.13
Valine	5.6	0.85
Phenylalanine	3.8	0.98
Isoleucine	3.6	0.91
Leucine	7.6	1.73
Essential AA[3]	*38.0*	*9.15*
Aspartic acid	8.8	3.27
Glutamic acid	8.9	2.93
Alanine	5.1	1.39
Glycine	4.8	0.86
Histidine	2.2	-
Proline	4.4	-
Serine	4.6	1.14
Tyrosine	3.8	0.69
Non-essential AA[3]	*42.6*	*10.28*
Total AA[3]	80.6	19.43
Essential/Non-essential AA ratio[3]	0.89	0.85
Essential/Total AA ratio[3]	0.47	0.47

Sources: [1]Heuze et al. (2019) - www.feedipedia.org; [2]Olteanu et al. (2015); [3]Calculated values.

Table 4. Fatty acids profile of mulberry leaves (*Morus alba*)[1]

Items (% fat)	Mulberry leaves
	meal
Myristic acid (C14:0)	1.37
Pentadecanoic acid (C15:0)	2.42
Palmitic acid (C16:0)	25.22
Heptadecanoic acid (C17:0)	0.44
Stearic acid (C18:0)	5.36
Arachidic acid (C20:0)	1.87
Saturated FA (SFA)[2]	*36.68*
Myristoleic acid (C14:1)	0.64
Pentadecenoic acid (C15:1)	7.24
Palmitoleic acid (C16:1)	2.35
Oleic acid (C18:1n-9)	3.37
Monounsaturated FA (MUFA)[2]	*13.60*
Linoleic acid (C18:2n-6)	13.50
α-linolenic acid (C18:3n-3)	29.07
Octadecatetraenoic acid (C18:4n-3)	0.20
Eicosatrienoic acid (C20:3n-6)	0.81
Arachidonic acid (C20:4n-6)	0.53
Polyunsaturated FA (PUFA)[2]	*44.11*
Σ n-6 PUFA[2]	14.84
Σ n-3 PUFA[2]	29.27
n-6: n-3 ratio[2]	0.51

Sources: [1]Olteanu et al. (2015); [2]Calculated values.

The minerals composition provided in Table 5 revealed that in mulberry leaves, the main macro-mineral was calcium, followed by potassium, magnesium, phosphorus and sodium in both fresh and meal form, whereas iron was the main micro-minerals, followed by the zinc, manganese and selenium in fresh mulberry leaves (Heuze et al., 2019 - www.feedipedia.org).

Table 5. Minerals composition of mulberry leaves (*Morus alba*)[1]

Items	Mulberry leaves	
	fresh	meal
Macrominerals (g/kg DM)		
Calcium	22.3	42.3
Phosphorus	3.2	4.2
Potassium	17.5	21.7
Sodium	2.0	1.2
Magnesium	4.9	4.7
Microminerals (mg/kg DM)		
Manganese	31	-
Zinc	55	-
Cooper	10	-
Iron	322	-
Selenium	0.10	-

Source: [1]Heuze et al. (2019) - www.feedipedia.org

The vitamins composition of mulberry leaves contains 14.0 mg/100 g β-carotene, 11.30-15.37 mg/100 g vitamin C, 0.58 mg/100 g vitamin B2, and 0.04 mg/100 g vitamin B3 (Khakwani et al., 2022), while Dhiman et al., (2020) reported 13.6 mg/100 g β-carotene, 15.20-24.42 mg/100 g vitamin C, 0.36 mg vitamin B2, and 0.88 mg/100 g vitamin B3. Mulberry leaves contain as the main active compound 1-Deoxynojirimycin (DNJ), known for its higher α-glucosidase inhibitory activity that suppresses postprandial blood glucose, prevents diabetes mellitus and decreased lipid accumulation (Tsuduki et al., 2009; Asai et al., 2011; Li et al., 2013). Several studies have found that the DNJ concentration of different mulberry leaves varieties varied from 0.08 to 1.12 mg/g dry weight (Hu et al., 2013; Yu et al., 2018) or from 0.03 to 1.7 mg/g dry weight (Vichasilp et al., 2012).

