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Review on artificial diets for silkworm (*bombyx mori*): advances, challenges, and perspectives

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Abstract
Silkworms (*Bombyx mori* L.) have long been recognized as indispensable insects, both economically, due to their role in silk production, and scientifically, as model organisms for biomedical and biotechnological studies. Traditionally, silkworms have been reared exclusively on mulberry leaves (*Morus alba* L.), which provide essential nutrients required for their growth and cocoon production. However, seasonal limitations, geographic constraints, and the rising demand for controlled experimental conditions have led to the development and refinement of artificial diets. In recent years, substantial progress has been made in understanding diet formulation, nutrient supplementation, gut microbiota interactions, and physiological consequences of artificial feeding in silkworms. The review evaluates artificial diets as alternatives to mulberry leaves, focusing on their formulations, effects on physiology and immunity, applications in sericulture and biomedical research, and future directions for improvement. By integrating these studies, we highlight the promise of artificial diets in advancing sericulture sustainability and expanding silkworms' utility in science while also identifying persistent challenges that require multidisciplinary solutions.

Keywords: Artificial diet, *Bombyx mori*, nutrition, sericulture, gut microbiota

1. Introduction

The silkworm (*Bombyx mori* L.) has been domesticated for more than 5000 years and remains one of the most economically important insects worldwide (Saviane *et al*., 2014) [28]. It is central to the sericulture industry, producing silk—a natural protein fiber valued for its luster, strength, and versatility in textiles. Today, silk is also finding applications in medicine, cosmetics, and biotechnology due to its biocompatibility and mechanical properties (Bhattacharyya *et al*., 2016) [1]. The global demand for silk and silk-derived products has sustained continuous research into silkworm rearing, disease management, and productivity enhancement. Traditionally, silkworm rearing has been entirely dependent on mulberry (*Morus alba*) leaves, which provide the full complement of nutrients necessary for larval development, metamorphosis, and cocoon formation (Zhou *et al*., 2015; Yu *et al*., 2018) [37, 36].

Mulberry leaves are rich in proteins, carbohydrates, amino acids, vitamins, minerals, and bioactive compounds such as flavonoids and alkaloids that influence larval immunity and silk production (Wang *et al*., 2010; Wen *et al*., 2020) [32, 233]. However, the reliance on mulberry as the sole feed source has long posed limitations. Mulberry is a perennial crop, but leaf availability and quality fluctuate seasonally, with significant variations in nutrient content across different times of the year, leaf maturity stages and cultivation conditions (Iqbal *et al*., 2012; Manjula *et al*., 2011; Lee & Choi, 2012) [1, 18, 15]. In regions with harsh winters, drought, or poor soil conditions, maintaining a consistent supply of high-quality mulberry leaves becomes especially challenging. Additionally, diseases of mulberry plants can further limit the availability of leaves, creating bottlenecks in sericulture operations (Naqvi *et al*., 2005) [20].

Another driving factor for developing artificial diets is the increasing recognition of *Bombyx mori* as a valuable model organism in life sciences. Compared with vertebrate models, silkworms offer several advantages: ease of handling, rapid life cycle, and fewer ethical concerns (Kaito & Sekimizu, 2007; Panthee *et al*., 2017) [13, 24]. Their metabolic and immune systems share key similarities with mammals, making them suitable surrogates for studying infectious diseases, immunity, toxicology, and pharmacokinetics. For example, silkworms respond to bacterial infections with innate immune mechanisms similar to humans, and therapeutic doses of antibiotics effective in silkworms are comparable to those used in mammalian models (Paudel *et al*., 2020) [23]. Their hemolymph can also mimic mammalian blood plasma in drug metabolism and distribution studies (Wu *et al*., 2024) [34]. These features have led to the adoption of silkworms for screening antimicrobial agents, antidiabetic drugs, and metabolic modulators, as well as for producing recombinant proteins and biomaterials (Tatematsu *et al*., 2012; Saviane *et al*., 2019) [29, 31].

In both sericulture and biomedical research contexts, artificial diets present multiple advantages. First, they provide independence from seasonal mulberry production, ensuring year-round rearing. Second, they allow the incorporation of specific nutrients, additives, or drugs for experimental purposes. For example, Paudel *et al*. (2020) [23] formulated a simplified artificial diet (MS-11) for disease model studies, while Wu *et al*. (2024) [34] supplemented artificial diets with valine to correct amino acid deficiencies and improve cocoon productivity. Third, artificial diets reduce the risk of pathogen contamination from mulberry leaves, providing cleaner rearing conditions that are especially valuable for pharmaceutical or laboratory use (Gheorghe *et al*., 2023) [9].

