Influence of Mulberry Leaf Fortified with Limiting Amino Acids on Biochemical Constituents and Economic Traits of Silkworm, *Bombyx mori* L.

Kahkashan Qayoom
(MSS/2018/63)

College of Temperate Sericulture
Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir

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Thesis

Submitted to

The College of Temperate Sericulture

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In partial fulfillment of requirements for the award of the degree of

Master of Science in Sericulture

2021

Dedicated

To

My Beloved Parents

And loving Brother

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
College of Temperate Sericulture, Mirgund

Certificate – I

This is to certify that the thesis entitled **"Influence of Mulberry Leaf Fortified with Limiting Amino Acids on Biochemical Constituents and Economic Traits of Silkworm, *Bombyx mori* L."** Submitted in partial fulfilment of the requirements for the award of **Master of Science in Sericulture,** to the College of Temperate Sericulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, is a record of bonafide research work carried out by **Ms. Kahkashan Qayoom (Regd. No. Mss/2018/63)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that information received during the course of investigation has duly been acknowledged.

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Chairman
Advisory Committee

Endorsed

Prof. & Head
Division of Sericulture Crop Improvement
College of Temperate Sericulture

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
College of Temperate Sericulture, Mirgund

Certificate – II

We, the members of the Advisory committee of **Ms. Kahkashan Qayoom (Regd. No. MSS/2018/63)**, a candidate for the degree of **Master of Science in Sericulture**, have gone through the manuscript of the thesis entitled, **"Influence of Mulberry Leaf Fortified with Limiting Amino Acids on Biochemical Constituents and Economic Traits of Silkworm, *Bombyx mori* L."** and recommend that it may be submitted by the student in partial fulfilment of the requirements for the award of degree.

Advisory Committee

Chairman

Dr. Firdose Ahmad Malik
Assistant Professor
CTS, Mirgund, SKUAST-Kashmir

Members

Dr. Shabir Ahmad Bhat
Assistant Professor
CTS, Mirgund, SKUAST-Kashmir

Dr. Imtiyaz Murtaza
Professor and Head
Division of Basic Science and Humanities,
SKUAST-Kashmir, Shalimar

Dr. Shakeel Ahmed Mir
Professor and Head
Division of Agri-statistics,
SKUAST-Kashmir, Shalimar

Dean's Nominee

Dr. A. H. Hakeem
Dean FOA, Wadura
SKUAST-Kashmir

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
College of Temperate Sericulture Mirgund

Certificate – III

This is to certify that the thesis entitled, **"Influence of Mulberry Leaf Fortified with Limiting Amino Acids on Biochemical Constituents and Economic Traits of Silkworm, *Bombyx mori* L."** submitted by **Ms. Kahkashan Qayoom (Regd. No. MSS/2018/63)** to the College of Temperate Sericulture Mirgund, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, in partial fulfilment of the requirements for the award of the degree of **Master of Science in Sericulture** was examined and approved by the Advisory Committee and external examiner on ………………

Chairman
Advisory Committee

External Examiner

Prof. & Head
Division of Sericulture Crop Improvement
College of Temperate Sericulture

Associate Dean
College of Temperate Sericulture Mirgund

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Temperate Sericulture Research Institute, Mirgund

Name of the student : **Kahkashan Qayoom**

Registration No. : MSS/2018/63

Major subject : Sericulture

Minor subjects : Biochemistry

Major advisor : **Dr. Firdose Ahmad Malik**
Assistant Professor
CTS, Mirgund, SKUAST-Kashmir

Title of the Thesis : **"Influence of Mulberry Leaf Fortified with Limiting Amino Acids on Biochemical Constituents and Economic Traits of Silkworm, *Bombyx mori* L."**

ABSTRACT

The investigation entitled "Influence of Mulberry Leaf Fortified with Limiting Amino Acids on Biochemical Constituents and Economic Traits of Silkworm, *Bombyx mori* L." was carried out at Division of Sericulture Crop Improvement in College of Temperate Sericulture, Mirgund, during spring in the year 2020. The study material comprised of pure race APS_4. The mass rearing of silkworms was done upto 3rd instar. Just after 3rd moult, three replications containing 100 worms were maintained for each treatment as well as for control batches. For the present study three amino acids viz. Methionine, Tryptophan and Lysine with three different concentrations i.e., 0.5%, 1.0% and 1.5% were selected to determine their effect on growth and development of silkworm, commercial cocoon characters and on biomolecules of haemolymph and silk gland. After 3rd moult, these amino acid solutions were sprayed @100 ml/200gm mulberry leaf with the help of an automizer for 100 larvae daily once as first feed during 4th and 5th instar. Two controls viz. control-I (worms fed with mulberry leaves treated with distilled water) and control-II (worms fed with mulberry leaves without any treatment) were also maintained along with other treated batches. The treatments include T_1 (0.5% Methionine), T_2 (1.0% Methionine), T_3 (1.5% Methionine), T_4 (0.5% Tryptophan), T_5 (1.0 Tryptophan), T_6 (1.5% Tryptophan),

T_7 (0.5% Lysine), T_8 (1.0% Lysine), T_9 (1.5% Lysine), T_{10} (control-I) and T_{11} (control-II). The shortest 5th instar and total larval duration of 147 and 627 hours, respectively were recorded when mulberry leaves were fortified with Methionine at 0.5% concentration and fed to the silkworms. The highest weight of 10 mature larvae of 54.03 grams, silk gland weight of 1.97 grams, silk gland somatic index of 36.53 per cent, cocoon yield of 9635 cocoons/10,000 larvae by number, cocoon yield of 18.78 kg by weight, silk conversion index of 24.53 per cent, silk productivity of 7.92 cg/day, single cocoon weight of 1.940 grams, single shell weight of 0.483 grams and shell percentage of 24.89 per cent were recorded in T_1 (0.5% Methionine). In case of post cocoon parameters longest filament length of 1110 meters, thinnest filament size (denier) of 2.60 and highest raw silk percentage of 17.37 per cent were also recorded in the same treatment. The highest fecundity of 590 eggs per layings was recorded when mulberry leaves were fortified with Tryptophan at 0.5% concentration (T_4). The highest total protein content of 70.59 mg/ml in haemolymph and 195.89 mg/g in silk gland, and total free amino acid content of 25.86 mg/ml in haemolymph and 157.76 mg/g in silk gland were also recorded in T_1 where silkworms were fed with mulberry leaves fortified with 0.5% concentration of Methionine. Thus results in this study clearly indicated that by improving the nutritional value of mulberry leaf by fortification supports good growth and development of the silkworm *viz-a-viz* improves the economic and biochemical parameters significantly.

Keywords: *Bombyx mori* L., Amino acids, Methionine, Tryptophan, Lysine, Economic traits, Biochemical parameters.

Signature of Student   Signature of Major Advisor

Dated: ___________   Dated: _______________

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Kahkashan Qayoom

Place: Mirgund

Dated: __________

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INTRODUCTION

Sericulture is a unique process of raising silkworms that combines modern technology and traditional knowledge for the production of silk. Known for its glamour and comfort, the fabric is worn by the people across the cultures and is cherished for its elegance. Sericulture is an agro based labour intensive industry which plays an important role in the transformation of Indian rural economy and is very much suited to the country like India where more than 80% of farmers are small and marginal. The nature of this industry with its rural based on-farm and off-farm activities and enormous employment and income generation potential makes it one of the most appropriate tools for socio-economic development. With the development of new technologies in mulberry cultivation and silkworm rearing, sericulture has now emerged as a sustainable and indispensable profession and cash crop for the rural folk of the country.

Silk, is considered the most elegant and valued textile fibres in the world with its natural sheen, fineness, inherent affinity for dyes, high durability, soft touch, elegance, strength, light weight and high absorbance among all other fibres and is thus known as “Queen of textiles”. The popularity of silk continues to grow as no other fabric has been able to match its reputation for luxury and royalty. Silk is the most hypoallergenic of all fabrics because of its protein structure. It has been rightly quoted by Oscar de la Renta, “Silk does for the body what diamond do for the hand”.

Sericulture is an economic resource for nearly 30 million families in countries like China, India, Vietnam, Japan, Brazil and Thailand among which 26 million are from China and India only (Ganai *et al.,* 2012). Although more than 40 countries deal with the production of silk, Asia remains the major producer of silk in the world contributing 98.5% to the total global output and bulk of the silk production comes from China and India, followed by Brazil, Uzbekistan, Thailand, Vietnam, Korea and Japan (Bhat, 2014).

India is the second largest raw silk producing country in the world after the People's Republic of China, with an annual raw silk production to about 36, 152 MT during 2019-20 which accounts for 19 per cent of the total global raw silk production besides providing employment to 9.25 million persons in 2019-20 compared to 9.17 million persons in 2018-19, indicating a growth of 3.8 per cent (Anonymous, 2020). The annual export earnings of silk and silk products from India during the year 2019-2020 were around Rs.1498.39 crores.

