JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

A REVIEW ON THE INFLUENCE OF DIETARY SUPPLEMENTATION ON SILK PRODUCTION IN SILKWORMS (*Bombyx mori*).

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Abstract : Supplementing the diet of silkworms with protein ingredients with high nutritional value and great digestibility may improve the development of the silkworms and cocoon production [1]. For example, a study aimed to evaluate the effect of silkworm larva (Bombyx mori) diet supplementation with two amino acids (threonine and valine) on the cocoon production and on the structural and mechanical properties of the silk produced. The study found that higher production (yield) of silk was obtained using threonine in the diet of the silkworm [1].
Silkworms are beneficial to humans, not only because of their high nutritional value, but also because of their several pharmacological properties.Serrapeptase is a dietary supplement that comes from the gut bacteria of silkworms. It's becoming popular as an anti-inflammatory and general pain reliever. Serrapeptase may also help with asthma, carpal tunnel syndrome, blood clots, and ear, nose, and throat disorders.
The development of the silkworms and cocoon production may be enhanced by adding protein sources with high nutritional value and excellent digestibility to the diet of the silkworm. However, due to the silkworm larvae's limited adoption of this kind of feeding, there is insufficient information on artificial diets for silkworms.

Index Terms - Silkworm, Sericulture, Dietary supplements, Silk production, Silk larvae.

I. INTRODUCTION

Several domestic and untamed insects have the ability to produce silk through their specialised glands [1]. The domesticated, monophagous silkworm, Bombyx mori L. (Lepidoptera: Bombycidae), is a significant economic insect. Fresh mulberry leaves that have been supplemented with lipids, proteins, moisture, and inorganic materials serve as its main food supply. Moreover, silkworms have been used to produce natural silk for thousands of years, and they play a vital part in rural agro-industry in tropical and subtropical regions of the world. Sericulture involves growing mulberry plants, raising silkworms, gathering, reeling, and weaving silk, as shown in Figure 1. Silk has long been referred to as the "Queen of Textiles" because of its beauty, opulence, and comfort properties [2]. The cocoon of a silkworm a structure known as a bave is made up of two fibroin cores, each of which is referred to as a brin, and a cementing layer of sericin surrounding them. Sericin is amorphous and serves as an adhesive binder to maintain the fine filaments of the fibroin core and the overall structural integrity of the cocoon, whereas silk fibroin is a semicrystalline polymer of a naturally occurring fibrous protein that mainly consists of two important phases: one is well known by the highly b-sheet crystals; the other is a non-crystalline form consisting of microvoids and amorphous structures. The structural protein in silk fibres is called fibroin, and the water-soluble proteinaceous glue known as sericin holds the fibres together. Fibroin (70–80%), sericin (19–27%), lipids and waxes (0.5%), and carbs (1–1.5%) make up the silk fibre. Insect pupae and spoil make up 75 and 0.6%, respectively, of the weight of the cocoons, which make up to 25% of the total weight [1].

Dietary supplements are meant to enhance or complement the diet which is different from regular food. Even though a product is marketed as a dietary supplement, it is still considered a medicine to the extent that it is meant to treat, diagnose,cure, prevent diseases, improve the quality or production. Several nutritional supplements include fish oil, echinacea, vitamin D, and multivitamins.

Commonly used supplements are:

Vitamins (such as multivitamins or specific vitamins like vitamin D and biotin) are typical dietary supplements, minerals (including iron, calcium, and magnesium), herbs or botanicals (like echinacea and ginger), botanical substances (like curcumin and caffeine), amino acids (such as glutamine and tryptophan),live microorganisms, often known as "probiotics".

According to previous studies, researchers tested various dietary supplements through experiments on silkworms some of them are amino acids, ascorbic acids, vitamins, zinc, selenium, Bovine milk, Honey, Probiotics, Secondary metabolites, etc.

Dietary supplements are used to improve the production or qualityof silk, larvae, cocoon weight or shell,etc. The significance of this study is to know how the dietary supplements help to improve the production and quality of silk

2.Literature Survey:

1 Size and Morphology of Silkworm

Silkworms go through a comprehensive metamorphosis that includes larval (caterpillar) and pupal stages (cocoon). The following section discusses the morphology of the Bombyx mori during various phases of its life cycle.

a) Egg:
Many strains have distinct egg characteristics, including size, weight, shape, color, and number laid per laying. A single silkworm egg weighs about 0.55 to 0.6 mg, which is quite small. The silkworm eggs are ellipsoid or oval in shape, flat on the ventral side, micropyle at the anterior pole, and slightly off. They range in size from 1 to 1.3 mm. The egg's surface is covered in numerous funnel-shaped respiratory canals that are widely spaced out. The racial character allows access to the air required for breathing. Whereas races that produce yellow cocoons lay deep yellow eggs, those that produce white cocoons lay pale yellow eggs. In both situations, the Japanese races lay eggs that are a little bit darker than the Chinese races.