Nevertheless, artificial diets face persistent challenges. They often lack certain amino acids, sterols, vitamins, or phytochemicals naturally present in mulberry leaves, leading to reduced larval growth, silk yield, or disease resistance (Eguchi *et al*., 1982; Katagiri *et al*., 1990) [6, 14]. Furthermore, gut microbiota composition changes dramatically when silkworms are shifted from mulberry leaves to artificial diets, resulting in reduced microbial diversity and functional capacity (Xin *et al*., 2024) [35]. These microbial shifts may impair digestion, immunity, and overall resilience. In addition, artificial diets can sometimes produce physiological responses that differ from natural feeding, such as enhanced immune responses in laboratory conditions (Costantin *et al*., 2022) [4], which may complicate ecological interpretations.

This review aims to synthesize recent progress in the development, application, and optimization of artificial diets for *Bombyx mori*. We focus on five interrelated themes:

1. **Formulation of artificial diets:** Including simplified research diets, commercial formulations, nutrient supplementation strategies, and additive approaches.
2. **Nutritional requirements and deficiencies:** Analyzing how artificial diets compare with mulberry leaves in amino acids, lipids, vitamins, minerals, and phytochemicals.
3. **Gut microbiota adaptation:** Evaluating how dietary shifts influence microbial diversity, functional metabolism, and potential roles for probiotics.
4. **Physiological and immunological impacts:** Assessing effects on growth, development, metabolism, and immune function.
5. **Applications, challenges, and future prospects:** Considering artificial diets in sericulture, biomedical research, and biotechnology, while highlighting sustainability and cost concerns.

This review underscores the importance of multidisciplinary approaches—including nutrition, microbiome research, metabolomics, and biotechnology—in refining artificial diets that can meet the needs of both industry and research.

2. Formulation of Artificial Diets

2.1 Simplified Diets for Research Applications

The first step toward establishing artificial diets for silkworms was motivated by the need for simplified feeding systems in research laboratories. Paudel *et al*. (2020) [23] described the development of a simple artificial diet called MS-11, formulated using mulberry leaf powder and soybean flour in a 1:1 ratio, solidified with agar. The goal of MS-11 was not to maximize silk yield but to provide a practical medium for disease modeling and drug screening. The study showed that silkworms fed MS-11 exhibited growth comparable to those fed natural mulberry leaves, and importantly, infection models using *Staphylococcus aureus* produced consistent outcomes across both diet types. These results demonstrated that simple diets could substitute mulberry leaves in controlled experiments without compromising model validity.

The MS-11 diet was further tested for pharmacological applications, such as evaluating hypoglycemic agents. Silkworms fed high-glucose artificial diets displayed elevated hemolymph glucose levels, which could be lowered with antidiabetic drugs like acarbose. Such experimental systems underscore the flexibility of artificial diets in establishing reproducible disease models (Paudel *et al*., 2020) [23]. Table 1 compares different artificial diet formulations and their intended applications.

Table 1: Comparative Formulations of Artificial Diets for Silkworms (*Bombyx mori*)

Diet Name / Reference	Main Components (per 100 g diet)	Target Application	Key Findings
MS-11 diet (Paudel *et al*., 2020) [23]	50 g mulberry leaf powder, 50 g soybean flour, agar, water	Research model for infections, diabetes	Supported normal growth; comparable infection outcomes to mulberry feeding
Commercial SilkMate 2S (Japan)	Mulberry powder, vitamins, minerals, binding agents	Standard rearing, infection assays	Widely used in Japan for experimental silkworm rearing
Valine-supplemented artificial diet (Wu *et al*., 2024) [34]	Mulberry leaf powder, soybean flour, amino acid supplements (2-4% valine)	Improved feed efficiency, silk yield	Increased cocoon efficiency by 11-25%; reduced food intake
Transition diets (Xin *et al*., 2024) [35]	Artificial diet → mulberry leaves	Microbiota adaptation studies	Transitional microbiota more diverse than full artificial diet
Review-recommended formulations (Gheorghe *et al*., 2023) [9]	Mulberry powder + protein source (soy, casein), vitamins, sterols, antioxidants	Sericulture and biomedical use	Stress importance of amino acids, sterols, vitamin balance

