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A novel approach to improve silkworm nutrition and silk quality using ayurvedic formulations and study it's impact on biochemical composition of silkworm, *Bombyx mori* L.

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Abstract
Sericulture, a significant contributor to the Indian economy, relies heavily on the quality and quantity of mulberry leaves for silkworm growth and silk production. This study investigates the impact of feeding mulberry silkworms (*Bombyx mori*) on leaves fortified with ayurvedic formulations, specifically Hingwastaka and Kapikacchu, on biomolecules. The fortified mulberry leaves were treated with solutions of Hingwastaka and Kapikacchu at concentrations of 0.5%, 1.0%, 1.5%, and 2.0%, which were sprayed onto fresh leaves and shade-dried. The bivoltine double hybrid silkworm (FC1 × FC2) was fed with treated mulberry leaves. The results showed significant enhancements in protein content in both the fat body and haemolymph, with Kapikacchu at 2.0% and Hingwastaka at 2.0% being the most effective treatments, respectively. Carbohydrate levels also increased, with higher concentrations yielding better results. These findings underscore the importance of nutritional fortification in enhancing silkworm growth, digestion efficiency, and silk protein synthesis, thereby improving cocoon quality and yield. These findings suggest a novel approach to enhancing sericulture productivity by leveraging traditional ayurvedic knowledge to optimize mulberry leaf quality for silkworm rearing. This method offers a sustainable and cost-effective strategy to improve silk quality and yield, contributing positively to the sericulture industry.

Keywords: Ayurvedic, haemolymph protein, Kapikacchu and Hingwastaka

Introduction
Sericulture is a welfare-oriented, technology-based art and science sector, immensely contributing to the economy of India. Considered the classic cottage industry because of its labor-intensive nature, it has an industrial superstructure and agricultural and forestry base [Singh *et al.*, 2005] [25]. Shedding light on the important source for silk-larvae of mulberry silkworm cocoon *B. mori* L.-produce large quantities of silk. The lepidopteran insect in histories bred for silk production is mulberry silkworm. Considered "Queen of Textiles," silk is a natural fiber produced by silkworms symbolizing comfort, elegance, wealth, and status. India stands as the top consumer of silk worldwide and ranks second as the producer [38,913 MT: CSB Report - 2024]. Yet being such an enormous project in nature, the silkworm really is only reared on mulberry leaves, and the amount of silk is proportional to the larval growth and development on the leaves. Various environmental conditions and technologies in the growth of silkworms and mulberries bring about variations in the amount and quality of mulberry leaves. As a monophagous insect, the silkworm depends on the mulberry for its entire life cycle. Silkworms require diverse quality leaves at different stages in their development, thus necessitating the focus on methods of mulberry production. The nutritional status of mulberry leaves determines the growth and development of larvae and the cocoon formation [Seidavi *et al.*, 2005] [23]. Various nutrients have been added to mulberry leaves and fed to silkworms to improve the quantity and quality of silk produced. Major stimulants in mulberry leaves that attract silkworms consist of the attractant, biting, and swallowing factors [Hamamura and Naito, 1961] [10]. The feeding of various leaves directly affects the silk-producing ability of *B. mori* [Muthukrishnan *et al.*, 1978] [19].

