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***Research Article** (Open Access)*

BIOCHEMICAL DYNAMICS IN THE HAEMOLYMPH OF SILKWORM *BOMBYX MORI* L. SUPPLEMENTED WITH COCONUT MILK

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ABSTRACT
The mulberry silkworm, *Bombyx mori,* is an economically significant, and delicate insect that consumes only mulberry leaves and secretes a lustrous silk thread in the form of a cocoon during the larval stage, contributing to the country's economic prosperity. The success of the cocoon crop is influenced by the quantitative and qualitative traits of mulberry leaves provided during the culturing of silkworms. One of the strategies to boost the silkworm's metabolism, growth rate, and commercial yield parameters of cocoons is to enrich mulberry leaves with nutrients. Therefore, nutrition supplementation to silkworm *Bombyx mori* is an important area to study for improving biological activities, larval growth, and silk production. The nutritional value of mulberry leaves can be increased by supplementing with coconut milk. It is anticipated that the extra nutritional supplements will support the general growth and development of silkworms as well as the biochemical parameters. Haemolymph play a key role and a variety of functions like storage, transportation of nutrients, excretion, defence, moulting and metamorphosis have been reported for haemolymph. Changes in biochemical parameters in haemolymph serve as indicators of an organism's physiological status. In view of the above, it is proposed to evaluate several biomolecules such as proteins, amino acids, protease, SOD, catalase, GST, and ascorbic peroxidase.

Keywords: *Silkworm Bombyx mori, Nutritional Supplements, Coconut Milk, Biochemical Modulations*

INTRODUCTION
Sericulture is a unique combination of art and science that involves raising silkworms for silk manufacture, the most valuable and glamorous commodity in the world. It is one of the most labour-intensive sectors, combination of agricultural and industrial activity. Silk production is seen as a vital tool for a country's economic growth since it is a labour-intensive and high-income-generating business that produces value-added products with economic significance. Developing countries, like India, rely on it to generate employment, particularly in the rural sector, as well as to gain foreign exchange (Syamaladevi, 2022).

The silk business relies mainly on *Bombyx mori* Linnaeus, an economically significant insect with a short life span and reliant on humans for survival. The silkworm, *Bombyx mori,* is a soft, small, and delicate sericigenous insect that feeds entirely on mulberry leaves to generate a long, lustrous silk fiber known as the "Queen of Textiles" (Gautam *et al.,* 2022). Mulberry leaves' nutritional content has a considerable impact on larval growth and development, as well as the quality and amount of raw silk produced. Silkworm nutrition plays a prime role on silk industry, as it enhances the expression of the economic characteristics of cocoons (Muzamil *et al.,* 2023). The food additives help to improve biological activities, in turn elevating the qualitative and quantitative commercial characteristics of silk cocoons. Thus, feeding mulberry leaves enriched with nutritional supplements can increase metabolism, silkworm growth rate, and commercial cocoon qualities (Shahzadi *et al.,* 2022). The nutritional value of mulberry leaves can be increased by supplementing with coconut milk. Coconut milk provides bioactive compounds such

as proteins, fats, carbohydrates, minerals, vitamins, phenols, enzymes etc. that can positively modulate several regulatory processes of the organism.

Insect blood, known as haemolymph, is an internal circulating fluid that exists in a bound, non-vascular condition and comes into direct contact with tissue and organs, filling the body cavity. The haemolymph runs freely throughout the insect's body, soaking various tissues (Jones 1979; Begum and Savithri, 2023). Insect haemolymph is similar to vertebrate blood in that it contains carbohydrates, proteins, lipids, salts, water, hormones, and other substances in addition to cellular components (Kerenhap *et al.,* 2005). Haemolymph has been shown to perform a range of tasks, including storage, nutrient transfer, excretion, defense, moulting, and metamorphosis (Begum and Savithri, 2023).

Studying biochemical parameters in silkworm haemolymph is crucial for understanding physiological processes, identifying stress factors, optimizing rearing conditions, and improving silk production. The haemolymph provides insights into the silkworm's overall health, metabolism, and response to environmental changes during different developmental stages, allowing researchers to identify potential issues such as nutritional deficiencies or disease early on and devise mitigation techniques. Studying biochemical parameters in the haemolymph of silkworms is important for understanding their physiological processes, identifying potential biochemical changes, optimizing rearing conditions, and improving silk production. Considering the importance of biomolecules for the vital physiological processes of silkworms, the investigation has been carried out with the objective mentioned hereunder.