2.2 Nutritional Foundations
A broader understanding of artificial diets requires dissecting the nutritional requirements of silkworms. Gheorghe *et al*. (2023) [9] emphasized that silkworm performance is highly dependent on diet composition, particularly protein quality, amino acid balance, and vitamin content. Mulberry leaves naturally provide these nutrients, but artificial diets must be carefully formulated to replicate them. For example, carbohydrates like glucose, sucrose, and maltose are primary energy sources that stimulate feeding (Ito, 1967), while proteins directly contribute to silk fibroin synthesis (Bhattacharyya *et al*., 2016) [1]. Deficiencies in any category can lead to stunted growth, abnormal molting, or reduced cocoon quality.

The review also highlighted that mulberry leaves are rich in antioxidants such as flavonoids and alkaloids, which have immunoregulatory and protective roles (Wang *et al*., 2010; Wen *et al*., 2020) [32, 33]. Artificial diets, while nutritionally balanced, often lack these phytochemicals, potentially compromising silkworm resilience to stress.

2.3 Amino Acid Supplementation for Feed Efficiency
A notable development in artificial diet optimization is the supplementation of specific amino acids. Wu *et al*. (2024) [34] investigated metabolic differences between silkworms reared on mulberry leaves versus artificial diets and identified valine as a key regulator of feed efficiency. Hemolymph metabolomics revealed lower amino acid levels in artificial-diet-fed silkworms, indicating deficiencies. Supplementing valine at 2% and 4% improved cocoon-production efficiency by 11.3% and 25.1%, respectively, despite reduced food intake. This suggests that artificial diets may be improved not by increasing total nutrient levels but by strategic supplementation of limiting amino acids.

Other studies have reported similar benefits from supplementing methionine, glycine, and essential fatty acids (Dong *et al*., 2021; Li *et al*., 2020) [5]. Together, these findings highlight the importance of metabolomics-guided formulations to fine-tune artificial diets for maximum productivity. However, deficiencies remain across amino acids, vitamins, sterols, and bioactive compounds when comparing artificial diets with mulberry leaves. These nutritional gaps are outlined in Table 2.

Table 2: Nutritional Deficiencies of Artificial Diets Compared to Mulberry Leaves

Nutrient / Compound	Mulberry Leaves (Natural Diet)	Artificial Diets (General)	Consequence of Deficiency	Reference
Essential amino acids (e.g., valine, methionine, glycine)	Balanced profile	Often deficient	Reduced silk protein synthesis; poor feed efficiency	Wu *et al*. (2024); Dong *et al*. (2021) [34, 5]
Lipids & sterols	Present in natural form	Often absent or limited	Impaired molting, delayed growth	Eguchi *et al*. (1982) [6]
Vitamins (B complex, C, E)	Adequate supply	Deficient without supplementation	Reduced immunity, poor larval health	Katagiri *et al*. (1990); Gheorghe *et al*. (2023) [9, 14]
Flavonoids & antioxidants	Abundant (e.g., rutin, quercetin)	Rarely included	Lower stress resistance, immune suppression	Wang *et al*. (2010); Wen *et al*. (2020) [32, 33]
Minerals (Zn, Fe, Mg, Ca)	Naturally occurring	Deficiency common	Reduced cocoon weight, poor silk fiber strength	Singh *et al*. (1994) [30]

2.4 Probiotics and Additives
Xin *et al*. (2024) [35] proposed that probiotics such as *Lactobacillus* and *Weissella* could be incorporated into artificial diets to improve palatability and digestive efficiency. Probiotics may also counteract dysbiosis caused by the absence of natural mulberry phytochemicals. Similarly, Gheorghe *et al*. (2023) [9] suggested fortifying artificial diets with vitamins, sterols, and antioxidants to mimic the bioactive profile of mulberry leaves. Such additive strategies provide a path to balancing cost-effectiveness with biological realism.