A silkworm adds to its body weight as it eats leaves, and thus, the amount of silk it produces is also affected [Sumioka *et al*., 1982] [27]. One of the important traits of the silkworm is superior conversion of plant protein into silk protein [Ude *et al*., 2014] [29]. Silk proteins are used commercially and medically in many applications [Khyade, 2016; Ageitos *et al*., 2019] [14, 1]. Mulberry leaves, which have within them morin, are the food for the silkworm larvae. Essentially, the growing rate of the *B. mori* larvae and, therefore, the production of silk depend much upon the nutritional constitution of mulberry leaves. Fakuda [1960] [9] claimed that the protein content in mulberry leaves accounts for at least 70% of the silk protein that *Bombyx mori* L. can generate. Kumararaj *et al*. [1972] [15] affirmed that the diet substantially influences the growth and yield of the silkworm. The silk gland expansion for the production of more eggs and silks in the silkworm, *B. mori*, requires those particular critical minerals, proteins, amino acids, fatty acids, sterols, and carbohydrates [Ito, 1978] [12]. For silkworm larval instars to produce more silk, the appetite or the food-quality improvement must take place both [or either]. The basis of silk manufacture according to Legay [1958] [16] is the silkworms' consumption of mulberry and nutritionally necessary leaves for creating better cocoons. Silkworms require proteins for their growth and for the synthesis of silk proteins. There are free amino acids in the bodily fluid and others synthesized in the cells of the posterior silk gland supplying the amino acids required for living. The silkworm requires all the ten essential amino acids for its growth and development [Ito, 1978] [12]. Fibroin proteins are secreted by different portions of silk gland and have varied properties. The middle portion of silk gland secretes sericin while the posterior portion secretes fibroin. The amino acids constituting fibroin are produced by the cells of the posterior silk gland. The quality of sericin is the most important characteristic of cocoon. Each strain of *B. mori* has different amounts of sericin in the cocoon. Research on silkworm nutrition is quite helpful. Nutritional processes alter food into bodily constituents via complex biochemical and physiological processes. Diet is the primary determinant of growth and development in insects. The principal macromolecules required by silkworms for proper nourishment, growth, development, and even forming a cocoon are proteins, carbohydrates, and lipids. Different levels of protein in the diet were found to influence not only hemolymph, growth, silk production, but also the proteinous constituents of the hemolymph [Seol, 1982] [24]. There have been some studies focusing on supplementing mulberry leaves with silkworms in order to increase cocoon yield and production. The listed supplements include ascorbic acid, thiamin, niacin, vitamin C, and folic acid, to name a few, and some of these elements have shown remarkable results in regards to feed efficiency [Nirwani and Kaliwal, 1998; Saha and Khan, 1996; Etebari and Fazilati, 2003; Etebari *et al*., 2008] [20, 22, 6, 8]. *B. mori* displayed improved physiological traits as a result of adding copper sulphate, nickel chloride, and potassium iodide to its diet [Magadum, 1987] [18]. Furthermore, an increase in ascorbic acid in the diet of the silkworm led to a decrease in yield of the cocoon [Etebari *et al*., 2004] [7]. The carbohydrate content, along with other relevant biomolecules, determines directly the quality of mulberry leaves as they also serve as energy for the formation of fats and proteins. The addition of reinforcing factors on silkworms resulted in an increase in biochemical and economical indicators. Based on this, a study was conducted to determine how the feeding of silkworms with mulberry leaves boosted with ayurvedic preparations affects the biomolecules and marketable traits of the cocoons.

Materials and Methods

The experiment was conducted in a mulberry garden maintained by the Department of Sericulture Science at Manasagangotri Campus, Mysuru. Tender and medium mulberry leaves of the S-36 variety were used for young-age silkworm rearing followed V1 variety was used to rear late age silkworm. To ensure a pathogen-free environment, the rearing house and appliances were thoroughly cleaned using water, followed by disinfection with a 2% bleaching powder solution and 0.5% Serifighter solution at a rate of 1.5 liters per square meter (Dandin and Giridhar, 2010) [4]. Disease-free layings (eggs) of the popular bivoltine double hybrid silkworm strain *FC1* × *FC2* were procured from the cold storage facility of the National Silkworm Seed Organization (NSSO), Mysuru. The eggs were subjected to black boxing for about 48 hours at pin head stage (Benchamin and Nagaraj, 1987) [2] later Brushing and rearing was conducted in model rearing house DoS, Sericulture science.

Fortification Agents Used: *Hingwastaka churna* and *Kapikacchu churna*.

***Hingwastaka churna*:** Hingwastaka churna is a poly herbal ayurvedic formulation sold in the form of powder. It is composed of main ingredients, namely Shunti-*Zingiberofficinale* [Rz], pippali-*Piper longum* [Fr], Maricha-*Piper nigrum* [Fr], Saindavalavana-*Rock salt*, jeeraka-*Cuminumcyminum* [Fr], Krishnajeeraka- *Negella sativa* [Fr.] each 13.79g and Hingu-*Ferula asafetida* 3.49g. According to ayurveda, hingwashtaka churna has a depan [appetizer] and panchan [digestion] properties which help digest food, providing relief form indigestion. These properties also help with the loss of appetite.