b) Larva:
As it first hatches, the silkworm larva is either black or dark brown. Its body is heavily coated in bristles and has a huge head, giving it the appearance of a hairy caterpillar. The subdorsal, supra spiracular, infra spiracular, and basal tubercles, which each carry three to six setae, are among the four pairs of tubercles. Due to the rapid stretching of the cuticular skin during the several instars of the larval stage, the larva develops smoother and lighter in colour. The head, thorax, and abdomen of a larva are the same segments that make up an insect's body. A thin, elastic chitinous cuticle covers the entire body and can be expanded significantly to let the larva to grow quickly in any instar. The epidermis and cuticle that cover it make up the integument.

c) Head:
The head is made up of six bodily segments that are joined by a skull. The second, fourth, fifth, and sixth segments all have appendages that can be changed into various body parts, including antennae, mandibles, maxillae, and labium. The large and well-developed medial epicranial suture. The clypeus and the labrum are also noticeable on the outside. The six pairs of ocelli, also known as larval eyes, are situated just above and behind the base of the antennae. There are two antennal segments, which serve as sensory organs (feelers). The mandibles are strong and well-developed, and they are designed for mastication [8].

d) Abdomen:
There are eleven body segments in the abdomen, but only nine of them can be identified since the last three combine to produce the anal plate and the caudal legs, which appear to be the ninth segment. Each of the third through sixth and final abdominal segments has a pair of abdominal legs, which are fleshy, loosely connected protuberances of muscle. They resemble a disc with a series of inwardly curving, semicircular hooks at the extremity. The larva carries the caudal horn on the dorsal side of the ninth abdominal segment. The sexual marks, which appear in the eighth and ninth segments on the ventral side in the fourth and fifth instars, are carried by the abdominal segments. On the eighth and ninth segments of the female, there are two milky white spots that are the sexual marks. Ishiwata's fore glands are the pair of spots on the eighth segment, while Ishiwata's hind glands are the pair of spots on the ninth segment. Between the eighth and ninth segments of the ventral side of the male, a little milky white body (Herold's gland) can be seen. On either side of the body, there are nine pairs of spiracles that are positioned laterally. They are located on the first eight abdominal and first thoracic segments. These pores are used for breathing or respiration. The cuticle and hypodermis make up the larval skin, or integument.
According on the silkworm variety, these nodules are distributed differently; they are less common in kinds with glossy body surfaces, such the Chinese bivoltine and multivoltine varieties. Over areas of the body where the larval marks are often found, the nodules are more prevalent. Skin pigment is what gives silkworm larvae their distinctive markings.

e) Pupa:
Generally speaking, the pupal stage is referred to as the silkworm's dormant, inactive state, during which time it is unable to eat and seems quiescent. This name is incorrect. The pupal stage is a stage of transition during which there are noticeable changes. The larval body and its internal organs completely alter throughout this phase of biological activity to take on the new form of the adult moth. Prior to becoming a pupa, the mature silkworm larva transits through a brief pre-pupa stage. The breakdown of the larval organs occurs during the pre-pupal stage, and the pupal stage is when the adult organs are formed. The fore- and hind-wings, the legs, a pair of huge compound eyes, and a pair of large antennae are the most noticeable morphological components. Only nine of the ten abdominal segments are visible on the dorsal side, compared to ten on the ventral side. The abdomen also has seven pairs of spiracles, the last pair of which is non-functional.

f) Adult:
Due to its extensive domestication over a period of more than 4,000 years, the adult moth that emerges from the pupa is unable to fly. Throughout the brief adult stage, it does not eat. Similar to a caterpillar, a moth's body is made up of three different segments that are shared with other moths and butterflies and are located on either side of the head. Ocelli are not present. Bipectinate antennae are present. The thorax is divided into three parts, the largest of which is the mesothorax, which is pentagonal. Thoracic legs come in three pairs, one on each of the three thoracic segments. The thoracic legs are divided into five parts each. While the moth is in its resting position, the front pair of the meso's two sets of wings overlap the hind pair. Eight abdominal segments are visible in males, but they are only present in females laterally on either side of the body. In the adult stage, the male and female can be easily distinguished morphologically. The female moth's body

and abdomen are fatter than the male's, while the female's antennae are smaller in comparison. The female moth has a knob-like projection with sensory hairs dividing the sexes for the preparation of hybrid eggs at the caudal end of the mal