India has a unique distinction of being the only country in the world producing all four kinds of commercially exploited natural silks namely Mulberry, Eri, Muga and Tasar. Among all the varieties of silk produced in 2019-20, Mulberry accounted for 70.21 per cent (25, 384 MT), Tasar 9.3 per cent (3, 370 MT), Eri 19.80 per cent (7, 157 MT) and Muga 0.66 per cent (240 MT) of the total raw silk production, which is an increase of 1.9 per cent over the production achieved during the last year (Anonymous, 2020). The mulberry silk production was marginally up by 0.2 per cent during 2019-20 over previous year. Vanya silk, which includes Tasar, Eri and Muga silks, has growth of 13.1 per cent, 3.6 per cent and 3 per cent, respectively, growth during 2019-20 over 2018-19 (Anonymous, 2020). India holds the world monopoly in the production of Muga silk with golden yellow glitter, which is produced only in Assam (Anonymous, 2020). During 2018-19, over 52, 360 villages are involved in sericulture throughout all the states across India for silk production covering over 25 states. The highest silk producing states in India are, Andhra Pradesh, Karnataka, Manipur, Maharashtra, Nagaland, West Bengal, Jharkhand, Tamil Nadu, Meghalaya and union territory of Jammu and Kashmir (Anonymous, 2020).

Union Territory of J&K is the only territory in the country which is at the same altitude in which the leading bivoltine sericulture countries of the world lie. Sericulture has an important place in the economy of Jammu and Kashmir because of its low investment and high return. The climate of Jammu and Kashmir is temperate and congenial for rearing of bivoltine silkworm species and for producing fine bivoltine cocoons. These cocoons are superior to the multivoltine ones produced in other parts of the country and are comparable to international quality. Jammu and Kashmir is famous for producing fine and beautiful silk fabric. There are 2, 800 villages in union territory of Jammu and Kashmir where sericulture is an important economic activity and about 30, 300 families are engaged in silkworm rearing and raw silk production (Anonymous, 2019). The annual cocoon production in the union territory of Jammu and Kashmir during 2019-20 is 800 MT with the total raw silk production of 117 MT (Anonymous, 2020). Sericulture is one of the traditional occupations of Jammu and Kashmir and is practiced in 20 districts of J&K. The major silk producing districts are Udhampur (29.6%), Rajouri (29.5%), Kathua (17.9%), Baramulla (8.5%), Anantnag (8.1%), Pulwama (7.6%), Reasi (3.6%), and Ganderbal (2.4%) (Anonymous, 2019). In order to increase the production and productivity as per the enormous potential of the union territory of Jammu and Kashmir, collective efforts are made by Sericulture Development Department J&K, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir and Central Silk Board, due to which the union territory of Jammu and Kashmir ranks 1st among the north-western states and occupies 4th position at national level in-terms of production of quality bivoltine cocoons and raw silk.

Basically, silk a protein being secreted by sericigenous insect belonging to family Lepidoptera. The silkworm, *Bombyx mori* L. is a lepidopteran sericigenous insect of considerable economic importance. Hence, it remained a challenge to explore secret behind the stoniness and lusture of the silk, besides enhancing the production through various agrochemical, genetically and biotechnological tools. Considering, the importance of biochemistry in the growth of other biological sciences, sericulture is no exception and hence appropriate biochemical methodologies and laboratory techniques will unravel the mysteries behind the process of silk production.

Silkworm, *B. mori* L. is a monophagous insect, so its survival is entirely dependent on quality mulberry leaf. In addition to the metabolic processes, quality of cocoons is also largely dependent on the nutritional status of the mulberry leaf. Therefore, the leaf should contain all the essential elements required for normal growth and development. Nutritional quality of leaves play a vital role for robust growth of larvae and superiority of the silk produced by *Bombyx mori* L. (Hiware, 2006).Therefore, in order to further boost the production of bivoltine silk in the union territory of Jammu and Kashmir, it is important that research must be done in various fields of sericulture so that quality and quantity of raw silk is improved to a greater extend. This can be done by producing improved varieties of mulberry and also by supplementing the mulberry leaves with extra nutrient elements.

Miyashita (1986) observed that leaf quality alone contributes about 38.2% to success of cocoon crop. Significant seasonal variations occur in the nutritional value and composition of mulberry leaves depending on the factors such as weather conditions, nutritional status of soil, infestation by pests and diseases as well as agricultural practices (Ito, 1978). These have profound effect not only on nutritional quality, palatability and cocoon parameters, of a silkworm but also hinder the larval development. Components like water, amino acids, carbohydrates, proteins, fats, mineral elements, secondary metabolites and vitamins in feed play a central role in determining the quality of feed (Thirumalaisamy *et al.,* 2009). The biochemical substances in the leaves highly influence the growth and development of silkworm. Better cocoon production has been observed to be directly associated with evolving successful rearing techniques, which typically depend upon fulfilling the dietary needs of the silkworm.

Since there’s a robust correlation between nutrition and also the physiology of silkworm, the standard of leaf features a larger influence on the quantity of food eaten, the biology of the silkworm nutrition, as significantly it relates to the physiology of digestion. Among all the essential elements, amino acids are of vital importance in sericulture as cocoon shell itself is a protein and these amino acids are the structural units that makeup this protein. Silkworms acquire 72–86% of their amino acids from mulberry leaves and out of which 60% is used for the production of silk (Lu and Jiang, 1988). Methionine and Tryptophan plays an important role in increasing the various parameters like fecundity, fertility, adult emergence, developmental periods and longevity of adult *Bombyx mori*. L. (Laz, 2010). Cystine which also plays an important role in attaining the optimum growth and development of silkworm *Bombyx mori*. L. is formed from methionine (Ito, 1976). Tryptophan and Methionine increases the robustness of silkworm *Bombyx mori*. L. (Laz *et al.,* 2006). Essential amino acids are very important for the organisms, not only they carry out a number of processes themselves but also help in formation of other non- essential amino acids which in turn contribute towards the growth of silkworm *B. mori*. L. They help in improving the commercial cocoon parameters as well. Increase in total proteins and total free amino acid implies increased metabolic activities and activation of silk production. Haemolymph acts an amino acid reservoir between midgut and silk glands and supplies amino acids to silk gland for silk synthesis. For the biosynthesis of silk, silkworms utilize the proteins obtained from mulberry leaves that they feed on during their larval period. It is therefore clear that mulberry is the sole source of nutrition to the silkworm which plays a dominant role in cocoon production. Hence nutrition of silkworm is of primary importance in improving the growth and development of the silkworm, *Bombyx mori* L. as the success of cocoon crop production is directly influenced by the nutritive status of mulberry leaves.

Nutrition of proteins and amino acids is of particular importance for silkworm because of their active utilization of nitrogen substances involved in the synthesis of silk protein. The optimal level of dietary protein for growth of the silkworm is in the range of 22 to 26% and nutritive values of various kinds of proteins are greatly dependent on their composition of amino acids. Protein and amino acids of the silkworm are largely derived from the mulberry foliage (Fukuda and Kameyan, 1960). Monophagy is strengthened by the high correlation between amino acid composition of proteins of the silkworm, *B. mori* and its host plant, *Morus*. Mulberry leaf should not contain less than 3-3.5% of the total nitrogen to support larval growth (Arsenev and Bromelei, 1957). Many studies on amino acid metabolism in silkworm have been concerned with the synthesis of silk protein. It goes without saying that amino acid nutrition of the silkworm is closely related to the synthesis of silk protein as well as to the growth of silk gland. These studies have in part dealt with the nutritional aspects of amino acids, but information on absolute amino acid requirements has been inconclusive. The silkworm, *Bombyx mori* L. requires essential, non-essential and acidic amino acids for its growth and development.

1. Essential amino acids: The essential amino acids or indispensable amino acids, are those amino acids which are not synthesized by the organisms itself and must be supplied to the organism through diet. These are Arginine, Histidine, Isoleucine, Leucine, Methionine, Phenylalanine, Theorine, Valine, Lysine and Tryptophan. If diet is insufficient in any essential amino acid, protein synthesis can't continue beyond the rate at which that amino acid is available and such amino acids are called as limiting amino acids.

2. Semi essential amino acids: Semi essential amino acids are present to a certain limit in the organism itself but its requirement is to be checked as it is also an important constituent for growth and development as organisms usually grow slowly in their absence. This includes Proline.

3. Non-essential amino acids: Non-essential amino acids are present within the body of an organism. The organism can synthesize all non-essential

amino acids but their deficiency needs to be checked. These are Alanine, Serine, Glycine, Cysteine and Tyrosine.

4. Acidic amino acids: Acidic amino acids include Aspartic acid and Glutamic acid. Single omission of these acidic amino acids from a diet affects larval growth slightly, but double omission resulted in a marked suppression of growth.

The lack of the supply of any essential amino acid may result in a cessation of the protein synthesis. In each instar larvae failed to grow in the absence of the respective amino acid (Inokuchi and Yoshitake, 1978). Apparently, they seem to be hardly synthesized from other amino acids or simpler precursors, or the quantity which is synthesized by the larva is entirely inadequate for supporting growth. The digestible foliar proteins contain 82% useful amino acids, 49% essential amino acids and 33% non-essential amino acids. The proportion of essential amino acids: non-essential amino acids are 60:40, which meets the growth and other metabolic requirements of silkworm (Reddy, 2011).