***Kapikacchu churna*:** *Mucuna pruriens* [Kapikacchuchurna] In tropical regions, kapikacchu is a widely used fodder plant. Animals consume the whole plant as hay, silage, or seeds. In Vietnam and Benin, kapikacchu is used as a biological control against deleterious *Imperata cylindrica* grass.

Formulation and application of ayurvedic supplements in silkworm rearing

Understanding how to prepare and add Hingwastaka and Kapikacchu churna to Ayurvedic formulations was the primary aim of the research. After dissolving the formulations in distilled water, dilutions of 0.5%, 1.0%, 1.5%, and 2.0% were prepared. The solutions obtained were sprayed onto fresh mulberry leaves, shade-dried for a day, and then fed to silkworm larvae at every morning feeding from the start of the fourth instar until it was a fully developed fifth instar. A distilled water control and an absolute control were prepared for comparison. Each treatment was replicated three times, which included 50 larvae. A completely randomized design was used to arrange the experiment.

T_1: Hingwastaka @ 0.5% T_2: Hingwastaka @ 1.0% T_3: Hingwastaka @ 1.5% T_4: Hingwastaka @ 2.0% T_5:

Kapikacchu @0.5% T_6: Kapikacchu @1.5% T_7: Kapikacchu @1.5% T_8: Kapikacchu @2.0% T_9: Distilled WaterT_{10}: Absolute Control

Estimation of biomolecules

Collection and preservation of tissue samples

Biochemical analysis was carried out in the fat body and haemolymph in respective treatment and control batches. The fat body and haemolymph tissues were collected by dissecting fifth instar fifth day old larvae and preserved at -20ºC until further use. One per cent tissue homogenate was prepared and samples were used for the estimation of biomolecules**.**

Estimation of protein

We used the approach of Lowry *et al*. [1951] [17] to determine the protein content of hemolymph and fat body. After reacting 5 ml of protein reagent with 1 ml of tissue homogenate the reaction mixture was held at room temperature for 10 minutes, after which an additional 0.5 ml of protein reagent was added. Following careful mixing, the reaction mixture was allowed to develop colour for an additional 30 minutes. As a standard, we used 1 ml of bovine serum albumin (BSA) and blanked using 1 ml of distilled water. We used a spectrophotometer to determine optical density (OD) at 660 nm, using the blank as a reference. Optical density was expressed as mg/ml for hemolymph and mg/g for fat body.

$$Amount of protein = \frac{OD of the sample X mg of BSA standard}{OD of the standard X mg of tissue taken} X1000$$

Estimation of carbohydrate

Using glucose as a standard, total carbohydrates were calculated using the Anthrone method [Dubois *et al*., 1956] [5]. Four milliliters of Anthrone reagent were added to one milliliter of sample, and heated for eight minutes in a water bath, and cooled under running water. After absorbance at 630 nm was measured with a spectrophotometer against a blank, the carbohydrate content was expressed in mg/g for fat body and mg/ml for hemolymph.

$$Amount of Carbohydrate = \frac{OD of the sample X mg of Glucose standard}{OD of the standard X mg of tissue taken} X1000$$

Analysis of the data

The data obtained in the investigation was statistically analyzed using SPSS statistical software [ver. 21.0] and standard error values were presented along with and mean values. Further, percent change in values was depicted for treatments over absolute control.

Results and Discussion

Protein content

1. Haemolymph

In insects, the haemolymph proteins play an important role for their enzyme action as well as the transport functions of various metabolites. Fortification of ayurvedic formulations *viz*., Hingwastaka and Kapikacchuat different concentrations significantly increased the protein content in the haemolymph [*p≤0.01, F-value: 30.13***]. The highest protein content was recorded in T_4: Hingwastaka @ 2.0% [30.04±0.408 mg/ml], followed by T_3: Hingwastaka @ 1.5% [27.24 ± 1.106 mg/ml] and T_8: Kapikacchu @ 2.0% [27.21 ± 0.312 mg/ml]. Subsequently, T_7: Kapikacchu @ 1.5% and T_2: Hingwastaka @ 1.0% registered 24.01 ± 1.107 and 22.21±0.902 mg/ml, respectively found next best compared T_{10}: Absolute control [20.16 ± 0.529 mg/ml] and found in close proximity with T_{10}: Distilled water control [4.120 ± 0.040 mg/ml]. During larval stage conversion of haemolymph protein to silk protein takes place in the developing silk glands. Thus higher protein content in the haemolymph and fatbody suggest higher silk yield.