The bioactive compounds concentrations of mulberry leaves determined by Khakwani et al. (2022) were 660 mg/100 g alkaloids, 226 mg/100 g rutin, 763 mg/100 g quercetin, 432 mg/100 g catechins, 33.89 mg/100 g total flavonoids, 12.26 mg/100 g total phenols, and 0.2 mg/100 g hemicellulose. These bioactive compounds possess health-promoting effects such as antioxidant, antibacterial, anticancer, antidiabetic, hepato-, cardio-, neuro-protective, antihypertensive, and antiinflammatory antiapoptosis, antiarteriosclerosis, antiviral, and antidepressant properties (Hassan et al., 2020; Wang et al., 2022). Moreover, the phytochemicals compounds from various mulberry plant components, including the fruits (caffeic acid, catechin hydrate, chlorogenic acid; Yuan and Zhao, 2017), roots (kuwanon S, mulberry side A, mulberry side C, cyclomorusin; Singh et al., 2014), and woods (chlorogenic acid, maclurin, oxyresveratrol; Ahn et al., 2017) were also studied for their antioxidative, antidiabetic, antiinflammatory and antitumor biological properties (Dhiman et al., 2020).

Artificial diet composition

Horie (1995) stated that the artificial diets for silkworms required at least three conditions: i. to cover the nutritional requirements of silkworms; ii. to have adequate physical characteristics; iii. do not contain contaminants; iv. do not contain substances which can affect silkworms.

The artificial-based diet in silkworm-rearing technologies has been developed by researchers in Japan and China (Cui et al., 2016, cited by Dong et al., 2017). Japan applied the artificial-based diet in silkworm rearing from the 1980s to the 1990s at a large-scale (Hamamura, 2001, cited by Dong et al., 2017). Still, in China, the largest sericulture country in the world, artificial diet silkworm-rearing needs to be implemented (Dong et al., 2017).

In Bulgaria, Tzenov and Georgiev designed in 2010 an artificial diet that could be used during the entire larval cycle and for all-year seasons (Avramova et al., 2020).

The artificial diet formulation was based on the nutrient profile of mulberry leaves as the “gold standard food” (Qin et al., 2020). The formulation of optimal silkworm diets involves several practical issues and considers that the nutritional requirements depend on the silkworm's productivity (Cappellozza et al., 2005).

The composition of the artificial diets is based on different amounts of dried mulberry leaf powder, defatted soybean meal, wheat meal, corn starch, soybean fibre, ascorbic acid, citric acid, vitamin mixture, salt mixture, agar, sorbic acid, propionic acid, chloramphenicol and β-sitosterol (Cappellozza et al., 2005). According to Bhattacharyya et al. (2016), the dry components of an artificial diet are mixed with antibiotics (chloramphenicol or dihydro-streptomycin) or other substances (ascorbic or propionic acids) that have anti-microbiological activity and support the developmental cycle of silkworms. Even over time, the artificial diet formulae for silkworms were improved, and the metabolic utilization of the artificial diet was lower compared to mulberry leaves (Dong et al., 2017).

Studies have shown that several productive silkworm breeds, which initially had a low response to artificial diet, were gradually adapted to it through selective breeding across generations. Trivedy et al. (2001; 2003) created five multi- and six bivoltine strains that readily accepted the artificial diet and exhibited favourable economic characteristics comparable to those of their counterparts raised on mulberry leaves.

Avramova & Grekov (2013) conducted trials on hybrids, and due to the confirmed resilience of silkworm to breeding with artificial diet, they proposed formulating diets with different levels of dried mulberry leaves.

It has been stated that the silkworms can detect the mulberry odour and are drawn to β-γ-hexenol and α-β-hexenal elements present in the mulberry. This scent is perceived by the GR66 gene, which encodes a hypothetical gustatory receptor that is believed to be bitter, that are responsible for the silkworms' particular feeding preference for mulberry (Zhang et al., 2019).

Table 6 shows some examples of artificial diet composition used in silkworms *B. mori*.