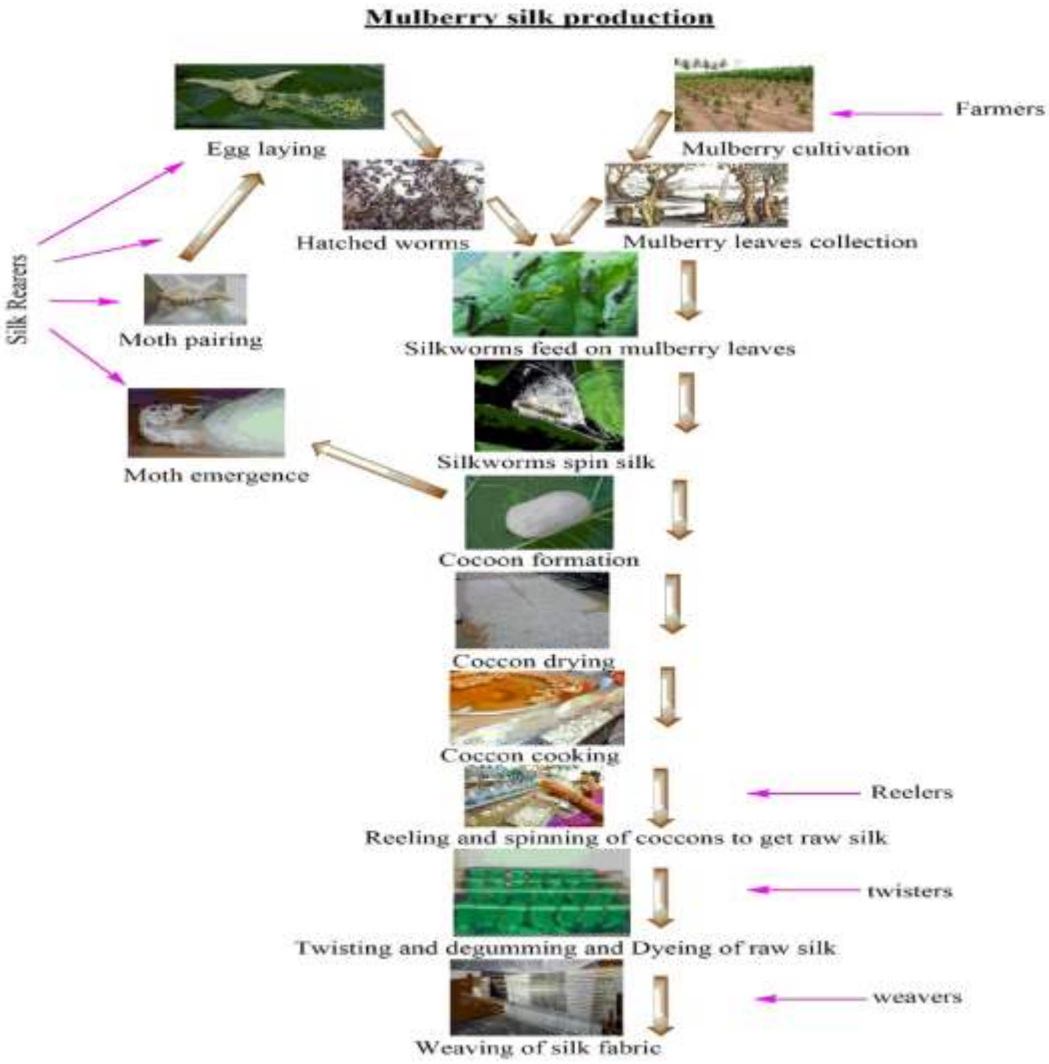


Fig 1: Phases involved in producing and processing mulberry silk (Naila Shahzad et.el 2022)

2.1 Immune system and disease resistance of Silkworm:

The genetic makeup of the host, which symbolizes an organism's immunity to diseases, determines the silkworm's vulnerability or resistance to pathogens. The silkworm's innate immune responses include cellular and humoral responses, as well as the production and release of antimicrobial peptides for the inhibition and eradication of foreign substances. All immunological responses in silkworms are either mediated by the Toll and Immunodeficiency signaling pathways or by the Janus Kinase/Signal Transducer and Activator of Transcription signalling pathway, depending on the type of pathogen present.

Any silkworm breed's immune capacity can be identified by the natural immune responses the host mounts against an encroaching infection. The ability of an organism to provide resistance against a particular pathogen so as to avoid the occurrence of disease can be broadly defined as immunity or the defence system. The immune system can be generally understood as a network of interconnected and correlated cells, tissues, and organs that participate in following processes to give the host resistance against invading pathogens. The immune system typically provides three stages of protection, namely;

a) Physical barriers, such as those made of skin, mucous membranes, saliva, tears, etc.
b) Innate immunity, such as phagocytosis and antimicrobial proteins.
c) Adaptive (specific) immunity, including antibodies, cytokines, T-cells, and B-cells. The majority of insects, including Bombyx mori L., are thought to have innate immunity, and insect immune responses can be divided into two major categories: cellular immune responses and humoral immune responses.

Although the silkworm Bombyx mori L. lacks a fully formed acquired immune system, it does have a highly developed innate immune system that protects the host from pathogens. The cellular and humoral responses that make up a silkworm's innate immune system include the production and release of antimicrobial peptides, phenoloxidase, phagocytosis, nodule formation, encapsulation, and melanization. By utilising the IMD and Toll pathways, innate immune responses are triggered, allowing the host to modulate gene expression in response to the invasion of foreign pathogens[9].