2. Fat body

Mulberry leaves fortified with two ayurvedic formulations namely Hingwastaka and Kapikacchuat different concentrations [0.5, 1.0, 1.5 and 2.0%] on protein content in the fat body was found highly significant [*p≤0.01, F-value: 61.09***]. The highest protein content in the fat body was observed in the treatment T_8: Kapikacchu @ 2.0% [6.733 ± 0.077 mg/g] closely followed by T_4: Hingwastaka @ 2.0% [6.593 ± 0.186 mg/g], T_7: Kapikacchu @ 1.5% [6.167 ± 0.189 mg/g] and T_3:Hingwastaka @ 1.5% [6.127 ± 0.125 mg/g]. On the other hand, T_6: Kapikacchu @ 1.0% [5.437 ± 0.041 mg/g] and T_2: Hingwastaka @ 1.0% [5.303 ± 0.190 mg/g] were found next best. However, However, both T_{10}: Absolute control [4.037 ± 0.050 mg/g] and T_9: Distilled water control [4.120 ± 0.040 mg/g] recorded lowest protein content in the fat body. Similarly, application of 2% aqueous honey has positive impact on the larval growth and cocoon yield of *B. mori* (Bhatti *et al*, 2019) [3]. Kajiura and Yamashita (1989) [13] suggested direct stimulatory effect of the exogenous applied compound during rearing on protein synthesis in silk gland. Shanshan Tao *et al*, 2021 [28] had similar views on the use of artificial diet and mulberry leaf together for rearing silkworms showing high efficiency growth of body weight of silkworms.

Carbohydrate content

1. Haemolymph

The haemolymph's carbohydrate content [p≤0.01, F-value: 88.82**] was significantly influenced by various proportions of Hingwastaka and Kapikacchuat. The carbohydrate range was varied from 27.02 ± 0.304 to 19.20 ± 0.382 mg/ml. T8: Kapikacchu @ 2.0% [27.02 ± 0.304 mg/ml] had the highest carbohydrate content, T7: Kapikacchu @ 1.5% [25.79 ± 0.185 mg/ml] and T4: Hingwastaka @ 2.0% [25.03 ± 0.224 mg/ml] were not different from each other followed with T6: 1.0% Kapikacchu [24.66 ± 0.223 mg/ml], T5: 0.5% Kapikacchu [24.19 ± 0.291 mg/ml], and T3: 1.5% Hingwastaka [23.16 ± 0.324 mg/ml] for carbohydrate ranks. Nevertheless, there were minimal differences between T2: Hingwastaka @ 1.0% and T1: Hingwastaka @ 0.5%, which had carbohydrate contents of 21.95 ± 0.092 and 21.63 ± 0.035 mg/ml, respectively. albeit T10: Absolute control had the lowest carbohydrate content (19.20 ± 0.382 mg/ml) which was similar to T9: distilled water control (20.90 ± 0.295 mg/ml).

2. Fat body

The fat body carbohydrate content was significantly increased by supplementing ayurvedic compositions of various ratios of Hingwastaka and Kapikacchuat [p≤0.01, F-value: 53.63**]. T8: Kapikacchu at 2.0% [3.317±0.095 mg/g], at the high carbohydrate content, followed closely by T4: Hingwastaka at 2.0% [3.073±0.040 mg/g], but were not far apart. Next best were T3: Hingwastaka @ 1.5%

[2.643±0.089 mg/g] and T7: Kapikacchu @ 1.5% [2.893±0.066 mg/ml]. The carbohydrate content achieved was 2.507 ± 0.112, 2.333 ± 0.037, 2.183 ± 0.026, and 2.110 ± 0.060 mg/g for T6: Kapikacchu @ 1.0%, T2: Hingwastaka @ 1.0%, T1: Hingwastaka @ 0.5%, and T5: Kapikacchu @ 0.5%. The lowest carbohydrate content were recorded by T10: Absolute control and T9: Distilled water control 1.883 ± 0.051 and 1.913 ± 0.023 mg/g respectively.