Table 6. Examples of artificial diet composition for *B. mori* silkworms

Ingredients	Reference
30% mulberry leaves powder, 28% defatted soybean meal, 15% cellulose powder, 6.1% corn starch, 3.7% citrate, 4% salt mixture, 4% sucrose, 7% agar, 0.4% ascorbic acid, 0.4% vitamin B mixture, 0.3% phytosterol, 1.3% soybean oil refined, 1.3, and 1% antiseptic.	Shinbo and Yanagaw (1994)
54.6% dried tofu cake, 25% defatted soy bean powder, 15% dried mulberry leaf, 2% citric acid, 1% ascorbic acid, and 2.4% others.	Sasaki et al. (2000)
25 g dried mulberry leaf powder, 36 g defatted soybean meal, 15 g wheat meal, 4 g corn starch, 5 g soybean fibre, 4 g citric acid, 2 g ascorbic acid, 3 g salt mixture, 4.2 g agar, 399 mg vitamin mixture, 200 mg sorbic acid, 691 mg propionic acid, 10 mg chloramphenicol, and 500 mg β-sitosterol per 100 g dry weight.	Cappellozza et al. (2005)
28% mulberry powder, 25% soya powder, 4% salt mixture, 0.3% sterol, 3% sugar, 3% cellular power, 7% jellying agent, and 3% preservative.	Rajaram et al. (2012)
36 g dried mulberry leaf, 30 g defatted soybean meal, 4 g wheat meal, 4 g rice meal, 4 g corn starch, 4 g citric acid, 2 g ascorbic acid, 3 g salt mixture, 8 g agar, 1 g aloe vera gel, 4 g potato starch and water.	Bhattacharyya et al. (2017)
35% mulberry leaf powder, 35% soybean powder, 15% green twig powder, 9.4% starch, 1.5% vitamin C, 1.5% vitamin B complex, 2% citric acid, 0.4% crotonic acid, and 0.2% choline chloride.	Dong et al. (2017) Dong et al. (2018)
20 g mulberry powder, 0 g soybean flour, 2.5 g agar/100 ml water. 16 g mulberry powder, 4 g soybean flour, 2.5 g agar/100 ml water. 10 g mulberry powder, 10 g soybean flour, 2.5 g agar/100 ml water. 4 g mulberry powder, 16 g soybean flour, 2.5 g agar/100 ml water. 0 g mulberry powder, 20 g soybean flour, 2.5 g agar/100 ml water.	Paudel et al. (2020)

Effect of using the artificial diet in silkworm

As it provides adequate nutrition and disease-free conditions throughout the year, silkworm raising on the artificial diet has numerous benefits over current practices; the first, second, and third instars are crucial for this (Nair et al., 2011). Sasaki et al. (2000) evaluated the effect of silkworm diets (artificial versus mulberry leaves) on the raw silk proteins dyeing properties. The authors found higher sericin levels, higher dye uptake and slower dyeing rate in raw silk samples, while silk fibroin exhibited similar equilibrium dye absorption and dyeing speed as effect of fed artificial than mulberry leaves.

Cappellozza et al. (2005) studied the effects of the artificial diet without or with 2% vitamin C on *B. mori* silkworm larvae throughout larval life or only in some larval instars. These authors reported that complete vitamin C deprivation during the larval cycle affects larval growth and cocoon production. Moreover, vitamin C deprivation from the diet during the first and last larval instars has positively affected cocoon production without influencing the mortality rate or delaying the larval cycle.

Tzenov & Georgiev (2010), cited by Avramova et al. (2020), noticed that using an artificial diet with a content of 38% mulberry meal shorter the silkworm larvae period during the fifth instar of the whole rearing period without altering the other production characteristics when compared to silkworm reared on mulberry leaves.

Nair et al. (2010) highlighted the possibility of using chawki rearing to produce breeding resource material for potential hybrids of multivoltine silkworms, using only artificial feed.