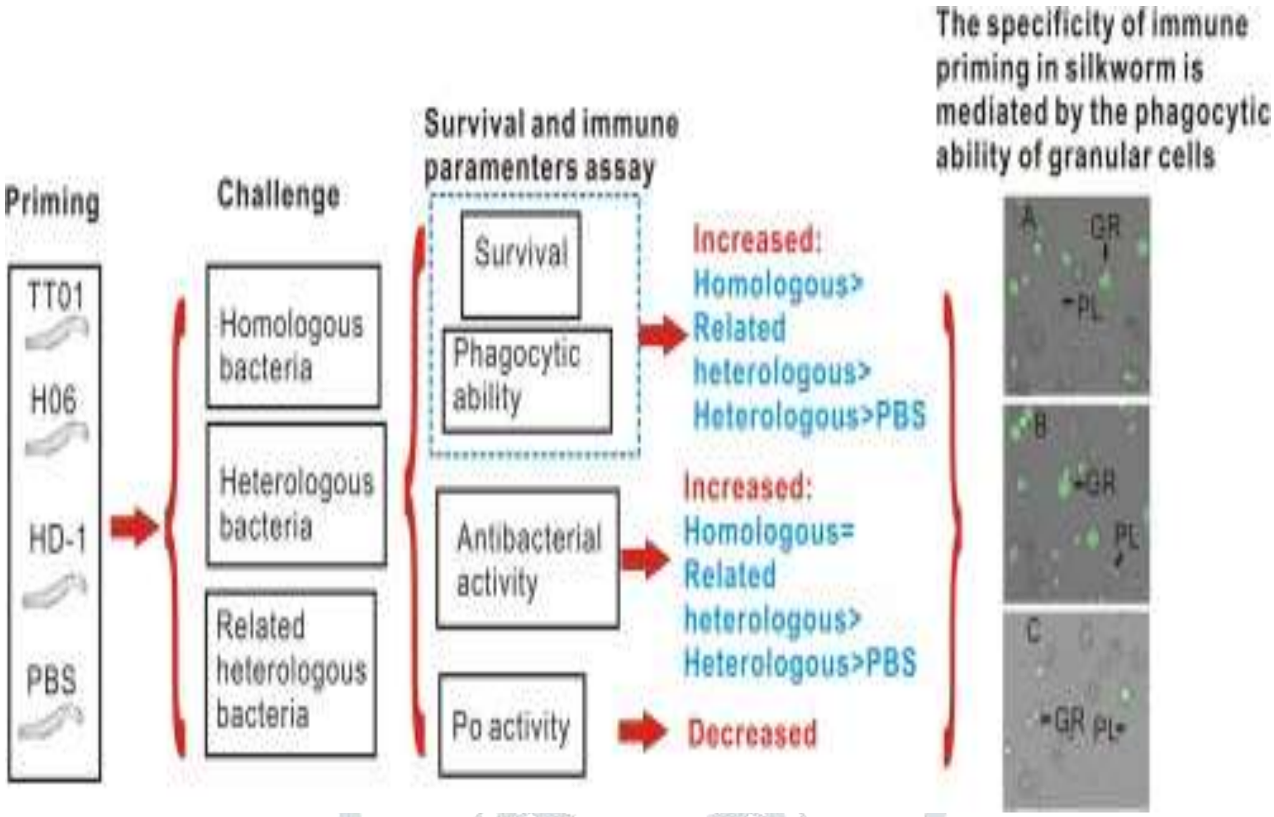


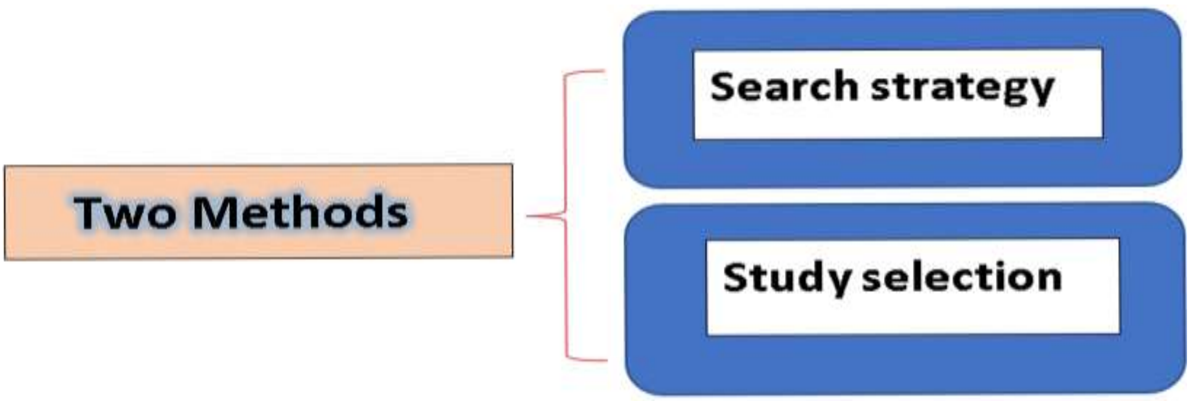
Fig 2: The specificity of immune priming in silkworm, *Bombyx mori*, is mediated by the phagocytic ability of granular cells (Gongqing et.al. 2015).