The ratio of essential amino acids over total amino acids in the diet (E/TA) optimal for growth is in the range of 0.43 to 0.54 (Horie *et al.,* 1970). It is interesting that this upper limit of E/TA ratio is corresponding to that calculated from mulberry leaves. There is optimal combination of the amounts of acidic and non-essential amino acids for growth acceleration in the silkworm and this quantitative ratio alters according to larval development. Furthermore, the female larvae are more susceptible to amino acid imbalances in the diet than male larvae (Arai & Ito, 1964). Methionine (0.4 mg/gm dry matter), Tryptophan (1.34 mg/gm dry matter) and Lysine (11.7 mg/gm dry matter) are considered to be the 3 limiting amino acids in mulberry leaves (Bose and Mujamder, 1989). The dietary supplementation of limiting amino acids markedly improves the nutritive value of gluten and zein (Ito and Arai, 1965). However, not much information is available on mulberry leaves supplemented with these limiting essential amino acids on economic traits and their utilization for protein synthesis in the silkworm.

In the light of the above considerations, the present investigation was envisaged to study the impact of supplementation of limiting essential amino acids of mulberry *viz*. Methionine, Tryptophan and Lysine on economic traits and biochemical constituents of the silkworm, *B. mori* L. with the following objectives:

- To study the influence of limiting amino acids on commercial traits of silkworm, *Bombyx mori* L.
- To study the influence of limiting amino acids on total protein and total free amino acids in haemolymph and silk gland.

Chapter-2

REVIEW OF LITERATURE

Keeping the objectives of the present study in view, the available literature on the subject has been reviewed and presented as under:

Bheemeshwar and Sreenivasaya (1952) reported that Aspartate and Glutamate play active role in amino acid nutrition as they acts as donors of amino groups in transamination reactions in the digestive tract of silkworm, *Bombyx mori* L.

Anderson (1956) studied the developmental changes in protein content and amino acid pools in the larval fat body and haemolymph of *Calliphora erythrocephala* and reported that the changing nutritional conditions, the proteolytic action of the molting fluid on the old cuticle, age, nutrition, and climatic conditions are the various factors through which the concentration of free amino acids is influenced.

Kirimura (1962) studied amino acid composition and chemical structure of silk protein by microbiological determination and reported that silk gland attains maximum growth towards the end of the 5^{th} instar owing to fibroin synthesis and also, reported that the silk gland weight is one of the important parameters for assessing the silk production potential of the larvae.

Ito and Tanaka (1962) studied the effect of different concentrations of sugars and proteins added to the artificial diet in silkworm, *Bombyx mori* L. and reported that, nutritional supplements have a significant impact on larval growth and cocoon production.

Ito and Arai (1965), while analyzing the amino acid requirements and nutritive effects of various proteins on silkworm, reported that both proteins as well as amino acids are of particular importance for the silkworm so far as synthesis of silk protein is concerned. They further reported that the optimal level of dietary protein for growth of the silkworm is in the range of 22-26% and nutritive values of various kinds of proteins are greatly dependent on the composition of amino acids.

Inokuchi *et al.* (1967) reported that silkworm *B. mori* fed with leaf deficient with any of the 10 essential amino acids fail to grow and ultimately died, and justified the importance of amino acids with regard to the growth and development of silkworm.

Nimrick *et al*. (1970) reported that Lysine and Methionine are limiting amino acids in microbial protein while performing quantitative assessment of supplemental amino acids which are needed by the growing lambs.

Horie *et al*. (1971) reported that elevation of dietary protein to an optimal level and supplementation of low nutritive proteins with their limited amino acids has accelerated the growth of the silkworm.

Ito (1972) while studying amino acid nutrition of the silkworm *Bombyx mori* L. has observed that amino acids are closely related to the biosynthesis of silk proteins as well as to the growth of silk gland in silkworm, *Bombyx mori* L.

Sengupta *et al.* (1972) while studying the enrichment of mulberry leaf with various sugars, proteins, amino acids and vitamins for vigorous growth of silkworm *B. mori* and increased cocoon crop production, found that silkworm requires certain essential sugars, proteins, amino acids and vitamins for its growth, survival and also for the growth of the silk gland.

Tazima (1978) declared silkworm as an important laboratory tool and further reported that the growth of silk gland is marked by the accumulation of organic compounds particularly proteins in the lumen of the silk gland during the larval growth period of silkworm, *Bombyx mori* L.

Horie and Watanabe (1983) had confirmed that the supplementation of soyabean protein increased the protein and amino acid content in the haemolymph of silkworm larvae which resulted in marked improvement in silk yield.

Ahmad (1983) studied the effect of phosphorus and potassium treated mulberry leaves on larval development and silk yield of silkworm, *B. mori* and found that the monophagous feeding habit of silkworm requires improvement in the diet in order to enhance the silk production.

Mathavan *et al.* (1984) while performing some experiments reported that various nutrients like proteins, amino acids, vitamins, minerals, harmones etc. have been supplemented to mulberry leaf and fed to the larvae of silkworm, *Bombyx mori* L. in order to improve cocoon as well as post-cocoon parameters, which in turn will improve silk production.

Levenbook (1985) has reported that in the larvae of holometabolous insects, storage proteins are the major haemolymph proteins which play an important function as reservoir for amino acids that are utilized for larval and adult development.

Krishnappa (1987) studied influence of amino acid supplementation on growth and development of mulberry silkworm, *Bombyx mori* L. and reported that supplementation with amino acids reduces the total larval duration.

Lu and Jiang (1988) reported that amino acids have multiple metabolic functions in the living cells. Diversity in the amount of free amino acids of haemolymph is generally affected by diet. Silkworm absorbs 72-86% of amino acids from the mulberry leaves and in females more than 60% of absorbed amino acids are from the mulberry leaf.

Eid *et al*. (1989) has reported that in non-mulberry silkworms excess amino acids increased the cocoon and raw silk weight when fed on green castor leaves. The strength and elongation of silk fibers also increased by administration of excess amino acids and green castor leaves. They further indicated in their study that activities of silk gland GTP, GOT enzymes, the accumulation of RNA in the silk gland and the incorporation of amino acids in the silk fibers were promoted by applying excess amino acids.

Mahmood (1989) demonstrated that mulberry leaves dipped in 0.2 per cent of nitrogen solution when fed to silkworm, *Bombyx mori* L. produced larvae with maximum weight as compared to other doses when he was trying to analyse the effect of nitrogen on the larval development and silk yield of *Bombyx mori* L.

Prasad and Mohan (1990) analysed the behaviour of amino acids, aminotransferases and proteins in the metamorphosing silkworm, *Bombyx mori* L. and found that the total free amino acid levels declined from the early 4^{th} instar to mid 5^{th} instar and were elevated significantly during the late 5^{th} instar in CNS, muscles, silk gland and haemolymph.

Kumarilalitha *et al.* (1992) has reported that mulberry leaf supplemented with amino acids when fed to silkworm larvae resulted in the development of heavy females with more number of eggs.

Kabila *et al.* (1994) indicated an improvement in the economic parameters of silkworm *B. mori* when fed on mulberry leaves enriched with Aspartic acid, while studying the impact of supplementation on growth and economic characters of silkworm, *Bombyx mori* L.

Kamili (1994) reported that the silk gland weight is highly correlated to silk production and different silkworm strains have different silk gland weight influencing their silk production ability. It was further reported that the cocoon yield exhibit significant positive association with ERR, pupation rate, cocoon weight, shell weight, shell ratio, hatching percentage, filament length, larval weight and larval duration.

Zaman *et al.* (1996) studied the effect of feeding Mg and N treated mulberry leaves on larval development of silkworm, *Bombyx mori* L. and demonstrated that mulberry leaves enriched with 0.2% nitrogen contributed to the significant increase in silkworm larval weight.

Chapman (1998) reported that Proline of silk is very much essential for development of insects and it also acts as a source of energy in certain insects.

Isairasu and Ganga (2000) while studying the influence of dietary Glycine supplementation on the mulberry silkworm *Bombyx mori* L. found that weight and size of cocoon, shell ratio and fibrion content of the shell showed a significant increase.

Unni *et al*. (2000) analysed that silkworm needs large quantity of nitrogen during last larval instar for synthesis of fibrion protein which comprises more than 70 to 75 per cent of silk filament. It has been further reported that silkworm *Bombyx mori* L. ingests more than 65 per cent of nitrogen for silk production during 5^{th} instar.

Tazima (2001) while studying the improvement of biological functions in the silkworm reported that, 75% of mulberry protein is directly converted into silk protein and 25% goes to the body tissues of the silkworm larvae.

Leonardi *et al*. (2001) while observing the modulation of Leucine absorption in the larval midgut of *Bombyx mori* L. demonstrated that Leucine can greatly increase the activity of the transport system which in turn is responsible for the absorption of most essential amino acids in the larval midgut. They further reported that nutrient absorption and its modulation is critical for the animal growth.

Etabari (2002) has demonstrated that mulberry leaves treated with 0.5% glycine showed 12.3% increase in larval weight while studying the effect of enrichment of mulberry leaves (*Morus alba*) with some vitamins and nitrogenous compounds on some economic traits of silkworm, *Bombyx mori* L.

Gill (2003) while working on value added ingredients and amino acids in pig and poultry, explained that Methionine and Lysine are first and second limiting amino acids in most of plant protein sources.

