Potential Applicability of Whey Protein Supplementation to Mulberry Leaves in Silkworm *B. mori* Feeding

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Abstract
Mulberry leaves serve as the primary feed source for the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae), an insect of significant economic importance to the sericulture industry, particularly concerning global silk production. The nutritional quality of mulberry leaves is critical, as it directly influences both the quality and quantity of silk yield. Research has focused on evaluating various alternative nutrients, including proteins, carbohydrates, amino acids, and vitamins, to enhance the nutritional profile of mulberry leaves and subsequently improve the productivity response of silkworms. This review aims to provide an overview of the nutritional value and bioactive substances of whey as high-value-added protein sources. Whey, a primary by-product of cheese production, emerges as a rich natural reservoir of proteins such as β-lactoglobulin, α-lactalbumin, bovine serum albumin, immunoglobulins, and lactoferrin, as well as bioactive peptides boosting valuable nutritional and functional properties. Furthermore, whey encompasses additional essential nutrients, including lactose, fats, vitamins, and minerals. The review also discusses the potential applicability of whey protein as a mulberry leaves supplement on silkworm production traits and health.

Keywords: B. mori, mulberry leaves, production traits, whey protein.

1. Introduction

Mulberry leaves serve as the primary feed source for the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae), an insect of significant economic importance to the sericulture industry, particularly concerning global silk production. The nutritional quality of mulberry leaves is critical, as it directly influences both the quality and quantity of silk yield [1, 2]. Research has focused on evaluating various alternative nutrients, including proteins, carbohydrates, amino acids, and vitamins, to enhance the nutritional profile of mulberry leaves and subsequently improve the productivity response of silkworms [3-5].

Bovine milk contains two major protein sources: casein, an insoluble fraction that represents 80%, and whey protein, a soluble fraction that accounts for 20% [1, 6]. Casein is rich in glutamic acid, and B. mori larvae require both glutamic and aspartic acid for optimal growth and development [7 ,8]. Casein also has higher content of fatty acids, cholesterol, sugars, vitamins, and minerals. Earlier studies have demonstrated that dietary supplements, including fatty acids and carbohydrates, influence the regulation of fatty acids production during the larval stages of B. mori [9]. Sterols, such as cholesterol, positively impacted the feed efficiency in *B. mori* [10]. Whey, the primary by-product of the dairy industry, is produced worldwide in amounts of around 200 million tonnes [11], of which the

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European Union produced about 40 million tonnes [12].

While about 50% of the remaining whey is utilized as a source of valuable compounds in the food and pharmaceutical industries, a significant amount still goes to waste, contributing to environmental pollution due to its high biological oxygen demand and considerable organic load [13, 14].

Whey is a rich natural source of proteins (β-lactoglobulin, α-lactalbumin, bovine serum albumin, immunoglobulins and lactoferrin) and peptides with valuable nutritional, functional and bioactive properties. Additionally, whey contains other nutrients, i.e., lactose, fats, vitamins, and minerals [15, 16]. Due to its versatility, whey is considered a valuable raw material with extensive applications in various industrial fields, including animal feeding [12, 17].

This review aims to provide an overview of the nutritional value and bioactive substances of whey as high-value-added protein sources, and to summarise the effects of using mulberry leaves supplemented with whey protein on silkworm production traits and health.

2. Materials and methods

Databases including ScienceDirect, Web of Science, Google Scholar, Springer, PubMed, MDPI, and Elsevier were searched. The keywords used were: “dietary supplements”, “silkworm *B. mori*”, “mulberry leaf”, “milk or cheese by-product”, “nutrients composition”, “bioactive substances”, “silkworm productivity and health”.

3. Chemical composition and nutritional value of whey

The composition of whey exhibits variability influenced by several factors, including the source of milk, its chemical constituents, processing techniques, and the methods employed in cheese-making. Additionally, factors such as genetic background, seasonal changes, feed composition, and lactation phase significantly affect milk and whey composition [17, 18].