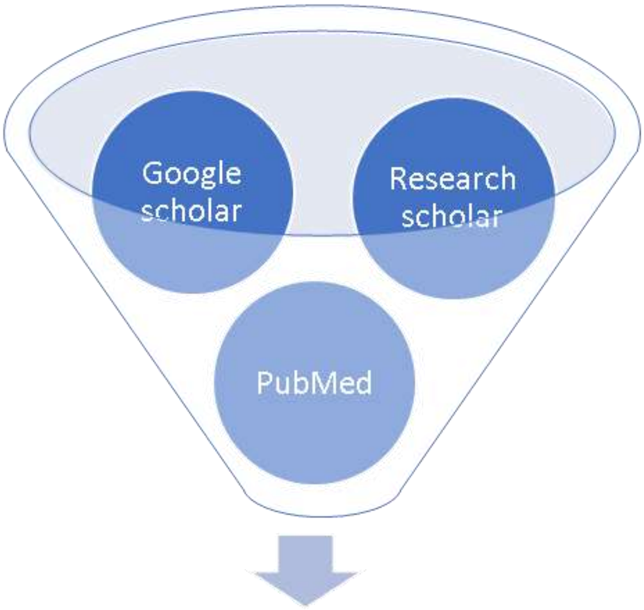
3. Quantity and Grade of Silk Fiber:

It has long been acknowledged that silkworm silk is an excellent source of inspiration for developing new materials with tailored properties, improved performance, and high added value for specific applications. This has opened up exciting new prospects in the field of materials science and related technological fields, including the bio-friendly integration, miniaturisation, and multifunctionalization of devices for biomedical applications. Engineering these novel biofunctional materials is quickly becoming a reality thanks to nanoscience and nanotechnology. A wide range of characterisation techniques, as well as a thorough understanding of chemistry, physics, and biology, are needed for research on silk. The attraction of silk to chemists, physicists, and biologists has always been strong[10]

4.METHODOLOGY:

These are the two approaches that can be used to conduct a review of the topic:





SEARCH STRATEGY

Search Strategy: A systematic research of this database is conducted by above mentioned strategies.

Study Selection: Databases searches include Google scholar, PubMed, Research scholar. The keywords used for the electronic search were silk, silkworm, dietary supplements, silk production, cocoon, larvae. The relevant articles, abstracts and chapters from books were identified and studied. The tables were made on the basis of the data collected from various sources, keeping in mind the objectives and the relevance of the data to the topic. Pictorial representation was done to help understand the broad view of the topic in a simplified manner.

5.OBJECTIVES:

1. To study the size and morphology of silkworm.
2. To study the immune system of silkworm and their ability to resist disease.
3. To study the longevity and reproductive potential of silkworm.
4. To study the number and quantity of cocoon.
5. To study the amount and quality of silk fibre.

6.RESULT AND DISCUSSION

Table 1a: The effect of Threonine and Valine on silk and silkworms.

Food Supplement	**Threonine**	**Valine**
Concentration **1%** **2%**	8.29 6.86	8.00 10.57
Cocoon weight	1.66g 1.70g	1.36g 1.53g
Cocoon shell	O.35g 0.36g	0.28g 0.33g
Reference	Daniel Nicodemo et.al (2014)	Daniel Nicodemo et.al (2014)

Control

Cocoon weight: 1.61g

Cocoon shell: 0.34g

Threonine and Valine effect: The morphological differences in the silk fiber threads produced by threonine and valine supplements were hardly noticeable. Threonine was added to the silkworm's diet to increase silk production (yield). The tensile strength, toughness (T), and maximum deformation of the silk fibre threads have all increased significantly as a result of the threonine treatments. Threonine shows best results in silk production.

Table 1b: The effect of Selenium and Zinc silk and silkworms.

Food Supplement	**Selenium**	**Zinc**
Consequences	Influenced the growth and production by extending the larval stage, increasing the weights of the larvae, cocoons, and pupae, and increasing the amount of eggs deposited by the female moth.	After being fed to silkworms, zinc-fortified mulberry leaves increase the silk moths' fecundity as well as cocoon quality indicators like higher shell-to-body ratios, raw silk percentages, denier, and renditta. In addition, it lowers the floss-shell ratio by reducing the protein synthesis in the floss
Dose	50µM	-
Reference	Li Jiangl et.al(2020)	M Younus Wani et.al (2018)

Se effect on Growth and Reproduction: Se's effects on the Lepidoptera insect silkworm, B. mori L., were assessed with regard to growth and reproduction. Due to dose-dependent effects, B. mori L. showed improved larval weight at Se concentration ranges between 25 and 125µM and reduced B. mori growth outside of this range. While the pupal stage did not significantly differ from the control group following treatment with Se, the larval stage was prolonged in the treated larvae. Regarding cocoon creation, there was a negative affect on cocoon shell weight and cocoon shell ratio, and there was an increase and downward trend in cocoon weight and pupal weight at various concentrations of Se.Based on a variety of growth and quality measures, B. mori L.'s development and reproduction were negatively impacted by the maximum dose of 200 M Se. In conclusion, low Se concentrations may promote the development and reproduction of B. mori L., whereas excessive Se concentrations may be harmful [5].