Kochi and Kaliwal (2005) have reported that feeding silkworms with various food additives helped to increase the economic characters and played a vital role in their larval development.

Etabari (2007) reported that dietary supplements of Glutamic acid, Glycine and Urea when fed to silkworm larvae showed significant changes in larval parameters such as larval weight and nutritional indices. They further reported that the amount of cholesterol and triglycerol in the haemolymph increased significantly and the supplementations of amino acids in these groups had a great influence on the account of glucose in the larval haemolymph.

Mondal *et al.* (2007) reported that the silkworm, *Bombyx mori* L. during the final stage of their larval development produces massive amount of silk proteins. It was further mentioned that these proteins are stored withinside the middle silk gland and they're discharged via the anterior duct and spinneret at the end of the 5^{th} instar.

Rufaie and Ganie (2008) studied the effect of fortification of proteins (casein and soyabean) at different concentration on mulberry leaf. It was observed that various commercial characters like larval weight, cocoon weight, average filament length got enhanced significantly as compared to control.

Stuart *et al.* (2009) reported that free amino acids particularly Proline and Glycine act as main phagostimulatory chemical that restricts movement of female insects, while analyzing the free amino acids as phagostimulents.

Rufaie *et al.* (2010) also studied the effect of various minerals (ferrous sulphate and zinc chloride) on growth, development and other commercial characters of cocoon. It was observed that cocoon weight and raw silk percentage was improved by fortification of these minerals with mulberry leaf.

Laz (2010) reported that mulberry leaves treated with amino acids like Methionine and Tryptophan when fed to silkworms resulted in significant increase in various parameters like fecundity, fertility and adult emergence, developmental periods and longevity of *Bombyx mori* L. adults. It was further reported that in adults there was decrease in mortality.

Rouhalla (2010) has reported that supplementation of amino acids on mulberry leaves was found effective for improvement in various cocoon characters and growth and development of silkworm. However, a higher level of these amino acids beyond a certain limit has no positive effect on these parameters.

Chakraborty and Kaliwal (2012) analyzed that oral supplementation of Arginine and Histidine to silkworm *Bombyx mori* L. has resulted in significant increase in cocoon and post cocoon parameters. They further reported that moth emergence, hatching percentage and fecundity were significantly increased in all the treated groups.

Saad *et al.* (2014) while studying the effect of mulberry leaves supplemented with Glycine on some biological aspects of silkworm, *Bombyx mori* L. found that the cocoon parameters and economic parameters were enhanced by feeding 1.0 per cent Glycine treated leaves to silkworm *Bombyx mori* L.

Srinivas (2014) while studying the impact of supplementation of amino acids, Valine and Leucine to Eri silkworms, *Philosamia ricini* on its cocoon characters, found that there is a significant effect of Valine and Leucine on castor leaves fed to 5^{th} instar larvae of Eri silkworm.

Aparupa (2015) reported that there are number of artificial foods such as royal jelly, dietary proteins, amino acids and vitamin B_3, that are effective in increasing the protein and lipid content as well as the growth and development of silkworm larvae.

Borgohain (2015) while reviewing silk protein and its biosynthesis in silkworm *Bombyx mori* L. reported that synthesis of silk protein starts after 4^{th} moult when posterior silk gland cells get increased in size even though total number of cells remains constant throughout the post embryonic life.

Thulasi and Sivaprasad (2015) observed that while feeding silkworm, *Bombyx mori* L. with amway nutrilite (which contains proteins, carbohydrates, fats, calcium, iron and nine essential amino acids) supplemented mulberry leaves enhanced growth rate in the larval body and silk gland during 5^{th} instar.

Hamzah *et al.* (2016) observed that the cocoon weight, cocoon shell weight and shell ratio improved significantly when silkworm larvae were fed on mulberry leaves supplemented with pollen grains, royal jelly, amino acids and bee honey. Also such feeding led to shorter larval duration and decreased larval mortality percentages.

Bhat and Rufaie (2017) while reviewing the impact of amino acid fortified mulberry leaf on silk gland and commercial characters of silkworm, *Bombyx mori* L., demonstrated that 0.5 per cent Histidine was superior in its efficacy in improving growth and development of silk gland and other commercial cocoon characters of silkworm, *B. mori* L.

Gorissen *et al.* (2018) reported that the essential amino acid content and amino acid composition of dietary protein source contribute to the differential muscle protein synthetic response to the ingestion of different proteins and further stated that lower essential amino acid content and specific lack of certain amino acids like Leucine, Lysine and Methionine may be responsible for the lower anabolic capacity of plant based proteins.

Kumar and Kumar (2018) while analyzing the influence of mulberry leaf fortified with Methionine on the economic traits and aminotransferase activity in *Bombyx mori* L., reported that the free amino acid content in haemolymph was increased significantly due to the additional supplementation of Methionine. They also recorded higher larval weight, cocoon weight, fecundity, shell ratio, filament length, shell weight and fecundity at 0.5 per cent of Methionine supplementation as against the absolute control.

Ghoreyshi *et al.* (2019) reported that as per nutritional and nutraceutical interest, Lysine and Methionine are essential amino acids and these are used as dietary supplements to enhance the feed characteristics in broiler chicken while analyzing the effect of supplementation of limiting amino acids on growth of broilers.

Borah and Boro (2020) demonstrated that the protein is one of the essential materials of the body which can adjust and control the substance for metabolism and physiological function of the silkworms by combining active substances like enzymes, harmones etc. with other substances. It was further reported that adult female silkworm needs protein to mature their ovaries and eggs.

Shivkumar *et al.* (2020) reported that silkworm fed with amino acid treated mulberry leaves showed significant increase in terms of body weight, cocoon weight, shell weight and cocoon yield as compared with control and included fortification as recent techniques in order to enhance the cocoon yield and silk content.

Chapter-3

MATERIALS AND METHODS

The silkworm rearing was conducted at Division of Sericulture Crop Improvement in College of Temperate Sericulture, Mirgund and biochemical analysis was carried out at Proteomics Laboratory, Division of Biotechnology, SKUAST-K, Shalimar, during spring (May-June) in the year 2020.

3.1 Mulberry variety

Mulberry varieties namely Ichinose (for chawki rearing) and Goshoerami (for late age rearing) available at the mulberry farm of College of Temperate Sericulture, Mirgund were utilized for the present investigation.

3.2 Silkworm race

The disease free layings of silkworm breed APS_4, were incubated, brushed and reared by following the recommended package of practices (Anonymous, 2003). For this purpose mass rearing of three disease free layings of APS_4 bivoltine silkworm breed was done up to 3^{rd} instar by feeding Ichinose mulberry leaf without any treatment. Just after 3^{rd} moult, three replications containing 100 worms were maintained for each treatment as well as for control batches.

3.3 Preparation of amino acid solution

The 0.5%, 1.0% and 1.5% concentrations were prepared by dissolving 0.5 grams, 1.0 grams and 1.5 grams of fine powder of amino acids *viz*. Methionine (HiMedia Laboratories Pvt. Ltd. Mumbai, Maharashtra), Tryptophan (HiMedia Laboratories Pvt. Ltd. Mumbai, Maharashtra) and Lysine (HiMedia Laboratories Pvt. Ltd. Mumbai, Maharashtra) in 100 ml of distilled water by (W/V).

3.4 Fortification of mulberry leaf

For the present study three amino acids *viz*. Methionine, Tryptophan and Lysine with three different concentrations i.e., 0.5%, 1.0% and 1.5% were