3. Role of Gut Microbiota in Diet Adaptation
3.1 Microbial Diversity Across Diets
Gut microbiota plays a central role in silkworm digestion, immunity, and overall health. Xin *et al*. (2024) [35] used 16S rRNA sequencing to compare silkworms fed mulberry leaves, artificial diets, and transitional diets. Results showed stark differences in microbial diversity. Mulberry-fed silkworms (SY group) harbored rich microbial communities dominated by Proteobacteria and Firmicutes, while artificial-diet-fed silkworms (SL group) had reduced microbial complexity. The transitional group (ZS), which switched from artificial diet to mulberry leaves, displayed an intermediate microbiota profile, suggesting adaptability and resilience in microbial communities.

The presence of *Lactobacillus* and *Weissella* in both artificial and transitional diet groups indicated their potential role in facilitating adaptation. These bacteria are known to aid carbohydrate fermentation, produce antimicrobial metabolites, and regulate host immunity (Xin *et al*., 2024) [35]. The detailed differences are summarized in Table 3.

Table 3: Gut Microbiota Differences in Silkworms Fed Mulberry vs. Artificial Diets

Diet Group	Dominant Microbial Taxa	Diversity Index	Functional Traits Observed	Reference
Mulberry diet (SY group)	Proteobacteria, Firmicutes, *Enterococcus*, *Klebsiella*	High	Supports vitamin synthesis, amino acid metabolism	Xin *et al*. (2024) [35]
Artificial diet (SL group)	Reduced diversity; dominance of *Weissella*, *Lactobacillus*	Low	Efficient simple carbohydrate metabolism; fewer complex pathways	Xin *et al*. (2024) [35]
Transition diet (ZS group)	Intermediate diversity; mix of mulberry- and artificial diet microbes	Medium	Adaptive community aiding transition to mulberry leaves	Xin *et al*. (2024) [35]
Probiotic-supplemented (suggested)	Enrichment of *Lactobacillus*, *Weissella*	Expected higher stability	Potential improved digestion, immunity	Xin *et al*. (2024); Gheorghe *et al*. (2023) [35, 9]

3.2 Functional Implications of Microbial Shifts
Functional profiling revealed that dietary transitions induced metabolic reconfigurations in silkworms. Artificial diets favored microbial taxa involved in simple carbohydrate metabolism but lacked the diversity needed for complex polysaccharide digestion. This could explain lower feed efficiency and metabolic imbalances observed in artificial-diet-fed silkworms (Wu *et al*., 2024) [34]. By contrast, mulberry-fed silkworms benefited from a richer microbiota capable of synthesizing vitamins and amino acids.

3.3 Implications for Silk Production
Gut microbiota also influences silk quality. Studies have suggested that shifts in microbial composition can affect nitrogen metabolism, energy allocation, and fibroin synthesis. Thus, artificial diets that disrupt microbial communities may indirectly compromise silk yield or quality unless corrective measures are taken. Xin *et al*. (2024) [35] advocate for microbial inoculants as a low-cost strategy to improve artificial diets without altering their base composition.

4. Physiological and Immunological Effects of Artificial Diets
4.1 Immune Responses to Artificial Feeding
Artificial diets not only influence nutrition but also impact immune responses. Costantin *et al*. (2022) [4] studied the velvetbean caterpillar *Anticarsia gemmatalis* and found that larvae reared on artificial diets had 20.5% more circulating hemocytes and exhibited prolonged survival against viral infections compared to those fed natural leaves. Although not conducted on *Bombyx mori*, these findings are relevant, as they demonstrate that artificial diets can enhance baseline immune function.

However, such immune enhancements may not fully reflect natural conditions. The simplified nutrient profiles of artificial diets may overestimate immune parameters by eliminating the trade-offs silkworms face in natural environments where phytochemicals and secondary metabolites influence immunity (Costantin *et al*., 2022) [4].

4.2 Metabolic Consequences
Wu *et al*. (2024) [34] showed that artificial diets caused disruptions in amino acid, lipid, and carbohydrate metabolism, leading to reduced feed efficiency. Metabolomic analysis revealed deficiencies in pathways linked to energy production and silk protein synthesis. Supplementation with valine partially restored these pathways, but other essential nutrients may also be limiting.

4.3 Growth and Developmental Effects
Paudel *et al*. (2020) [23] reported that silkworm larvae reared on MS-11 displayed normal development, indicating that basic formulations can sustain growth. However, long-term dependence on artificial diets may result in delayed molting, lower cocoon shell weights, or reduced fecundity, as shown in other studies (Qin *et al*., 2020; Dong *et al*., 2021) [5]. These findings suggest that while artificial diets are effective substitutes, they still require optimization to match the performance of mulberry-based feeding.