Zinceffect on Larval growth and Quality of cocoon: As zinc plays a number of roles in the synthesis of lipids, proteins, and carbohydrates as well as shortening the duration of the larval and pupal stages, it is important to determine the significance of mineral nutrition. These research offer proof that zinc and other microelements can be applied practically to improve the quality and quantity of silk production.The neurological system, the muscular system, and the glutamatergic and cholinergic neurotransmitters all play a part in the action of spinning the cocoon. Since the larval stage of silkworm growth is the only eating stage, consuming a balanced diet is crucial for the creation of silk. The many functions of the hormonal system, neuromuscular system, reproductive system, etc., can be efficiently controlled by adding minerals, vitamins, and trace elements to the diet. When fed to silkworms, mulberry leaves treated with zinc improve fertility. In addition to raising the raw silk percentage, shell cocoon ratio, silk-body ratio, denier, and renditta ratios, zinc also lowers the floss-shell ratio by reducing the protein synthesis in the floss, which is eliminated as waste during the silk reeling process[7].

Table 2: The effect of various food supplements and food additives on silk and silkworms.

Food Supplements	Vitamin C and E	Ascorbic acid+ Lemon juice	Spermidine	Cowpea seeds powder
Consequences	As comparison to vitamin E, vitamin C produces superior results in the concentration of silk gland protein. Moreover, vitamins C support the silkworms' metabolism and have an impact on their larval development and growth throughout metamorphosis.	Better growth and economic metrics in silkworms are due to ascorbic acid. The denier, protein, and weight of the shell are improved by lemon juice.	Spermidine enhances silkworm development and productivity.	enhance the length and weight of the silk, as well as food digestion and silk production.
Duration	7 days remain until the cocoon forms.	On a daily basis	three times every day	five times per day
Dose	1%	1%	25µM-75µM	7.5%
Larval stages in Bombyx mori	5th stage	Fourth and fifth stages	5th stage	three to five instars
Reference	Naila Shahzad et.al (2022)	Naila Shahzad et.al (2022)	Naila Shahzad et.al (2022)	Naila Shahzad et.al (2022)

Table 3: The effect of various food supplements and food additives on silk and silkworms.

Food supplement	Ascorbic acid	AgNPs and Spirulina	Royal Jelly	Secondary metabolites(p henols flavonoids, phenols total amino acids and proline)
Consequences	Improve economic metrics and the amount of protein in the body's fat, muscles, and silk glands.	Increase the body's protein intake and the level of protein in its fat, muscles, and silk glands.	Intake of protein should be increased, as should the amount of protein in the body's fat, muscles, and silk glands.	responsible for enhanced renditta and reelability as well as higher shell ratio, cocoon, and shell weight.
Duration	-	4 times daily till the development of the cocoon	-	Every four hours
Dose	1% and 2%	300ppm AgNPs	20ml of distilled water and 0.5g of dry powder	
Larval Stages in Bombyx mori	first stage	3rd stage	till the fourth instar	every larval stage
Reference	Naila Shahzad et.al (2022)	Naila Shahzad et.al (2022)	Naila Shahzad et.al (2022)	Naila Shahzad et.al (2022)

Probiotics:

The enzymatic profiles (increased amylase and invertase activity, which are critical for better food utilisation with increased larval growth) and prompt disease resistance with improved quantitative economic parameters of the silkworm have been influenced by the enrichment of mulberry leaves with probiotic microorganisms like Saccharomyces cerevisiae [10]. Probiotics increase host resistance and the production of dietary vitamins. They also give silkworms the ability to compete with bacteria (pathogens) by synthesising organic and antibiotic compounds[11]. Mulberry leaves mixed with Spirulina (blue green algae) were shown to be very successful in improving the features of the larva and cocoon when fed to silkworms [12] [13]. When compared to *Lactobacillus acidophilus* (Moro), previous studies showed that blue green algae (Spirulina) and *S. cerevisiae* (yeast) produced superior fibroin content, indicating the production of high-quality silk [14].