5^{th} instar larvae of silkworm race APS_4

Cocoons of silkworm race APS_4 harvested during study

Plate 1: Larvae and cocoons of silkworm race APS_4 used for the research trial

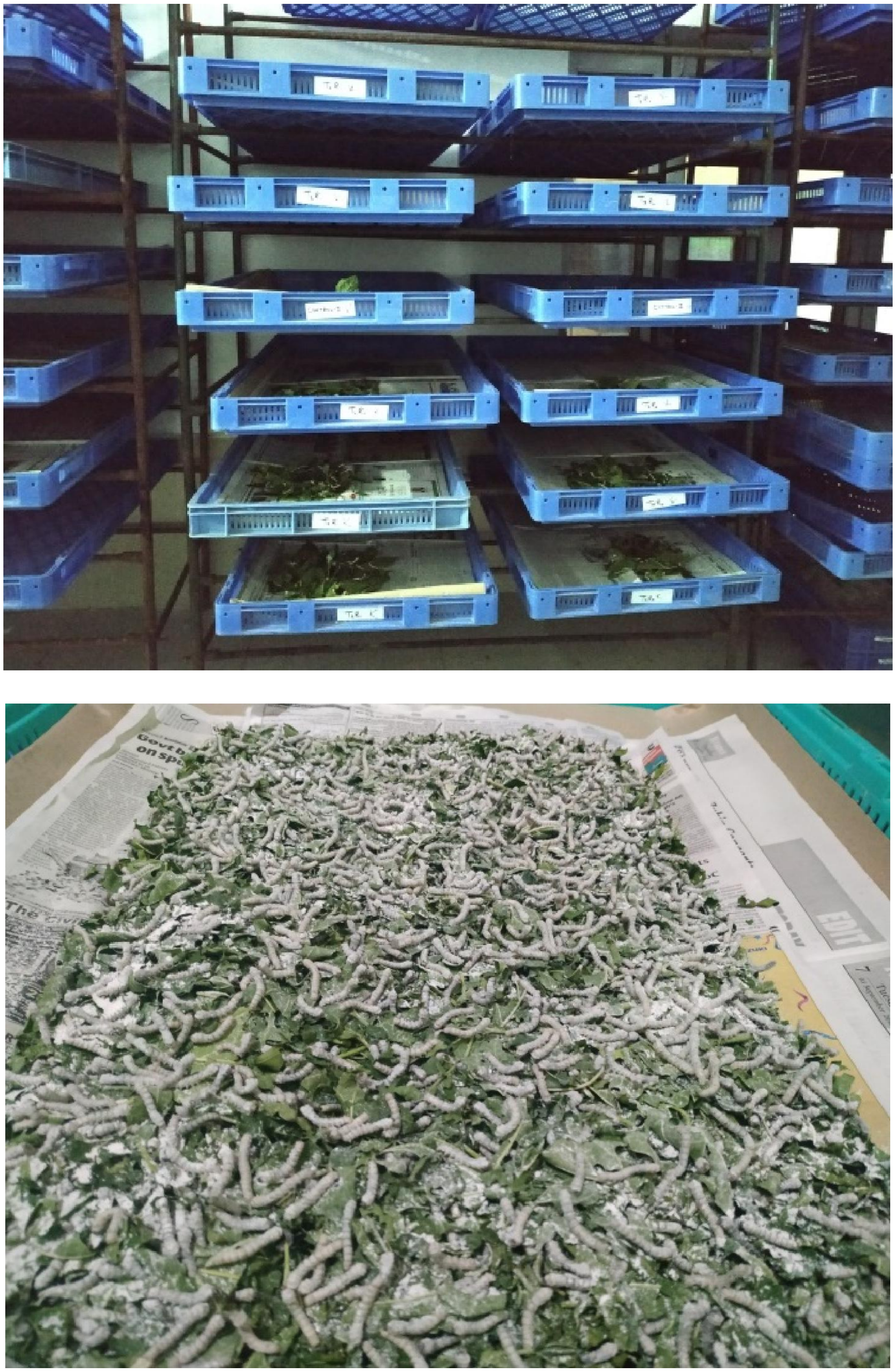


Plate 2: Silkworm rearing at research site

selected. After 3rd moult, these amino acid solutions were sprayed on fresh mulberry leaves with the help of an automizer and the treated leaves were air dried for 10-15 minutes before feeding them to the silkworm, *Bombyx mori* L. daily once as first feed during 4th and 5th instar.

3.5 Technical programme

The silkworm rearing was conducted by following the recommended package of practices (Anonymous 2003). For this purpose mass rearing of silkworms was done up to 3rd instar by feeding mulberry leaf without any treatment. Just after 3rd moult, three replications containing 100 worms for each treatment was maintained and fed with mulberry leaf fortified with different concentrations of amino acids *viz*. 0.5%, 1.0% and 1.5% @100 ml/200gm leaf for 100 larvae daily once as first feed during 4th and 5th instar. Two controls *viz*. control-I (worms fed with mulberry leaves treated with distilled water) and control-II (worms fed with mulberry leaves without any treatment) were maintained for comparisons. The experiment was accomplished using a Completely Randomized Design with 3 replications for each treatment.

The experimental details of the study are as under:

3.5.1 Experimental Layout

Target crop	:	Silkworm
Silkworm breed	:	APS_4
No. of treatments (amino acids)	:	03

- A_1 : Methionine
- A_2 : Tryptophan
- A_3 : Lysine

Weighing of amino acids

Preparation of stock solution of amino acids in double distilled water

Plate 3: Preparation of different concentrations of amino acids

Spraying of amino acids on mulberry leaves

Feeding of amino acid treated mulberry leaves to silkworms

Plate 4: **Spraying and feeding of amino acid fortified mulberry leaves to silkworms**

No. of chemical concentrations : 03

- C_1 : 0.5%
- C_2 : 1.0%
- C_3 : 1.5%

Control treatment

- Control-I : T10 (distilled water)
- Control-II : T11 (without any treatment)

Total No. of treatment combinations **:** 11

3.5.2 Treatment combination details:

Code	**Symbol**	**Details**
T1	A_1C_1	0.5% Methionine
T2	A_1C_2	1.0% Methionine
T3	A_1C_3	1.5% Methionine
T4	A_2C_1	0.5% Tryptophan
T5	A_2C_2	1.0% Tryptophan
T6	A_2C_3	1.5% Tryptophan
T7	A_3C_1	0.5% Lysine
T8	A_3C_2	1.0% Lysine
T9	A_3C_3	1.5% Lysine
T10	Control-I	Distilled water
T11	Control-II	Normal leaf without any treatment

No. of silkworms per replication	:	100
Stage of treatment	:	4^{th} & 5^{th} instar
Design of experiment	:	CRD (Completely Randomized Design)

3.6 Parameters recorded

The various cocoons and post cocoon parameters which were recorded during this study are as under:

1. Fifth age larval duration (hours):

The 5^{th} age larval duration was calculated by recording the time in terms of total number of days taken from the first day of 5^{th} instar till the commencement of spinning.

2. Total larval duration (hours):

The total larval duration was calculated by recording the total number of days taken from date of brushing to mounting.

3. Weight of 10 mature larvae (g):

The weight of ten mature larvae was recorded on 5^{th} day of 5^{th} instar by randomly taking 10 silkworm larvae from each replication and weighed separately on digital balance.

4. Silk gland weight (g):

Ten larvae were randomly collected from each replication and were dissected in order to take silk gland. The weight of silk gland was taken by using the formulae:

$$\text{Silk gland weight} = \frac{\text{Total weight of silk gland}}{\text{No. of larvae taken for dissection}}$$

Dissecting silkworm larva

Dissected out silk gland

Plate 5: Dissection of silkworm to obtain silk gland from 5th instar larva

5.

5. Silk gland somatic index (%):

Silk gland somatic index is the ratio between the weight of silk gland and larval body weight in grams. Silk gland somatic index was calculated as:

$$\text{Silk gland somatic index} = \frac{\text{Silk gland weight (g)}}{\text{Mature larval weight (g)}} \times 100$$

6. Cocoon yield/10,000 larvae by number:

The number of larvae retained and the resultant number of cocoons harvested from a standard unit of 10,000 larvae was calculated by using the following formula:

$$\text{Cocoon yield by number} = \frac{\text{Number of cocoons harvested}}{\text{No. of worms retained after } 3^{rd} \text{ moult-worms taken for dissection}} \times 10{,}000$$

7. Cocoon yield/10,000 larvae by weight (kg) :

The quantum of viable cocoons as expressed in kilograms procured from a standard unit of 10,000 larvae was calculated as:

$$\text{Cocoon yield by weight} = \frac{\text{Weight of cocoons harvested}}{\text{No. of worms retained after } 3^{rd} \text{ moult-worms taken for dissection}} \times 10{,}000$$

8. Silk conversion index (%):

The silk conversion index (SCI) is the ratio of shell weight to silk gland weight and was calculated as:

$$\text{Silk conversion index} = \frac{\text{Shell weight (g)}}{\text{Silk gland weight (g)}} \times 100$$

9. Silk productivity (cg/day):

The silk productivity (cg/day) gives the quantity of silk synthesized per day and was calculated by using the formula of Malik and Reddy (2009).

10. Single cocoon weight (g):

In order to calculate single cocoon weight, 10 male and 10 female cocoons were randomly selected from each replication of each treatment and weighed on digital balance to determine average cocoon weight.

11. Single shell weight (g):

In order to calculate single shell weight, 10 male and 10 female cocoons were taken randomly from each replication and cut open to take their shells. These 20 cocoon shells were weighed on a digital balance in order to calculate average single cocoon shell weight.

12. Shell percentage:

Shell percentage was calculated by using the formula:

$$\text{Shell percentage} = \frac{\text{Single cocoon shell weight (g)}}{\text{Single cocoon weight (g)}} \times 100$$

13. Average filament length (m):

Average filament length was calculated by selecting 10 cocoons from each replication which was simultaneously reeled to get total length of raw silk spun by the larvae.

14. Filament size (denier):

It was calculated as the weight of 9,000 meters of raw silk in grams by using the following formula:

$$\text{Filament size (denier)} = \frac{\text{Conditioned weight of raw silk (g)}}{\text{Total length of raw silk (m)}} \times 9{,}000$$

15. Raw silk percentage:

Raw silk percentage was calculated for the same cocoons used in filament length by using the following formula:

$$\text{Raw silk percentage} = \frac{\text{Weight of silk reeled (g)}}{\text{Weight of green cocoons}} \times 100$$

16. **Fecundity:**

Fecundity was calculated by counting the number of eggs laid by a particular female moth.

3.6.1 Biochemical analysis of silkworm tissues

1. Estimation of total protein

The quantitative estimation of protein in silk gland was carried out by Lowry *et al.* (1951) using crystalline bovine serum albumin (BSA) as standard and the values were expressed in terms of mg of protein per gram of silk gland or per ml of haemolymph.