Whey can be categorized into two primary types, sweet whey and acid whey, based on the coagulation method utilized during milk processing [6]. The most prevalent type, sweet whey, is produced through rennet-induced coagulation during the manufacture of various cheese types, including hard, semi-hard, and soft cheeses [6].

The chemical composition and nutritional value of sweet whey are presented in Tables 1 to 3, according to the literature database [19]. As shown in Table 1, sweet whey is a rich source of lactose, protein, essential macro- and micro-minerals, B-complex and C vitamins.

The predominant component of whey, lactose, is the primary carbohydrate, and it has been demonstrated that *B. mori* possesses a β-glucosidase enzyme that acts on both cellobiose and lactose [1, 20]. Due to the existence of this enzyme, lactose does not present any digestive issues for *B. mori* larvae [1].

Whey is considered a valuable protein source due to its higher protein content with an excellent amino acids (AAs) profile. The AAs are fundamental components of proteins and act as signalling molecules that regulate feed consumption, protein phosphorylation, gene expression, and cell communication [21].

According to the literature database [19], the amino acid profile (Table 2) shows that whey protein contains 18 AA and all 9 essential AA (EAA) with key roles in protein and lipid metabolism. Due to their elevated concentration in EAA (lysine, threonine, leucine, isoleucine, valine), the EAA/NEAA ratio of whey protein represents 0.89%, and the EAA/total AA ratio is 0.47%.

Whey protein is a balanced source of sulphury AA, methionine and cysteine, with a higher protein efficiency ratio (3.2) compared to casein (2.6) [22-24]. Moreover, has been found that the content of available cysteine in whey protein is 3-4 times higher than in other protein sources, i.e., soya, corn, wheat gluten [22]. Cysteine is crucial for glutathione production, a tripeptide known for its antioxidant, anticarcinogenic, and immune-boosting effects, influencing the overall protein metabolism and organism growth [25, 26]. Whey protein is the richest source of branched-chain AA (BCAA), including leucine, isoleucine, and valine [22, 23]. Whey protein is the richest source of branched-chain AA (BCAA), including leucine, isoleucine, and valine [22, 23].

Table 1. Chemical composition of whey[1]

Items	Sweet whey, powder
Dry matter (DM % as fed)	97.0
Crude protein (% DM)	12.5
Ether extract (% DM)	1.3
Ash (% DM)	8.2
Lactose (% DM)	71.06
Gross energy (MJ/kg DM)	16.1
Minerals	
Calcium (g/kg DM)	11.5
Phosphorus (g/kg DM)	8.7
Potassium (g/kg DM)	21.0
Sodium (g/kg DM)	7.0
Magnesium (g/kg DM)	1.5
Chlorine (g/kg DM)	16.8
Sulphur (g/kg DM)	4.8
Manganese (mg/kg DM)	0.6
Zinc (mg/kg DM)	20
Copper (mg/kg DM)	2
Iron (mg/kg DM)	10
Selenium (mg/kg DM)	0.3
Cobalt (mg/kg DM)	0.1
Molybdenum (mg/kg DM)	5

Reference: [1] www.feedbase.com [19]

Table 2. Amino acids profile of whey protein[1]

Amino acids	% protein
Essential AA (EAA)	
Lysine	7.6
Methionine	1.4
Threonine	5.7
Arginine	2.2
Valine	5.1
Phenylalanine	2.9
Isoleucine	5.2
Leucine	8.8
Tryptophan	1.5
Non-essential AA (NEAA)	
Aspartic acid	9.1
Glutamic acid	15.1
Alanine	4.3
Glycine	1.9
Cystine	1.9
Histidine	1.6
Proline	5.3
Serine	4.4
Tyrosine	2.0
Total EAA[2]	40.40
Total NEAA[2]	45.60
Total AA[2]	86.00
EAA/NEAA ratio[2]	0.89
EAA/Total AA ratio[2]	0.47

Reference: [1]www.feedbase.com [19]; [2]Calculated values.