✓ To evaluate different biomolecules in silkworm *Bombyx mori* supplemented with coconut milk in different concentrations.

MATERIALS AND METHODS

For the experimentation young age (Chawki worms) silkworm of the bivoltine double hybrid (CSR2 X CSR27) X (CSR6 X CSR 26), were procured from chawki rearing centre, Jakkadhanna, Vedurkuppam mandal, Chittoor district of Andhra Pradesh. After procurement of chawki worms, silkworm stock was maintained by following the standard rearing techniques as suggested by Dandin *et al.,* (2003) by coordinating the primary requisites like suitable quality mulberry leaves, maintaining optimum ecological factors and a pathogen free atmosphere in the silkworm culture laboratory. Five days before to start the culturing of silkworm the silkworm culture laboratory and equipment were thoroughly disinfected and lime and bleaching mixture (95:5 ratio) was sprinkled in the surroundings of the laboratory to provide pathogen free atmosphere. Silkworm rearing beds were cleaned daily, and optimum spacing has been provided to obtain proper growth and development. For experimentation (coconut milk supplementation) silkworms were taken from the stock.

Preparation of coconut milk and supplementation

The fresh coconut milk was prepared by crushing 100 grams of raw coconut in a mixer grinder, after grinding the coconut, milk (stock solution) was collected by filtering through a muslin cloth. The stock solution was then diluted to the required concentration i.e., from 1% to 5% for the experiment.

After the fourth moult, the silkworms were taken for the supplementation of coconut milk. The mulberry leaves were supplemented with different concentrations of the coconut milk, i.e., from 1% to 5%. Freshly harvested mulberry leaves were dipped in different concentrations of coconut milk for 10 minutes and then shade-dried. After shade-drying, the coconut milk-supplemented mulberry leaves were fed to silkworms, i.e., one feed per day throughout the 5th instar silkworms. Four replications were maintained with 100 silkworms in each replication. Two controls were maintained: one is a normal control, and another one is mulberry leaves treated with distilled water.

Biochemical analysis was carried out in the haemolymph of 5th-day silkworms of the 5th instar silkworms of coconut milk supplemented and both the controls. On the fifth day of the fifth instar silkworm, the third pair of prolegs was clipped to collect hemolymph from both experimental and control silkworms. The haemolymph was collected into pre-chilled centrifuge tubes containing a pinch of

phenylthiourea before being tested for biochemical parameters. The samples were stored at -20°C for future use.

The haemolymph collected from the silkworms was used to analyse the various biomolecules such as proteins, amino acids, protease, superoxide dismutase, etc. The biochemical parameters analysed and methods followed were as follows.

Total proteins-Lowry *et al.,* (1951)
Amino acids-Moore and Stein (1954)
Protease-Davis and Smith, (1955)
Superoxide dismutase (SOD)-Beauchamp and Fridovic, (1971)
Catalase activity (CAT)–Clairbone, (1985)
Glutathione S transferase (GST)-Habig *et al.,* (1974)
Ascorbate peroxidase (APx)-Nakano and Asada, (1981)

RESULTS AND DISCUSSION

The investigation focused on evaluating the effect of coconut milk supplementation on various biochemical parameters. Changes in biochemical parameters were recorded and analysed and presented in Figures.

Biochemical parameters are the indicators to assess the physiological status of any organism. Therefore, in the current investigation, various biomolecules such as proteins, amino acids, protease, SOD, catalase, GST, and ascorbic peroxidase were estimated in the haemolymph of silkworms. Haemolymph is a fluid tissue that exists in species with open circulatory systems. The haemolymph is the major extracellular fluid in insects and is composed primarily of water, and it also contains proteins, carbohydrates, lipids, and ions. Haemolymph transports molecules and nutrients around the body and removes waste.