These statements were also supported by the observations (Ito and Arai, 1963) [11] of the consumption of and quality of the mulberry leaves had a notable effect on the deposition of biomolecules. In accordance with reports, silkworm nutrition factors (nutrients such as carbohydrates, proteins, amino acids, lipids, minerals and vitamins) play a role in physiological and biochemical processes in the silkworm body (Sridhar and Bhat, 1966; Ohmura *et al*., 1989) [26, 21].

Table: Impact of feeding mulberry leaf fortified with ayurvedic formulations at different concentrations on protein and carbohydrate contents in fat body and haemolymph of silkworm

Treatment	Protein		Carbohydrates	
	Fat body [mg/g]	Haemolymph [mg/ml]	Fat body [mg/g]	Haemolymph [mg/ml]
T_1 = Hingwastaka @ 0.5%	4.477±0.137 [10.89]	20.19±0.510 [0.158]	2.183±0.026 [15.93]	21.63±0.035 [12.66]
T_2 = Hingwastaka @ 1.0%	5.303±0.190 [31.65]	22.21±0.902 [10.17]	2.333±0.037 [20.90]	21.95±0.092 [14.32]
T_3 = Hingwastaka @ 1.5%	6.127±0.125 [51.77]	27.24±1.106 [35.12]	2.643±0.089 [40.36]	23.16±0.324 [20.63]
T_4= Hingwastaka @ 2.0%	6.593±0.186 [63.31]	30.04±0.408 [40.01]	3.073±0.040 [63.20]	25.03±0.224 [30.36]
T_5 = Kapikacchu @ 0.5%	4.863±0.099 [20.46]	20.33±0.450 [0.843]	2.110±0.060 [12.06]	24.19±0.291 [25.99]
T_6 = Kapikacchu @ 1.0%	5.437±0.041 [34.64]	20.65±0.580 [2.430]	2.507±0.112 [33.09]	24.66±0.223 [28.44]
T_7 = Kapikacchu @ 1.5%	6.167±0.189 [52.76]	24.01±1.107 [19.10]	2.893±0.066 [53.64]	25.79±0.185 [34.32]
T_8 = Kapikacchu @ 2.0%	6.733±0.077 [66.78]	27.21±0.312 [34.97]	3.317±0.095 [76.16]	27.02±0.304 [40.73]
T_9 = Distilled water control	4.120±0.040 [2.055]	20.45±0.215 [1.448]	1.913±0.023 [1.597]	20.90±0.295 [8.854]
T_{10} = Absolute control	4.037±0.050	20.16±0.529	1.883±0.051	19.20±0.382
F-value	61.09**	30.13**	53.63**	88.82**

±: standard error values **: $p \leq 0.01$ [ ]: per cent change values over absolute control

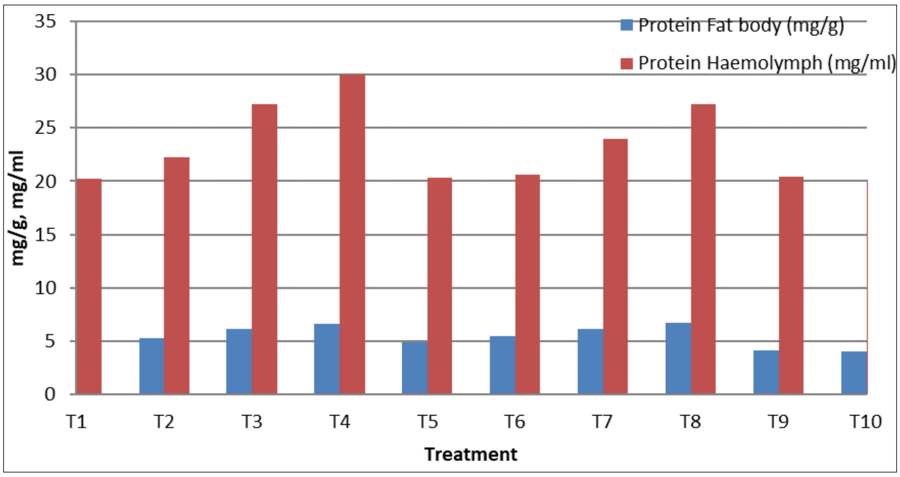

Fig. : Influence of feeding mulberry leaf fortified with ayurvedic formulations at different concentrations on protein content in fat body and haemolymph of silkworm