Leucine is a key player in tissue growth, and, together with isoleucine and valine, functions as a metabolic regulator in the maintenance of protein and glucose levels, as well as in lipid metabolism, controlling weight growth [22, 27-30].
In silkworms, the production and composition of silk fibroin are affected by the AA content of mulberry leaves or artificial diet fed [31, 32]. Glycine, alanine, serine, tyrosine and valine are the main AA found in silk fibroin [33, 34]. Generally, glycine, alanine, and serine represent about 82% of the mulberry silk composition, serine about 10%, tyrosine 5.5% and valine 2.5% [34].
Whey contains about 58.5% saturated fatty acids (FA), 24.4% monounsaturated FA, and 5% polyunsaturated FA from total fat, with an n-6: n-3 ratio of 4% (Table 3).

Table 3. Fatty acids profile of whey[1]

Items	% fat
Lauric acid (C12:0)	3.3
Myristic acid (C14:0)	10.8
Palmitic acid (C16:0)	29.3
Stearic acid (C18:0)	12.8
Arachidic acid (C20:0)	0.4
Behenic acid (C22:0)	1.1
Lignoceric acid (C24:0)	0.8
Saturated FA (SFA)[2]	58.5
Palmitoleic acid (C16:1)	2.7
Oleic acid (C18:1n-9)	21.7
Monounsaturated FA (MUFA)[2]	24.4
Linoleic acid (C18:2n-6)	4.0
α-linolenic acid (C18:3n-3)	1.0
Polyunsaturated FA (PUFA)[2]	5.0
n-6: n-3 ratio[2]	4.0

Reference: [1]www.feedbase.com [19]; [2]Calculated values.

4. Whey protein components, biological functions and properties

Table 4 shows that whey protein possesses unique biological and functional characteristics due to its valuable components, which include β-lactoglobulin, α-lactalbumin, bovine serum albumin, immunoglobulins, proteose-peptone, glycomacropeptide, and lactoferrin [15-16, 35-36]. Whey protein is a type of globular protein that features a higher number of alpha-helix structures, with a balanced arrangement of hydrophilic and hydrophobic amino acids, as well as both acidic and basic AA throughout its polypeptide chain [37]. The presence of α-lactalbumin, β-lactoglobulin, and a higher quantity of BCAA and glutamine improves the digestion rate compared to other protein sources [36, 38, 39]. One of the key functional properties of whey protein is its solubility across a wide pH range [36]. Moreover, as a soluble milk component, whey protein is abundant in AA, minerals, vitamins, lactose, and glycomacropeptides [36, 40]. Due to its higher lysine content, whey protein can supplement the plant-based proteins, particularly in cereal products. The presence of lactoferrin and lactoperoxidase promotes the growth of beneficial bacteria, having antibacterial, antiviral, antifungal, and antioxidant properties [6, 41, 42]. Additionally, due to the lactoferrin's higher content in sulphury AAs, it has a strong antioxidant capacity, can scavenge free radicals, chelate transition metals, and has significant antifungal activity [36, 43]. Additionally, due to the lactoferrin's higher content in sulphury AAs, it has a strong antioxidant capacity, can scavenge free radicals, chelate transition metals, and has significant antifungal activity [36, 43]. It has been noticed that whey proteins increase the activity of glutathione peroxidase, an important enzyme in the defense against oxidative stress [44]. The bioactive compounds contained in whey protein, i.e., hormones, growth factors, and cytokines, have the capability to control cell growth in both healthy and cancerous cells by inhibiting proliferation or promoting apoptosis. [45]. Table 5 summarises the major nutritional, techno-functional, and bioactive properties of whey protein [46].