Proteins

Significant elevation of protein content has been recorded (Figure 1) in the haemolymph of coconut milk-supplemented silkworms in different concentrations, from 1% to 5%. Protein levels recorded in different concentrations of coconut milk-treated silkworms were 22.8 mg in 1%, 23.01 mg in 2%, 23.48 mg in 3%, 23.51 mg in 4%, and 23.61mg in 5%, which were higher than the normal control (21.45 mg) and distilled

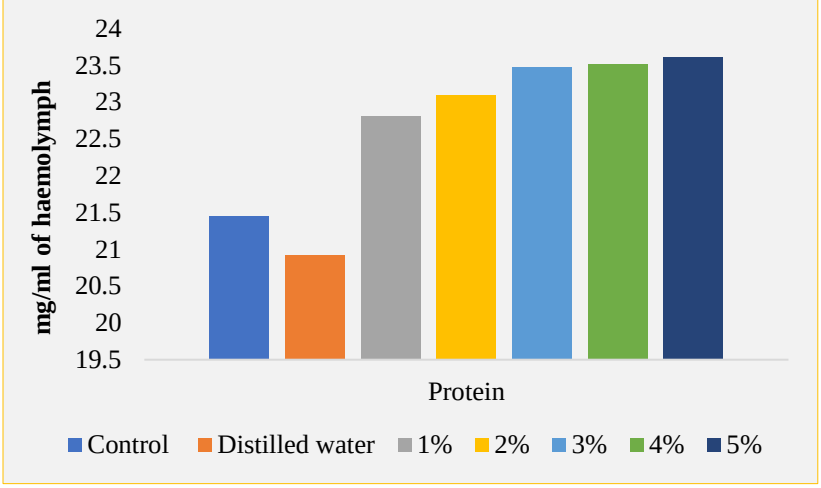

Figure 1. Proteins levels changes in haemolymph of silkworms supplemented with coconut milk in different concentrations (1% to 5%) (*significant P<0.05).

water control (20.92 mg). The highest protein level was observed in the 5% coconut milk supplementation group, indicating a dose-dependent response. The findings are in accordance with the previous studies carried out by Kumar *et al.*, (2018). Mohamed and Helaly, (2018) suggested that growth

and development of silkworm is may be due to the high protein content of cow milk. Madhavi and Prasad, (2020) noticed a significant elevation of total proteins in the silkworms fed with mulberry leaf supplemented with honey. Brahma (2018) investigated the effect of vitamin C and E on silkworm *Bombyx mori* and found that the vitamin C supplementation resulted in a higher concentration of silk gland protein than vitamin-E supplementation. The increased protein levels in the haemolymph of silkworms supplemented with coconut milk may be attributed to the presence of high-level protein content in coconut milk, and it also contains medium-chain triglycerides, as these lipids may convert to proteins.

Amino acids

The results in Figure 2 indicate the steady elevation of amino acid levels in coconut milk-supplemented silkworms in different concentrations. Amino acid levels were recorded in different concentrations of coconut milk-treated silkworms: 3.53 mg in 1%, 3.59 mg in 2%, 3.61 mg in 3%, 3.65 mg in 4%, and 3.69 mg in 5%, which were higher than the normal control (3.48 mg) and distilled water control (3.41 mg). Amino acids indispensable for the growth and survival of the silkworm are arginine, histidine, leucine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, aspartic acid, and glutamic acid (Borah and Boro, 2020). Coconut milk supplementation may enhance the nutritional quality of the silkworms' diet, leading to increased amino acid levels. Madhavi and Prasad, (2020) noticed a significant elevation of amino acids in the silkworms fed with mulberry leaf supplemented with honey. Kumar *et al.,* (2013) reported a significant elevation of amino acids in the silkworms fed with mulberry leaf supplemented with Spirulina. Muzamil *et al.,* (2023) enhances the amino acid levels in the silkworms fed with mulberry leaves fortified with amino acids. The current results are also in accordance with the above studies.

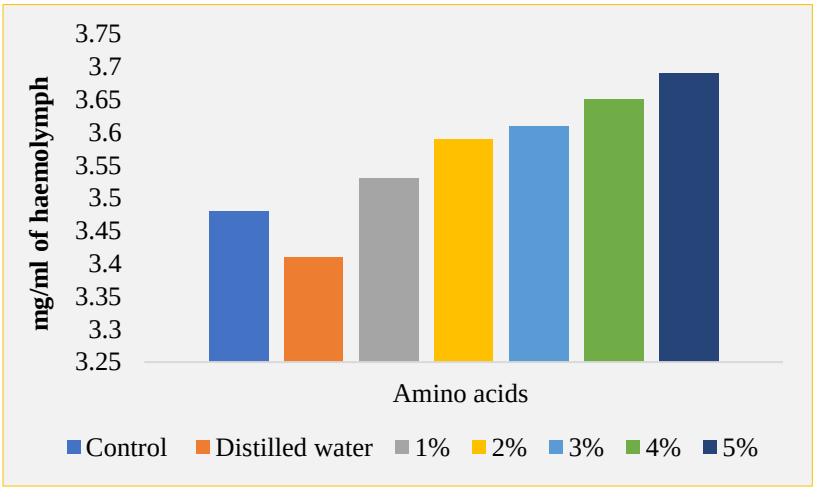

Figure 2. Amino acids levels changes in haemolymph of silkworms supplemented with coconut milk in different concentrations (1% to 5%) (*significant P<0.05).