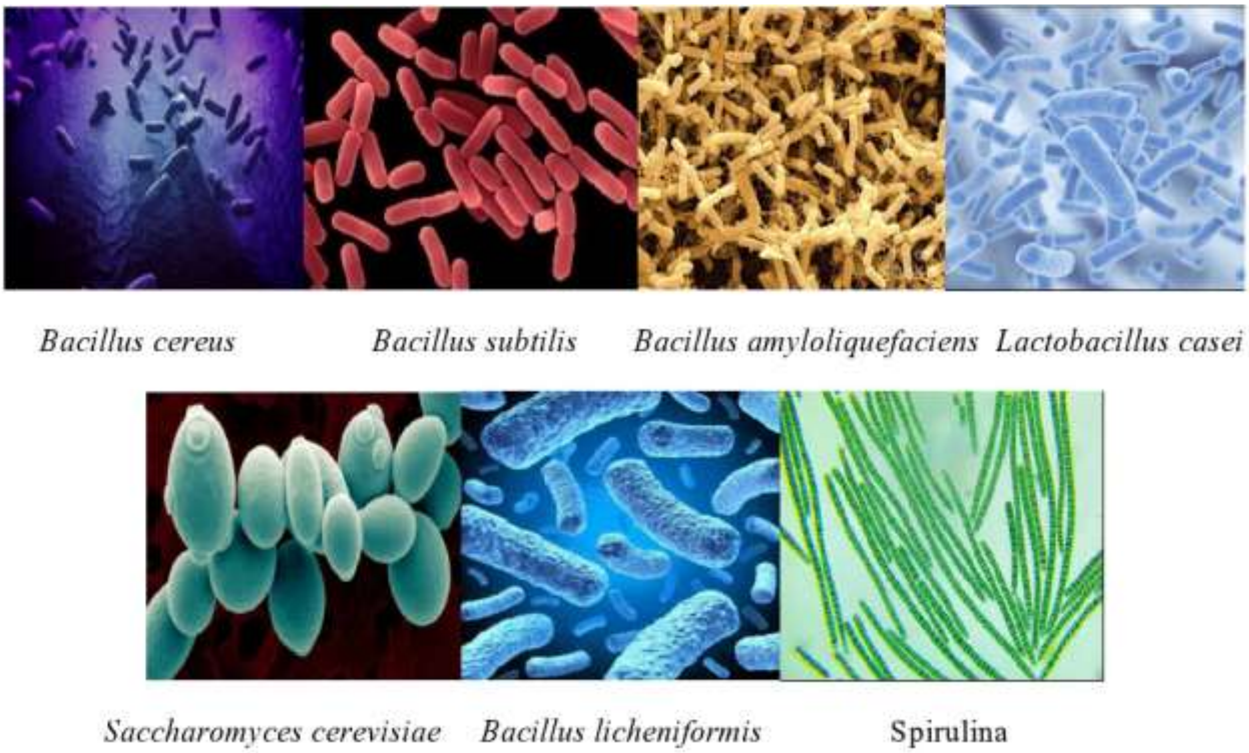


Fig.3: Commonly used probiotics as food additives foer improved sil production in silkworm (Naila Shahzadi et.al. 2022).

Honey:

A profitable dietary supplement is honey. In a previous study, it was discovered that feeding fifth instar larvae mulberry leaves that had been treated with 2% aqueous honey affected the larvae's growth and metabolism, decreased the amount of floss produced (a waste product of the silk industry), and increased the quantity and quality of the silk produced. As a result, honey, a low-cost and widely available food additive, not only encourages the production of silk proteins in the silk gland but also mobilises body protein reserves and boosts the economics of sericulture[15] [16].

Bovine Milk:

Multiple nutrients included in cow's milk support healthy growth. Because the fifth instar is when B. mori consumes voraciously and exhibits its greatest development potential among all of its larval stages, the therapy was given to fifth instar B. mori larvae. To ascertain whether milk played a part in increasing the weight of the larvae, treatments were administered on alternate days, and silkworms were weighed daily. Cocoon weights were measured because they provide an estimate of how much silk can be reeled. The findings showed that feeding milk-dipped mulberry leaves to larvae resulted in an 82.5% greater weight gain by the end of the fifth instar larval stage than feeding fresh mulberry leaves alone.

From day 1 to day 7 of the fifth instar, the larvae given milk-treated leaves gained 310% more weight than the larvae fed fresh leaves, which increased their weight by 153%. Additionally, when milk was added compared to when it wasn't, cocoon weight increased by 8%. These findings imply that feeding mulberry leaves to B. mori larvae after treating them with bovine milk can improve growth and boost silk production.

After the first treatment (day 1), larvae that ate the leaves treated with milk exhibited a considerable weight growth. Day 4 of the second treatment (day 3) led to an exponential rise in weight. The pattern persisted through day 7 (Figure 4). The weights stabilised on day seven. The fact that the larvae reached the spinning stage and ceased eating leaves by day 7 may have caused the weights to stabilise.