Solutions used:

- **Preparation of standard:**

Bovine serum albumin 1mg/ml was prepared in distilled water which acted as a stock solution. Then working solution was prepared by dissolving 10ml of this stock solution in 100ml of distilled water.

- **Preparation of protein reagent:**

Solution A: (alkaline solution)

For 500 ml mix 2.8598g of NaOH and 14.3084g of Na_2CO_3

Solution B:

For 100 ml take 1.4232g $CuSO_4.5H_2O$

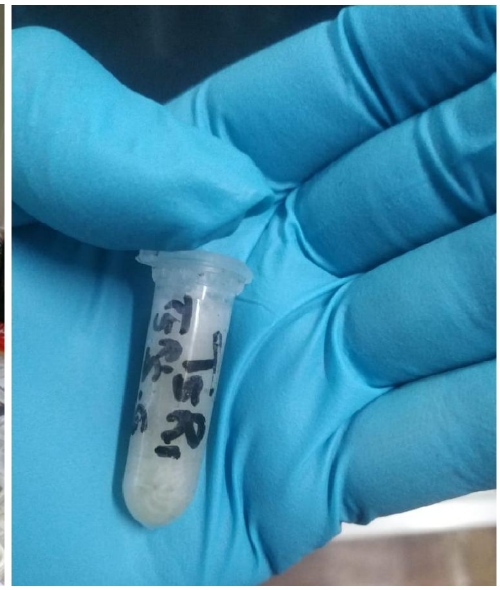
Solution C:

For 100 ml take 2.85299g of potassium sodium tartarate dihydrate.

Lowry solution:

It needs to be prepared fresh. For this solution A + solution B + solution C should be in the ratio (v: v) of 100:1:1.

Silk gland collection



Collected silk gland

Haemolymph collection

Collected haemolymph

Plate 6: Collection of samples for biochemical analysis

Folin reagent:

For this 5 ml of 2N folin and ciocalteu's phenol reagent is diluted in 6 ml of double distilled water.

This solution is light sensitive. So it should be prepared at the last 5 min. of the first sample incubation and should be kept in dark container. The dilution ratio for the folin and ciocalteu's phenol reagent is 1:1, resulting in a 1N folin reagent.

- **Total protein estimation in sample:**

0.1 ml of sample is diluted in 0.9 ml double distilled water. Then 5.0 ml of protein reagent was added to it and incubated for 15 minutes at room temperature. Then 0.5ml of diluted folin's reagent was added with thorough mixing and the mixture was allowed to stand for 30 minutes at room temperature for the colour development. Absorbency was measured at 660 nm using UV/VIS spectrophotometer. The quantity of proteins was calculated by plotting a standard curve by taking different concentrations of BSA as a standard (standard curve is prepared by taking different concentrations from working solution of standard BSA in the range from 0-100 micro-litre). The results were expressed in mg of protein per gram of silk gland or per ml of haemolymph.

2. Estimation of total free amino acid:

Total free amino acid content was estimated by Moore and Stein (1968) using Leucine as standard.

Solutions used:

- **Preparation of standard:**

Leucine was used as standard and was prepared by dissolving 0.05g of Leucine in 50ml of distilled water which acted as a stock solution. Then working solution was prepared by dissolving 10ml of this stock solution in 100ml of distilled water.

- **Preparation of reagent and diluent:**

A) **Ninhydrin reagent:** 0.8g of stannous chloride was dissolved in 500ml of 0.2M citrate buffer. Then to this solution 20 g ninhydrin in 500ml of methyl cellosolve was added. This is light sensitive so it should be prepared fresh and stored in brown bottle.

B) **Diluent:** The diluent was prepared by dissolving n-propanol and water in the ratio of 1:1.

- **Total free amino acid estimation in sample:**

The silk gland and haemolymph both were centrifuged at 12,000 rpm at $4^{0}C$ for 10 minutes. The silk gland was first de-proteinised with 10% TCA using liquid nitrogen and then centrifuged. Then to 0.1ml of supernatant of these samples, 1 ml of ninhydrin solution was added. The tubes with the contents were placed in boiling water bath for 20 minutes and cooled to room temperature. Finally, 5 ml of diluent was added to the contents and mixed. The purple colour developed was read at 570 nm against the reagent blank using UV/VIS spectrophotometer. The quantity of proteins was calculated by plotting a standard curve by taking different concentrations of working solution of Leucine *viz*. 20-100 micro-litre. The values were expressed in mg of free amino acids per ml of haemolymph or per gram of silk gland.

Plate 7: **Performing biochemical analysis of silkworm tissues at Proteomics Laboratory, Division of Biotechnology, SKUAST-K Shalimar**

Chapter-4

EXPERIMENTAL FINDINGS

The present chapter describes the experimental findings of the study entitled "Influence of Mulberry Leaf Fortified with Limiting Amino Acids on Biochemical Constituents and Economic Traits of Silkworm, *Bombyx mori* L.". The observations recorded during the study are presented in tables (1-20):

4.1 5th instar larval duration (hours):

A significant difference (at $p \leq 0.05$) was observed in 5th instar larval duration among different treatments and concentrations. The significantly shortest 5th instar larval duration of 150 hours was recorded in silkworm batches fed with Methionine supplemented leaves, which was followed by Lysine (158 hours) and Tryptophan (164 hours). However, among the concentrations of amino acids the significantly shortest 5th instar larval duration was recorded at 0.5% concentration (150 hours) followed by the concentrations, 1.0% (157 hours) and 1.5% (164 hours).

Among the 9 treatment combinations, the interaction effect of amino acid supplemented mulberry leaf with respect to control treatments showed a significantly shortest 5th instar larval duration of 147 hours in silkworm batches fed with Methionine at 0.5% concentration and longest 5th instar larval duration of 171 hours was recorded in Tryptophan at 1.5% concentration which was again significantly shorter to that of control-I (177 hours) and control-II (177 hours). (Table-1)

Table 1: Effect of amino acid supplemented mulberry leaf on 5th instar larval duration (hours) of silkworm, *Bombyx mori* L.

Concentration / Treatment	C1: 0.5%	C2: 1.0%	C3:1.5%	Mean
A1: Methionine	147	151	153	**150[a]**
A2: Tryptophan	153	169	171	**164[c]**
A3: Lysine	151	153	170	**158[b]**
Control-I	-	-	-	**177**
Control-II	-	-	-	**177**
Mean	**150[a]**	**157[b]**	**164[c]**	

*Means with different superscripts in rows and column are significantly different from each other

C.D ($p \leq 0.05$)

Amino Acid (A)	:	0.576
Concentration (C)	:	0.576
A×C	:	0.998
Control Vs. Rest	:	0.041

4.2 Total larval duration (hours):

A significant difference (at $p \leq 0.05$) was observed in total larval duration among different treatments and concentrations. The significantly shortest larval duration of 630 hours was recorded in silkworm batches fed with Methionine supplemented leaves, which was followed by Lysine (638 hours) and Tryptophan (644 hours). However, among the concentrations of amino acids the significantly shortest larval duration was recorded at 0.5% concentration (630 hours) followed by the concentrations, 1.0% (637 hours) and 1.5% (644 hours).

Among the 9 treatment combinations, the interaction effect of amino acid supplemented mulberry leaf with respect to control treatments showed a significantly shortest larval duration of 627 hours in silkworm batches fed with Methionine at 0.5% concentration and longest larval duration of 651 hours was recorded in Tryptophan at 1.5% concentration which was again significantly shorter than that of control-I (657 hours) and control-II (657 hours). (Table-2)

4.3 Weight of 10 mature larvae (g):

The larval weight showed significant difference (at $p \leq 0.05$) among different treatments and concentrations. The significantly higher larval weight of 52.18 grams was recorded in silkworm batches fed with Methionine supplemented leaves, followed by Lysine (51.34 grams) and Tryptophan (49.78 grams). However, among the concentrations of amino acids 0.5% concentration showed significantly higher larval weight of 52.67 grams followed by concentrations of 1.0% (50.91 grams) and 1.5% (49.73 grams).

Among the 9 treatment combinations, the interaction effect of amino acid supplemented mulberry leaf with respect to control treatments showed a significantly higher larval weight of 54.03 grams in silkworm batches fed with Methionine at 0.5% concentration, which was followed by 53.24 grams at 0.5% lysine and the lowest larval weight of 49.26 grams was recorded in Tryptophan at 1.5% concentration which was again significantly superior than that of control-I (47.83 grams) and control-II (47.33 grams). (Table-3)

Table 2: Effect of amino acid supplemented mulberry leaf on total larval duration (hours) of silkworm, *Bombyx mori* L.

Treatment \ Concentration	C1: 0.5%	C2: 1.0%	C3:1.5%	Mean
A1: Methionine	627	631	633	**630[a]**
A2: Tryptophan	633	649	651	**644[c]**
A3: Lysine	631	633	650	**638[b]**
Control-I	-	-	-	**657**
Control-II	-	-	-	**657**
Mean	**630[a]**	**637[b]**	**644[c]**	

*Means with different superscripts in column and rows are significantly different from each other

C.D ($p \leq 0.05$)

Amino Acid (A)	:	0.576
Concentration (C)	:	0.576
A×C	:	0.998
Control Vs. Rest	:	1.923

Table 3: Effect of amino acid supplemented mulberry leaf on weight of 10 mature larvae (g) of silkworm, *Bombyx mori* L.