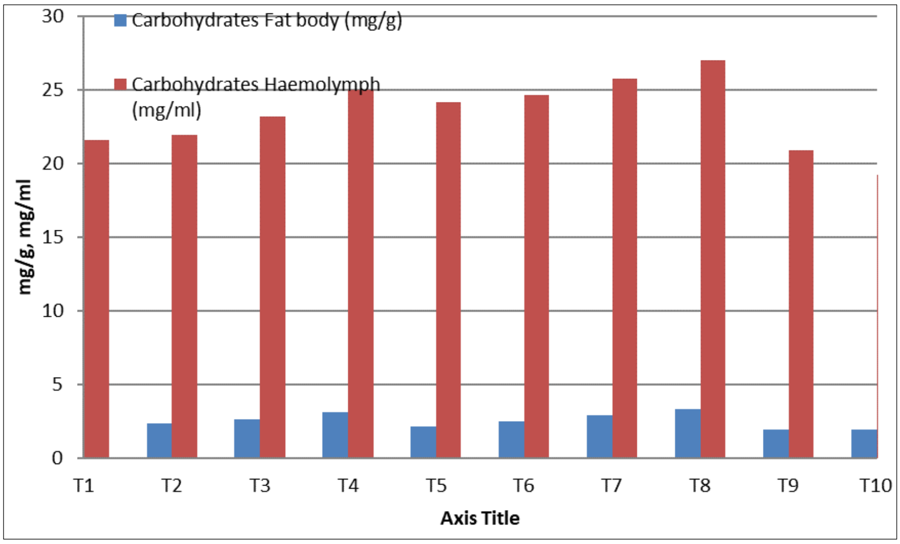

Fig. : Influence of feeding mulberry leaf fortified with ayurvedic formulations at different concentrations on carbohydrate content in fat body and haemolymph of silkworm

Summary
The current study showed a significant increase in the fat body and hemolymph protein content of the double hybrid silkworm, fed with mulberry leaves enriched with Hingwastaka and Kapikacchu. The protein content in the hemolymph was highest with Hingwastaka @ 2.0%, while for the fat body, Kapikacchu @ 2.0% was considered best for increasing protein-levels. Additionally, the overall carbohydrate content of fat body and hemolymph of the double hybrid silkworm significantly increased with Hingwastaka and Kapikacchu enhanced mulberry leaves. The highest amounts were observed for Kapikacchu at 2.0%, and levels of 2.0% and 1.5% were determined to be more effective than 1.0% and 0.5%. The study revealed that mulberry leaf fortified with ayurvedic formulation Hingwastaka @ 2.0% concentration enhanced the protein and carbohydrate contents in the fat body and haemolymph of the double hybrid silkworm and these ayurvedic formulations boost silkworm health and digestion leading to improved growth, development and commercial characters of silkworm.