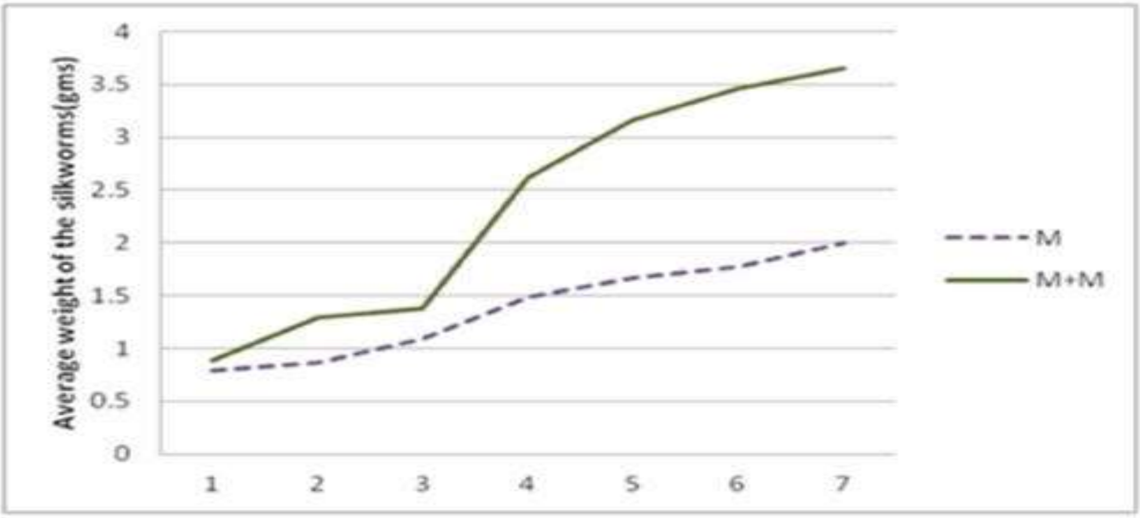


Fig4: Relationship between weight of the larvae during different days of the fifth instar when fed with fresh mulberry (M) and mulberry leaves dipped in milk (M+M). Milk treated leaves were provided to the larvae on alternate days (days 1, 3, 5, and 7). The X-axis in the graph indicates the days of the fifth instar. The Y-axis indicates the average weight of 20 larvae in each group.

By the end of the fifth instar, larvae given milk-dipped mulberry leaves gained 82.5% more weight than silkworms fed fresh mulberry leaves. From day 1 to day 7, the weight of the larvae fed milk-treated leaves increased by 310%, while the weight of the larvae given fresh leaves increased by 153% (Figure 5). The findings suggested that milk stimulated the growth of B. mori larvae.

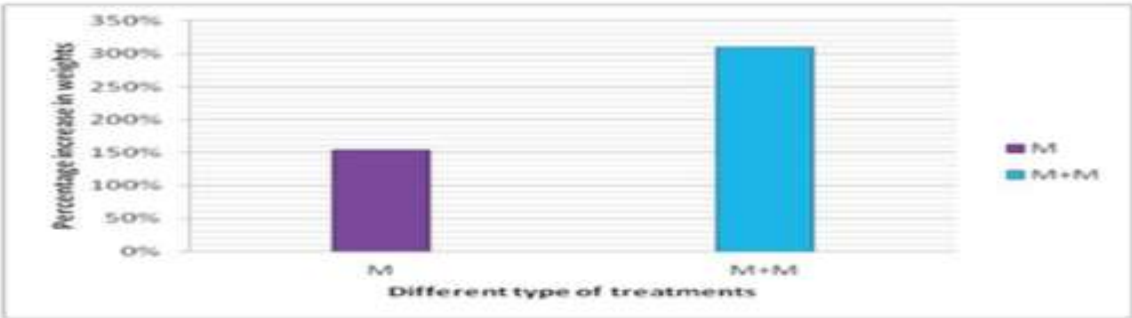


Fig 5: The larvae fed with milk treated leaves (M+M) gained 310% weight from day 1 to day 7 of the fifth instar, while the larvae fed with fresh leaves (M) gained 153% weight during th same period. The X-axis in the graph indicates the two treatments. The Y-axis indicates the percent increase in the body weights of the larvae.

7.Conclusion:

Dietary supplements are meant to enhance or complement the diet which is different from regular food. Even though a product is marketed as a dietary supplement, it is still considered a medicine to the extent that it is meant to treat, diagnose, cure, prevent diseases, improve the quality or production.

Dietary supplements are used to improve the production or quality of silk, larvae, cocoon weight or shell,etc. The significance of this study is to know how the dietary supplements help to improve the production and quality of silk.

Dietary supplements such as:

1) Zinc: Boost fertility. In addition to raising the ratio of shell cocoons to silk bodies, raw silk percentage, denier, and renditta, zinc also lowers the ratio of floss to shell by reducing the synthesis of the protein in floss, which is eliminated as waste during silk reeling[7].
2) Selenium: Low levels of selenium may promote the growth and reproduction of B. mori L., however large levels of selenium may be poisonous[5].
3) Threonine: The threonine treatments considerably improved the silk fibre threads' tensile strength, toughness (T), and maximal deformation[1]. The morphological changes in the silk fibre threads produced by threonine and valine supplementation were hardly noticeable.
4) Bovine milk: Boost growth and silk production of silkworm. Cocoon weight increased by 8%.

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