Proteases

The study shows a gradual drop in levels of protease enzyme in coconut milk-supplemented silkworms. A steady drop in protease enzyme activity was recorded in different concentrations: 0.036 µmoles in 1%, 0.031 µmoles in 2%, 0.030 µmoles in 3%, 0.029 µmoles. in 4%, and 0.028 µmoles in 5%, compared to both the controls, i.e., with the normal control (0.048 µmoles) and distilled water control (0.037 µmoles). Proteases play a crucial role in protein digestion and degradation, releasing amino acids for various physiological processes (López-Otín *et al.,* 2008). This decrease in protease levels is accompanied by an increase in amino acid levels, suggesting that coconut milk supplementation may enhance amino acid availability and utilization.

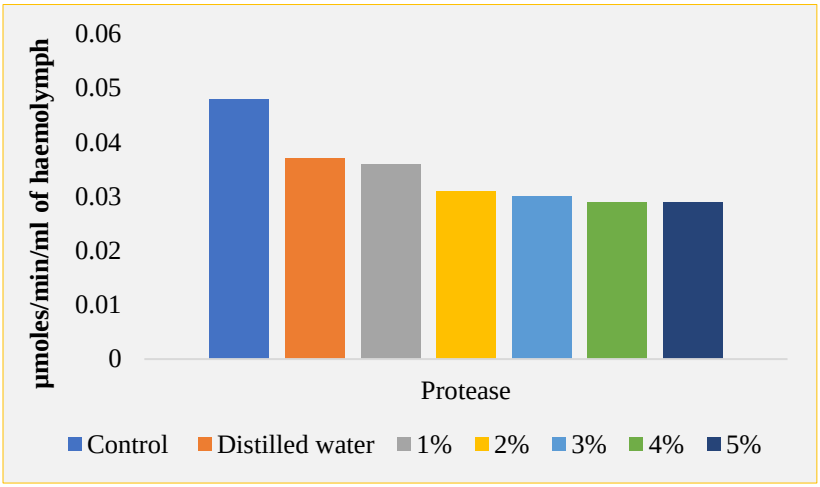

Figure 3. Protease levels changes in haemolymph of silkworms supplemented with coconut milk in different concentrations (1% to 5%) (*Not significant).

Superoxide Dismutase (SOD)

The data presented in Figure 4 show an increase in Superoxide Dismutase (SOD) levels in coconut milk-supplemented silkworms in different concentrations, from 1% to 5%. Increased levels of superoxide dismutase recorded in different concentrations of coconut milk-treated silkworms were 1.94 µmoles in 1%, 1.96 µmoles in 2%, 1.98µmoles in 3%, 1.98 µmoles in 4%, and 1.99 µmoles in 5% compared with the normal control (1.92 µmoles) and distilled water control (1.88 µmoles). Superoxide dismutase is one of the key enzymes that is involved in cellular defense against reactive oxygen species in living organisms; hence the elevation of the enzyme activity is an important indicator of antioxidant capacity. The elevation in SOD activity observed in coconut milk-supplemented silkworms may be attributed to coconut milk containing antioxidants, such as phenolic compounds and flavonoids, which can stimulate SOD activity, which may have implications for silkworm health, growth, and productivity.