References
1. Ageitos JM, Pulgar A, Csaba N, Garcia-Fuentes M. Study of nano-structured fibroin/dextran matrices for controlled protein release. Eur Polym J. 2019;114:197-205.
2. Benchamin KV, Nagaraj CS. Silkworm rearing technologies - Chapter 4. In: Jolly MS, editor. Appropriate Sericulture Techniques. Mysore: ICTRTS; 1987. p. 63-106.
3. Bhatti MF, Shahzadi N, Tahir HM, Ali S, Zahid MT, Khurshid R. Effect of honey (*Apis dorsata* [Hymenoptera: Apidae]) on larval growth and silk cocoon yield of *Bombyx mori* (Lepidoptera: Bombycidae). J Insect Sci. 2019;19(6):11.
4. Dandin SB, Giridhar K. Handbook of Sericulture Technologies. Bangalore: Central Silk Board; 2010. p. 420.
5. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem. 1956;28:350-6.
6. Etebari K, Fazilati M. Effect of feeding on mulberry's supplementary leaves with multi-mineral in some biological and biochemical characteristics of silkworm, *Bombyx mori* L. J Sci Technol Agric Natur Resour. 2003;7:233-44.
7. Etebari K, Ebadi R, Matindoost L. Effect of feeding mulberry leaves with ascorbic acid on some biological, biochemical and economical characters of silkworm, *Bombyx mori* L. Int J Indust Entomol. 2004;8:81-7.
8. Etebari K, Kaliwal BB, Matindoost L. Supplementation of mulberry leaves in sericulture: theoretical and applied aspects. Int J Indust Entomol. 2008;9:14-28.
9. Fakuda S. Biochemical studies on the formulation of silk protein. Part IX. The direct and indirect formulation of silk protein during the growth of silkworm larvae. Bull Agric Chem Soc Jpn. 1960;24:296-401.
10. Hamamura Y, Naito K. Food selection by silkworm larvae *Bombyx mori* L. Nature. 1961;190(3):879-80.
11. Ito T, Arai N. Food value of mulberry leaves for the silkworm, *Bombyx mori* L. determined by means of artificial diets. II. Comparison between soft and hard leaves. Bull Seric Expt Stn Tokyo. 1963;18:1-16.
12. Ito T. Silkworm Nutrition. In: Tazima Y, editor. The Silkworm: An Important Laboratory Tool. Tokyo: Kodansha Ltd.; 1978. p. 121-57.
13. Kajiura Z, Yamashita O. Super growth of silk glands in the dauer larvae of the silkworm, *Bombyx mori*, induced by a juvenile hormone analogue. J Seric Sci Jpn. 1989;58:39-46.
14. Khyade VB. Efficiency of silk sericin from the cocoons of silkworm, *Antheraea mylitta* [L.] and *Bombyx mori* [L.] for treating the hydrogen peroxide induced oxidative stress in feline fibroblasts. World Sci News. 2016;44:35-49.
15. Kumararaj S, Vijayaraghavan S, Krishnaswami S. Studies on fortification of mulberry leaves for feeding silkworms. Indian J Seric. 1972;11:68-72.
16. Legay JM. Recent advances in silkworm nutrition. Annu Rev Entomol. 1958;3:75-86.
17. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin Phenol Reagent. J Biol Chem. 1951;193:265-75.
18. Magadum SB. Studies on the effect of some chemicals and hormones on the gonads and the life cycle of the silkworm, *Bombyx mori* L. [PhD Thesis]. Dharwad: Karnataka University; 1987.
19. Muthukrishnan J, Madhavan S, Jothi VN. Effect of restriction of feeding duration on food utilization, emergence and silk production in *Bombyx mori* L. Monit Zool Ital. 1978;12:87-94.
20. Nirwani RB, Kaliwal BB. Effect of thiamine on commercial traits and biochemical contents of the fat body and haemolymph in the silkworm, *Bombyx mori* L. Sericologia. 1998;38:639-46.
21. Ohmura J, Jang DS, Pank EJ, Kang YH. The role of water in regulation of plant development. Can J Bot. 1989;65(7):1287-93.
22. Saha BN, Khan AR. The growth and development of the silkworm, *Bombyx mori* L. on feed supplemented with nicotinic acid. Bangladesh J Life Sci. 1996;1:103-9.
23. Seidavi AR, Bizhannia AR, Sourati R, Mavvajpour M. The nutritional effects of different mulberry varieties on biological characters in silkworm. Asia Pac J Clin Nutr. 2005;14:122-4.
24. Seol GY. Varietal difference in amylase activity of larval digestive fluid of the silkworm, *Bombyx mori* L. reared on artificial diet. Seri J Korea. 1982;24:73-80.
25. Singh KK, Chauhan RM, Pande AB, Gokhale SB, Hegde NG. Effect of Lactobacillus plantarum as a probiotic to improve cocoon production of mulberry silkworm, *Bombyx mori* [L.]. J Basic Appl Sci. 2005;1:1-8.
26. Sridhar S, Bhat JV. Trace elements in the silkworm, *Bombyx mori* L.: effect of trace elements. Proc Indian Acad Sci B. 1966;63(1):9-16.
27. Sumioka HS, Kunda S, Yoshitake N. Relationship among food ingestion, digestion and body weight gain in the silkworm larvae, *Bombyx mori* L., under restricted feeding. J Seric Sci. 1982;51:52-7.
28. Tao S, Wang J, Liu M, Sun F, Li B, Ye C. Haemolymph metabolomic differences in silkworms (*Bombyx mori* L.) under mulberry leaf and two artificial diet rearing methods. Arch Insect Biochem Physiol. 2021;98(4):e21838.
29. Ude AU, Eshkoor RA, Zulikifili R, Azahari CH. *Bombyx mori* silk fibre and its composite: A review of contemporary developments. Mater Des. 2014;57:298-305.