5. Applications of Artificial Diets
5.1 Sericulture and Productivity
Artificial diets provide independence from seasonal mulberry leaf supply, enabling year-round rearing. This is particularly useful in regions with limited mulberry cultivation or adverse climatic conditions. Moreover, artificial diets can standardize rearing conditions, reducing variability in silk yield. Gheorghe *et al*. (2023) [9] emphasized that pathogen-free artificial diets are advantageous in sericulture, as they reduce the risk of disease outbreaks.

Wu *et al*. (2024) [34] also noted that improved feed efficiency through amino acid supplementation could lower production costs by reducing nutrient waste. This highlights artificial diets' potential to increase profitability in sericulture while maintaining silk quality.

5.2 Biomedical Research and Disease Modeling
Silkworms are increasingly used in biomedical research due to their homology with mammalian metabolic and immune pathways. Artificial diets enable controlled feeding experiments necessary for studying diabetes, infections, and drug toxicity. Paudel *et al*. (2020) [23] demonstrated that silkworms fed glucose-enriched artificial diets developed hyperglycemia, which could be reversed with antidiabetic drugs. Similarly, antibiotic efficacy could be evaluated using artificial diets spiked with therapeutic compounds.

5.3 Biotechnological Platforms
Artificial diets are also critical for using silkworms as bioreactors to produce recombinant proteins, vaccines, and biomaterials. Pathogen-free rearing on artificial diets ensures sterility and consistency, making silkworms suitable for pharmaceutical production (Kaito & Sekimizu, 2007; Tatematsu *et al*., 2012) [13, 31]. By customizing artificial diets, it may even be possible to influence protein yield or composition.

6. Challenges and Future Directions
6.1 Nutritional Optimization
Despite progress, artificial diets still fall short of replicating mulberry leaves' complexity. Amino acid deficiencies, lack of phytochemicals, and imbalances in lipid or sterol content are common issues. Advanced metabolomic and proteomic analyses (Wu *et al*., 2024) [34] can guide supplementation strategies to address these deficiencies.

6.2 Microbiome Integration
Gut microbiota modulation presents a promising frontier. As Xin *et al*. (2024) [35] demonstrated, specific bacterial taxa facilitate adaptation to artificial diets. Incorporating probiotics or microbial inoculants could restore microbial balance and improve resilience. Future diets may combine traditional nutrients with targeted microbial consortia.

6.3 Physiological Realism
Artificial diets may exaggerate immune or metabolic responses compared to natural feeding (Costantin *et al*., 2022) [4]. Researchers must interpret results cautiously when extrapolating findings to ecological or biomedical contexts. Comparative studies between artificial and mulberry diets should continue to refine models.

6.4 Cost and Scalability

The widespread adoption of artificial diets in sericulture is limited by ingredient costs and preparation complexity. Mulberry remains cheaper and more accessible in many regions. Innovations in low-cost formulations using agricultural by-products such as rice bran, corn flour, or rapeseed meal (Xin *et al*., 2024) [35] may enhance scalability.

6.5 Toward Sustainable Diets

Future directions should emphasize sustainability, integrating locally available plant materials, renewable resources, and biotechnological advances. The integration of metabolomics, microbiome science, and nutritional modeling promises a new era of precision artificial diets tailored to specific rearing goals.

7. Conclusion

Artificial diets have transitioned from experimental substitutes to indispensable tools in sericulture and research. Simplified formulations such as MS-11 (Paudel *et al*., 2020) [23] enable disease modeling, while advanced approaches incorporating amino acid supplementation and microbiome management improve feed efficiency and adaptation (Wu *et al*., 2024; Xin *et al*., 2024) [34, 35]. Reviews such as Gheorghe *et al*. (2023) [9] emphasize the breadth of nutritional considerations necessary for sustaining silkworm productivity. Meanwhile, studies in related species (Costantin *et al*., 2022) [4] caution against overinterpreting enhanced immune responses in artificial-diet settings.

Despite challenges, the continued development of artificial diets represents a pivotal innovation for sericulture sustainability and silkworm-based biotechnological platforms. Through interdisciplinary research combining nutrition, microbiology, metabolomics, and immunology, artificial diets can be optimized to balance cost-effectiveness, biological realism, and productivity.

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