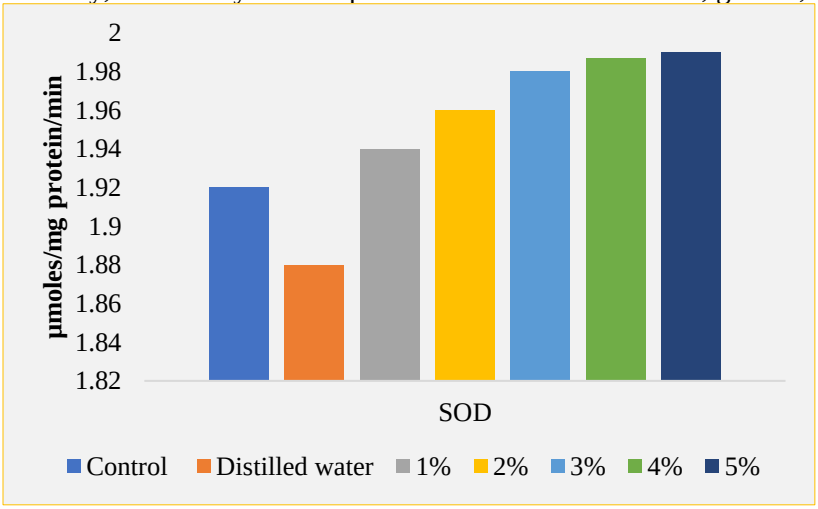

Figure 4. SOD levels changes in haemolymph of silkworms supplemented with coconut milk in different concentrations (1% to 5%) (*Not significant).

Catalase

Steady elevation of catalase enzyme activity was noticed in coconut milk-treated silkworms, but the level of elevation is non-significant compared to both the controls. Levels of catalase activity recorded in

different concentrations were 1.86 µmoles in 1%, 1.87 µmoles in 2%, 1.88 µmoles in 3%, 1.89 µmoles in 4%, and 1.9 µmoles in 5%. The levels of catalase activity in the normal control and distilled water control were 1.82 µmoles and 1.80 µmoles, respectively.

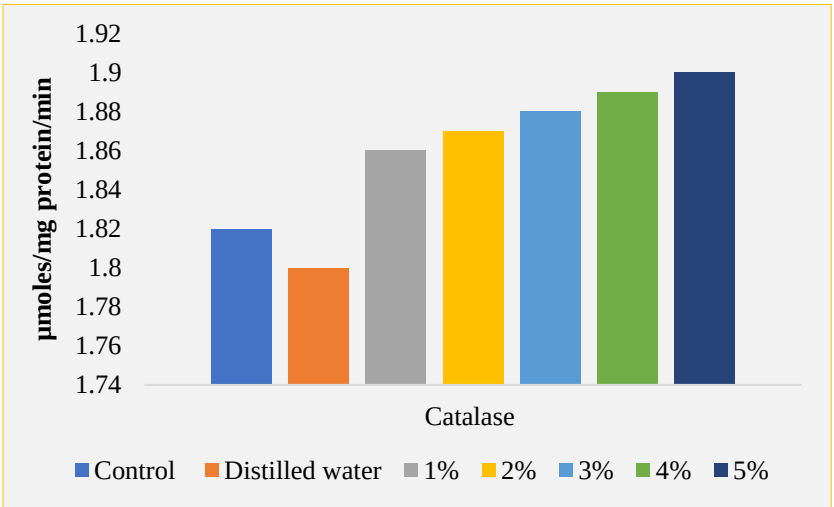

Figure 5. Catalase levels changes in haemolymph of silkworms supplemented with coconut milk in different concentrations (1% to 5%) (*Not significant).

Glutathione Stransferase (GST)

Non-significant enhancement of glutathione S-transferase (GST) enzyme activity was noticed in all 5 concentrations, i.e., from 1% to 5% of coconut milk-treated silkworms compared to both the controls. The level of Glutathione S-transferase (GST) activity recorded was 0.85 µmoles, 0.87 µmoles, 0.89 µmoles, 0.91 µmoles, and 0.92 µmoles in 1% to 5%, respectively, and in the normal control and distilled water control were 0.83 µmoles and 0.85 µmoles respectively. The glutathione S-transferase (GST) enzyme is one of the important enzymes involved in the detoxification of xenobiotics, regulating the balance between oxidants and antioxidants. The increase in GST activity may have triggered the cellular signaling pathway that promotes GST expression, enhances the detoxification process, and increases silk production and quality.