Treatment \ Concentration	C1: 0.5%	C2: 1.0%	C3:1.5%	Mean
A1: Methionine	54.03	52.04	50.47	**52.18[a]**
A2: Tryptophan	50.75	49.35	49.26	**49.78[c]**
A3: Lysine	53.24	51.33	49.46	**51.34[b]**
Control-I	-	-	-	**47.83**
Control-II	-	-	-	**47.33**
Mean	**52.67[a]**	**50.91[b]**	**49.73[c]**	

*Means with different superscripts in column and rows are significantly different from each other

C.D ($p \leq 0.05$)

Amino Acid (A)	:	0.827
Concentration (C)	:	0.827
A×C	:	0.662
Control Vs. Rest	:	1.282

4.4 Silk gland weight (g):

The silk gland weight showed significant difference (at $p \leq 0.05$) among different treatments and concentrations. The significantly higher silk gland weight of 1.81 grams was recorded in silkworm batches fed with Methionine supplemented leaves, which was followed by Lysine and Tryptophan with silk gland weight of 1.72 grams and 1.64 grams, respectively. However, among the concentrations of amino acids 0.5% concentration showed significantly higher silk gland weight of 1.87 grams followed by concentrations of 1.0% (1.73 grams) and 1.5% (1.57 grams).

The statistical analysis of the data revealed that among the 9 treatment combinations, the interaction effect of amino acid supplemented mulberry leaf with respect to control treatments showed a significantly higher silk gland weight of 1.97 grams in silkworm batches fed with Methionine at 0.5% concentration, which was followed by 1.87 grams at 0.5% Lysine and lowest silk gland weight of 1.50 grams was recorded in Tryptophan at 1.5% concentration which was again significantly superior than that of control-I (1.42 grams) and control-II (1.39 grams). (Table-4)

4.5 Silk gland somatic index (%):

A significant difference (at $p \leq 0.05$) was observed in silk gland somatic index among different treatments and concentrations. The significantly higher silk gland somatic index of 34.55 per cent was recorded in silkworm batches fed with Methionine supplemented leaves, followed by Lysine (33.63 per cent) and Tryptophan (33.08 per cent). However, among the concentrations of amino acids 0.5% concentration showed significantly higher silk gland somatic index of 35.59 per cent followed by 1.0% and 1.5% with silk gland somatic index of 34.03 and 31.63 per cent, respectively.

The statistical analysis of the data revealed that among the 9 treatment combinations, the interaction effect of amino acid supplemented mulberry leaf

Table 4: Effect of amino acid supplemented mulberry leaf on silk gland weight (g) of silkworm, *Bombyx mori* L.

Treatment \ Concentration	C1: 0.5%	C2: 1.0%	C3:1.5%	Mean
A1: Methionine	1.97	1.81	1.63	**1.81[a]**
A2: Tryptophan	1.78	1.64	1.50	**1.64[c]**
A3: Lysine	1.87	1.74	1.57	**1.72[b]**
Control-I	-	-	-	**1.42**
Control-II	-	-	-	**1.39**
Mean	**1.87[a]**	**1.73[b]**	**1.57[c]**	

*Means with different superscripts in column and rows are significantly different from each other

C.D ($p \leq 0.05$)

Amino Acid (A)	:	0.027
Concentration (C)	:	0.027
A×C	:	0.011
Control Vs. Rest	:	0.054

Table 5: Effect of amino acid supplemented mulberry leaf on silk gland somatic index (%) of silkworm, *Bombyx mori* L.

Concentration / Treatment	C1: 0.5%	C2: 1.0%	C3:1.5%	Mean
A1: Methionine	36.53	34.84	32.29	**34.55[a]**
A2: Tryptophan	35.07	33.37	30.79	**33.08[c]**
A3: Lysine	35.18	33.89	31.81	**33.63[b]**
Control-I	-	-	-	**30.00**
Control-II	-	-	-	**29.06**
Mean	**35.59[a]**	**34.03[b]**	**31.63[c]**	

*Means with different superscripts in column and rows are significantly different from each other

C.D ($p \leq 0.05$)

Amino Acid (A)	:	0.630
Concentration (C)	:	0.630
A×C	:	0.234
Control Vs. Rest	:	1.001

with respect to control treatments showed a significantly higher silk gland somatic index of 36.53 per cent in silkworm batches fed with Methionine at 0.5% concentration, which was followed by 35.18 per cent at 0.5% Lysine and the lowest silk gland somatic index of 30.79 per cent was recorded in Tryptophan at 1.5% concentration which was again significantly superior than that of the control-I (30.00 per cent) and control-II (29.06 per cent). (Table-5)

4.6 Cocoon yield/10,000 larvae by number:

A significant difference (at $p \leq 0.05$) was observed in cocoon yield/10,000 larvae by number among different treatments and concentrations. The significantly higher cocoon yield by number of 9501 was recorded in silkworm batches fed with Methionine supplemented leaves, followed by Lysine (9305) and Tryptophan (9208). However, among the concentrations of amino acids 0.5% concentration (9520) showed significantly higher cocoon yield by number followed by concentrations, 1.0% (9367) and 1.5% (9127).

The statistical analysis of the data revealed that among the 9 treatment combinations, the interaction effect of amino acid supplemented mulberry leaf with respect to control treatments showed a significantly highest cocoon yield/10,000 larvae by number in silkworm batches fed with Methionine at 0.5% concentration (9635), which was followed by Methionine at 1.0% concentration (9539) and lowest cocoon yield by number of 8942 was recorded at 1.5% concentration of Tryptophan which was again significantly superior than that of control-I (8679) and control-II (8673). (Table-6)

4.7 Cocoon yield/10,000 larvae by weight (kg):

Among the different treatments and concentrations there was a significant difference (at $p \leq 0.05$) in cocoon yield/10,000 larvae by weight. The significantly higher cocoon yield by weight of 17.29 kg was observed in silkworm batches fed with Methionine supplemented leaves, which was followed by Lysine (16.72 kg) and Tryptophan (15.95 kg). However, among the concentrations of amino acids

0.5% concentration (18.05 kg) showed significantly higher cocoon yield by weight followed by concentrations, 1.0% (16.79 kg) and 1.5% (15.12 kg).

The statistical analysis of the data revealed that among the 9 treatment combinations, the interaction effect of amino acid supplemented mulberry leaf with respect to control treatments showed a significantly highest cocoon yield by weight of 18.78 kg in silkworm batches fed with Methionine at 0.5% concentration, which was followed by 18.11 kg at 0.5% Lysine and lowest cocoon yield by weight of 14.38 kg was recorded in Tryptophan at 1.5% concentration which was again significantly superior than that of control-I (13.89 kg) and control-II (13.77 kg). (Table-7)

4.8 Silk conversion index (%):

There is a significant difference (at $p \leq 0.05$) among different treatments and concentrations in silk conversion index. The significantly higher silk conversion index of 23.81 per cent was recorded in silkworm batches fed with Methionine supplemented leaves, which was statistically at par with Lysine (23.45 per cent) and Lysine was immediately followed by Tryptophan (22.62 per cent). However, among the concentrations of amino acids, 0.5% concentration (23.86 per cent) showed significantly higher silk conversion index which was followed by 1.0% (22.96 per cent) which was statistically at par with the 1.5% (23.05 per cent).

The statistical analysis revealed that among the 9 treatment combinations, the interaction effect of amino acid supplemented mulberry leaf with respect to control treatments recorded a significantly higher silk conversion index of 24.53 per cent in silkworm batches fed with Methionine at 0.5% concentration, which was significantly superior than that of control-I (22.86 per cent) and control-II (22.83 per cent). (Table-8)

Table 6: Effect of amino acid supplemented mulberry leaf on cocoon yield/10,000 larvae by number of silkworm, *Bombyx mori* L.

Concentration / Treatment	C1: 0.5%	C2: 1.0%	C3:1.5%	Mean
A1: Methionine	9635	9539	9329	**9501[a]**
A2: Tryptophan	9440	9242	8942	**9208[c]**
A3: Lysine	9484	9320	9112	**9305[b]**
Control-I	-	-	-	**8679**
Control-II	-	-	-	**8673**
Mean	**9520[a]**	**9367[b]**	**9127[c]**	

*Means with different superscripts in column and rows are significantly different from each other

C.D ($p \leq 0.05$)

Amino Acid (A)	:	29.632
Concentration (C)	:	29.632
A×C	:	51.324
Control Vs. Rest	:	54.486

Table 7: Effect of amino acid supplemented mulberry leaf on cocoon yield/10,000 larvae by weight (kg) of silkworm, *Bombyx mori* L.