Table 4. Whey protein components, structures and biological functions[1,2,3]

Components	g/L[1]	Molecular weight (kDa)[2,3]	Number of AA residues[2,3]	Biological functions[1]
β-lactoglobulin (~50-60%)	3.2-3.3	18,277	162	Source of EAA and branched-chain AA (BCAA) - leucine, isoleucine, and valine; transporter for essential nutrients (retinol, fatty acids, palmitate, vitamin D and cholesterol); exhibits immunomodulatory properties; potential antioxidant and anti-carcinogenic activities.
α-lactalbumin (~25%)	1.2-1.3	14,175	123	Source of EAA and BCAA, lactose metabolism and synthesis, calcium transport, immunomodulator, and anticarcinogen.
Bovine serum albumin (BSA)	0.3-0.4	66,267	582	Lipid synthesis and fatty acids binding, antioxidant, antimutagenic, anticancer, immunomodulator, opioid agonist.
Immunoglobulins (A, M and C)	0.5-1.0	25,000 (light chain) and 50,000-70,000 (heavy chain)		Pathogen binding inhibition (chelates iron), antimicrobial, antifungal, activation of phagocytosis, anti-inflammatory, response to allergens.
Lactoferrin	0.02-0.1	80,000	700	Antioxidant, antibacterial, anticarcinogen, antiviral, antifungal, immunomodulatory activities, and growth-promoting of beneficial bacteria. Lactoferrin+β-lactoglobulin+α-lactalbumin⇒suppresses tumour development.
Lysozyme	trace	-	-	Antimicrobial, synergistic actions with immunoglobulins and lactoferrin.
Proteose-peptone	1.2	-	-	Structural protein.
Glycomacropeptide	1.2	6700	64	Antimicrobial, antiviral, bifidogenic, and immunomodulatory properties.
Lactoperoxidase	0.03	70,000	612	Antibacterial, antioxidant and immunomodulatory properties.
Others (transferrin)	0.8			Antibacterial and antioxidant.

References: [1]Alimoradi et al. [36]; [2]Madureira et al., [6]; [3]Eigel et al. [47]

Table 5. Whey protein properties[1]

Properties		
Nutritional	✓	β-lactoglobulin, α-lactalbumin, BSA, immunoglobulins, lactoferrin, EAA and BCAA.
Techno-functional	✓	Solubility, emulsification, gelling and foaming properties.
Bioactive	✓	Health-promoting effects due to the hormones, growth factors, and cytokines content.

References: [1]Zhao et al., [46]

5. Applicability of whey supplementation of mulberry leaves in silkworm

Few studies investigated the whey supplementation of mulberry leaves in silkworm; thus, below will also be discussed the effects of bovine milk on silkworms fed.

Previous studies [1, 2] have shown that bovine milk has a positive effect on silkworm growth rate, cocoon weight and silk production due to its potential to enhance silkworm development and immune response [48].

Helaly [5] suggests that the use of bovine milk as a mulberry leaves additive during the final instar of silkworm improves biological and biochemical traits, due to the high content of nutrients (proteins and lipids) that increases the growth and development of silkworm, and also the total protein and lipid in haemolymph.

Ruiz-Erazoise et al. [49] reported that fed larvae mulberry leaves supplemented with bovine milk in 4 and 5th instars improved larval growth rate and productive characters (cocoon weight, silk percentage, and pupal weight), increasing silk yield production.

Abdel-Rahman [50] evaluated the efficiency of three concentrations of liquid whey protein (4, 6, and 8%) and 5% whey protein powder in a 5th instar larvae feeding trial on economic traits of *B. mori* silkworm. The author concluded that the addition of whey protein to mulberry leaves increases body weight, growth index, cocoon and shell weight, while a higher level of supplementation (6 and 8%) does not positively affect silkworm growth performance.

Matsumoto et al. [51], using silkworms as an animal model to assess the orally administered whey protein hydrolysate, obtained via enzymatic treatment of whey protein, reported that whey protein hydrolysate includes a bioactive peptide that inhibits glucose absorption from the intestinal tract, thus alleviating sucrose-induced hyperglycemia.

6. Conclusions

The review highlighted that whey proteins represent a valuable source of nutrients and bioactive components, attributed to their superior composition of essential amino acids, branched-chain amino acids, and bioactive peptides. Whey proteins are a health-promoting functional feed due to their antioxidant, anti-inflammatory, antimicrobial, and immune stimulatory properties. The review also emphasized the potential applicability of whey protein as a natural way to enhance mulberry leaves, which could help sustain or improve the growth and development of silkworms and elevate silk production.

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