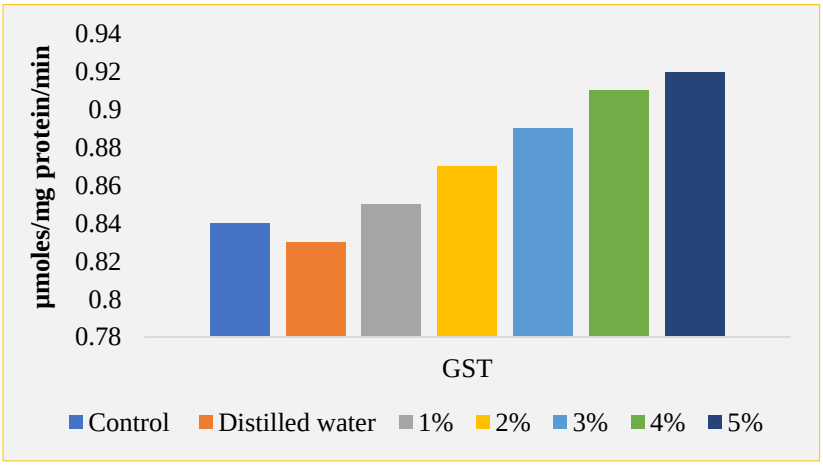

Figure 6. GST changes in haemolymph of silkworms supplemented with coconut milk in different concentrations (1% to 5%) (*Not significant).

Ascorbic Peroxidase (APX)

The results of the study (Figure 7) show an increase in Ascorbic Peroxidase (APX) activity levels in coconut milk-supplemented silkworms in different concentrations, i.e., from 1% to 5%, compared with the two controls. Ascorbic Peroxidase enzyme activity recorded in different concentrations, i.e., from 1% to 5% of coconut milk-treated silkworms, were 0.39 µmoles, 0.42 µmoles, 0.47µmoles, 0.49 µmoles µmoles, and 0.498µmoles, respectively, and in normal control 0.36 µmoles and distilled water control 0.35 µmoles.

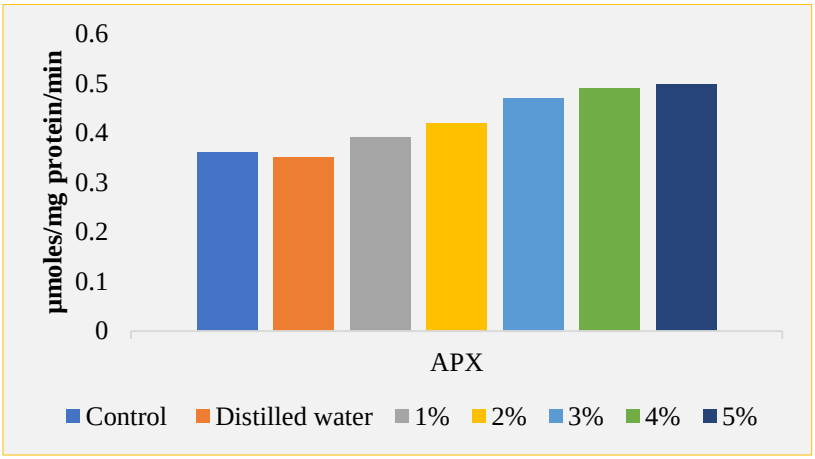

Figure 7. APx levels changes in haemolymph of silkworms supplemented with coconut milk in different concentrations (1% to 5%) (*Not significant).

CONCLUSION

Nutrition is the most important and essential component for all the living beings. Without nutrition or nutritious food it is impossible for any living organism to survive and attain a healthy disease free growth. Nutrition is the basic unit of silkworm for growth and development. Dietary efficiency of silkworm is a crucial factor in converting the feed ingested to produce silk of commercial importance. Fortification of mulberry leaves is a constructive technique to increase the nutritional value of the mulberry leaf, which improves silkworm health, cocoon, and silk quality.

The study focused on the importance of dietary supplement with coconut milk in different concentrations through mulberry leaves. Biochemical analysis indicated that significant elevation of protein and amino acid levels and non-significant elevation was noticed in other biomolecules such as protease, superoxide dismutase (SOD), catalase, glutathione S-transferase (GST), and ascorbic peroxidase (APX). Since the nutritional supplements improved the growth and commercial traits of silkworm, fortification of natural food supplements may be suggested for regular use during culturing of silkworms for better rearing performance and economic productivity. Coconut milk supplementation may offer a novel and sustainable approach to improving silkworm nutrition and overall health. Still there is a need to study comprehensively on nutrient supplements for silkworm *Bombyx mori* and dynamic biochemical changes is necessary to pave the way to enhance the qualitative and quantitative parameters and overall development of the silk industry.

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