Several studies reported that feeding a semi-synthetic artificial diet (SeriNutrid) in silkworm larvae up to chawki level did not affect the economical parameters of silkworm (Rajaram et al., 2012), or even increased the cocoon and shell weights, or shell ratio, thus obtaining a higher price (5-10%) for premium quality cocoons (Mondal et al., 2018).

Bhattacharyya et al. (2017) evaluated the antioxidant activity of artificial diet ingredients, i.e., defatted soybean meal, rice meal, wheat meal, corn starch, potato starch and Aloe vera gel along with mulberry leaf. These authors found that soybean has a higher impact on increasing cocoon shell weight and silk quality than mulberry leaves.

Avramova et al. (2020) reported that the silkworms reared on an artificial diet during the summer season in their first three instars, and mulberry leaves in their four and five instars obtained similar technological characteristics and optimal sanitary conditions compared to silkworms reared only on mulberry leaves.

Moise et al. (2020) fed different *B. mori* silkworm breed with an artificial diet with addition of 1% or 5% bee pollen noticed an insignificant improvement in biological parameters, especially in the case of 5% bee pollen addition.

Until now, studies on artificial diets have focused on improving silk production and quality. In the current context of rising interest in employing silkworms as drug discovery models, an artificial diet that is straightforward, usable by a large population, and suitable for the study is preferred. Several studies have also been conducted comparing the intestinal microbiota of silkworms fed on natural and artificial diets and their proteomic and metabolomic (Dong et al., 2017; Dong et al., 2018) profiles. The diversity of gut microbiota and the primary bacteria present in silkworms raised on an artificial diet exhibited significant variations as compared to those raised on mulberry leaves. This dissimilarity could be associated with factors such as growth, metabolic processes, and the ability to resist diseases in silkworms fed an artificial diet (Dong et al., 2018). Moreover, the alterations in the gut microbiota could also affect the silkworms' nutrient metabolism and immune resistance, which may be attributed to their adaptation to the long-standing evolutionary practice of consuming mulberry leaves (Dong et al., 2018).

When compared the metabolomics of silkworms raised on fresh mulberry leaves versus artificial diets, Dong et al. (2017) observed that the silkworms reared on artificial diets faced a severe deficiency of certain vitamins and experienced down regulation of glycolysis, the TCA cycle, and lipid metabolism. However, these larvae showed increased levels of various amino acids and amino acid-related metabolites. Dong et al. (2017) also stated that focusing on the balance of amino acids and developing a more efficient method for vitamin supplements in artificial diets could result in significant advancements.

Paudel et al. (2020) tested five simple artificial diets based on different amounts of soybean flour and mulberry leaf powder and the same agar content to screen drug candidates in silkworm hyperglycaemic and infection models. The authors concluded that using silkworms for biological, biotechnological, and pharmacological investigations depends on the accessibility of a simple artificial diet for feeding trials (Paudel et al., 2020). Comparing the fecal metabolome of silkworms fed mulberry leaves versus artificial diet, Qin et al., (2020) have shown that the silkworms raised on mulberry leaves increased the concentration of amino acids, carbohydrates, and lipids, which are essential for physical growth and silk protein synthesis. Conversely, the silkworms fed on artificial diets had a relatively higher level of urea, citric acid, D-pinitol, D-(+)-cellobiose, and N-acetyl glucosamine (Qin et al., 2020).

CONCLUSIONS

Based on the literature information available, it can be concluded that nutrition plays an essential role in silkworm and mulberry leaves, as traditional silkworm feed sources are valuable plants rich in nutrients (carbohydrates, protein, amino acids, fatty acids, minerals, vitamins), as well as nutraceuticals (morin, β-sitosterol) and antioxidants (flavonoids, anthocyanin and alkaloids). The present review highlighted that the effectiveness of an artificial diet on silkworms' productivity and health depends on the diet composition (especially the % of mulberry leaves and other protein sources). Though there is debate regarding the artificial diet's efficacy, it nevertheless attracts interest, particularly for research using models from the medical field. The artificial diets must give essential nutrients to meet the needs of the silkworms' larvae to have better-suited qualities. Due to the employment of silkworms as research models, it is necessary to include various compounds in artificial diets to explore specific mechanisms.

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