Treatment \ Concentration	C1: 0.5%	C2: 1.0%	C3:1.5%	Mean
A1: Methionine	18.78	17.38	15.71	**17.29^{a}**
A2: Tryptophan	17.27	16.22	14.38	**15.95^{c}**
A3: Lysine	18.11	16.77	15.29	**16.72^{b}**
Control-I	-	-	-	**13.89**
Control-II	-	-	-	**13.77**
Mean	**18.05^{a}**	**16.79^{b}**	**15.12^{c}**	

*Means with different superscripts in column and rows are significantly different from each other

C.D ($p \leq 0.05$)

Amino Acid (A)	:	0.163
Concentration (C)	:	0.163
A×C	:	0.112
Control Vs. Rest	:	0.256

Table 8: **Effect of amino acid supplemented mulberry leaf on silk conversion index (%) of silkworm, *Bombyx mori* L.**

Treatment \ Concentration	C1: 0.5%	C2: 1.0%	C3:1.5%	Mean
A1: Methionine	24.53	23.42	23.46	**23.81[a]**
A2: Tryptophan	23.18	22.12	22.54	**22.62[b]**
A3: Lysine	23.88	23.35	23.14	**23.45[a]**
Control-I	-	-	-	**22.86**
Control-II	-	-	-	**22.83**
Mean	**23.86[a]**	**22.96[b]**	**23.05[b]**	

*Means with different superscripts in column and rows are significantly different from each other

C.D ($p \leq 0.05$)

Amino Acid (A)	:	0.510
Concentration (C)	:	0.510
A×C	:	NS
Control Vs. Rest	:	0.827

4.9 Silk productivity (cg/day):

Among the different treatments and concentrations a significant difference (at $p \leq 0.05$) was observed in silk productivity. The significantly higher silk productivity of 6.87 cg was recorded in silkworm batches fed with Methionine supplemented leaves, followed by Lysine (6.20 cg) and Tryptophan (5.46 cg). However, among the concentrations of amino acids 0.5% concentration (7.16 cg) showed significantly higher silk productivity followed by concentrations, 1.0% (6.09 cg) and 1.5% (5.28 cg).

The statistical analysis of the data represented that among the 9 treatment combinations, the interaction effect of amino acid supplemented mulberry leaf with respect to control treatments showed a significantly higher silk productivity of 7.92 cg in silkworm batches fed with Methionine at 0.5% concentration, which was followed by 7.10 cg at 0.5% Lysine and the lowest silk productivity of 4.77 cg was recorded in Tryptophan at 1.5% concentration which was again significantly superior than that of control-I (4.41 cg) and control-II (4.30 cg). (Table-9)

4.10 Single cocoon weight (g):

A significant difference (at $p \leq 0.05$) was observed in single cocoon weight among different treatments and concentrations. The significantly higher single cocoon weight of 1.811 grams was recorded in silkworm batches fed with Methionine supplemented leaves, followed by Lysine (1.787 grams) and Tryptophan (1.707 grams). However, among the concentrations of amino acids 0.5% concentration (1.891 grams) showed significantly higher single cocoon weight followed by the concentrations, 1.0% (1.761 grams) and 1.5% (1.654 grams), respectively.

Among the 9 treatment combinations, the interaction effect of amino acid supplemented mulberry leaf with respect to control treatments showed a significantly higher single cocoon weight of 1.940 grams in silkworm batches fed with Methionine at 0.5% concentration, which was followed by 1.906 grams at 0.5% Lysine and the lowest single cocoon weight of 1.603 grams was recorded in Tryptophan at 1.5% concentration which was again significantly superior than that of control-I (1.596 grams) and control-II (1.576 grams). (Table-10)

4.11 Single shell weight (g):

A significant difference (at $p \leq 0.05$) was observed in single shell weight among different treatments and concentrations. The significantly higher single shell weight of 0.428 grams was recorded in silkworm batches fed with Methionine supplemented leaves, which was immediately followed by Lysine (0.405 grams) and Tryptophan (0.372 grams). However, among the concentrations of amino acids the significantly higher single shell weight was recorded at 0.5% concentration (0.446 grams) followed by the concentrations, 1.0% (0.397 grams) and 1.5% (0.362 grams).

Among the 9 treatment combinations, the interaction effect of amino acid supplemented mulberry leaf with respect to control treatments showed a significantly higher single shell weight of 0.483 grams in silkworm batches fed with Methionine at 0.5% concentration, which was followed by 0.446 grams at 0.5% Lysine and lowest single shell weight of 0.343 grams was recorded in Tryptophan at 1.5% concentration which was again significantly superior than that of control-I (0.326 grams) and control-II (0.320 grams). (Table-11)

Table 9: Effect of amino acid supplemented mulberry leaf on silk productivity (cg/day) of silkworm, *Bombyx mori* L.

Treatment \ Concentration	C1: 0.5%	C2: 1.0%	C3:1.5%	Mean
A1: Methionine	7.92	6.75	5.95	**6.87[a]**
A2: Tryptophan	6.46	5.17	4.77	**5.46[c]**
A3: Lysine	7.10	6.37	5.14	**6.20[b]**
Control-I	-	-	-	**4.41**
Control-II	-	-	-	**4.30**
Mean	**7.16[a]**	**6.09[b]**	**5.28[c]**	

*Means with different superscripts in column and rows are significantly different from each other

C.D ($p \leq 0.05$)

Amino Acid (A)	:	0.006
Concentration (C)	:	0.006
A×C	:	0.010
Control Vs. Rest	:	0.011

Table 10: Effect of amino acid supplemented mulberry leaf on single cocoon weight (g) of silkworm, *Bombyx mori* L.

Concentration / Treatment	C1: 0.5%	C2: 1.0%	C3:1.5%	Mean
A1: Methionine	1.940	1.810	1.683	**1.811**[a]
A2: Tryptophan	1.826	1.693	1.603	**1.707**[c]
A3: Lysine	1.906	1.780	1.676	**1.787**[b]
Control-I	-	-	-	**1.596**
Control-II	-	-	-	**1.576**
Mean	**1.891**[a]	**1.761**[b]	**1.654**[c]	

*Means with different superscripts in column and rows are significantly different from each other

C.D ($p \leq 0.05$)

Amino Acid (A)	:	0.015
Concentration (C)	:	0.015
A×C	:	0.012
Control Vs. Rest	:	0.023

Table 11: Effect of amino acid supplemented mulberry leaf on single shell weight (g) of silkworm, *Bombyx mori* L.

Treatment \ Concentration	C1: 0.5%	C2: 1.0%	C3:1.5%	Mean
A1: Methionine	0.483	0.423	0.380	**0.428[a]**
A2: Tryptophan	0.410	0.363	0.343	**0.372[c]**
A3: Lysine	0.446	0.406	0.363	**0.405[b]**
Control-I	-	-	-	**0.326**
Control-II	-	-	-	**0.320**
Mean	**0.446[a]**	**0.397[b]**	**0.362[c]**	

*Means with different superscripts in column and rows are significantly different from each other

C.D ($p \leq 0.05$)

Amino Acid (A)	:	0.0052
Concentration (C)	:	0.0052
A×C	:	0.0104
Control Vs. Rest	:	0.0097

4.12 Shell percentage:

A significant difference (at $p \leq 0.05$) was observed in shell percentage among different treatments and concentrations. The significantly higher shell percentage of 23.66 per cent was recorded in silkworm batches fed with Methionine supplemented leaves, which was immediately followed by Lysine (22.68 per cent) and Tryptophan (21.76 per cent). However, among the concentrations of amino acids the significantly higher shell percentage was recorded at 0.5% concentration (23.69 per cent) followed by the concentrations, 1.0% (22.60 per cent) and 1.5% (21.81 per cent), respectively.

Among the 9 treatment combinations, the interaction effect of amino acid supplemented mulberry leaf with respect to control treatments showed a significantly higher shell percentage of 24.89 per cent in silkworm batches fed with Methionine at 0.5% concentration, which was followed by 23.50 per cent at 0.5% Lysine and lowest shell percentage of 21.18 per cent was recorded in Tryptophan at 1.5% concentration which was again significantly superior than that of control-I (20.31 per cent) and control-II (20.13 per cent). (Table-12)

4.13 Average filament length (m):

A significant difference (at $p \leq 0.05$) was observed in average filament length among different treatments and concentrations. The significantly higher average filament length of 1060 meters was recorded in silkworm batches fed with Methionine supplemented leaves, followed by Lysine (1028 meters) and Tryptophan (916 meters). However, among the concentrations of amino acids 0.5% concentration showed significantly higher average filament length of 1040 meters which was followed by 1.0% (999 meters) and 1.5% (965 meters).

The statistical analysis of the data revealed that among the 9 treatment combinations, the interaction effect of amino acid supplemented mulberry leaf with respect to control treatments showed a significantly higher average filament length of 1110 meters in silkworm batches fed with Methionine at 0.5%

concentration, which was followed by 1079 meters at 0.5% Lysine and lowest filament length of 900 meters was recorded in Tryptophan at 1.5% concentration which was again significantly superior than that of control-I (833 meters) and control-II (831 meters). (Table-13)

4.14 Filament size (denier):

There was no significant difference (at $p \leq 0.05$) in filament size (denier) among different treatments and concentrations. The thinnest filament size (denier) of 2.63 was recorded in silkworm batches fed with Methionine supplemented leaves, which was statistically at par with Lysine (2.66) and it was followed by Tryptophan with denier of 2.79. However, among the concentrations of amino acids there was no significant difference and all the concentrations were statistically at par with each other but 0.5% concentration showed relatively lower filament size (denier) of 2.65 which was followed by 1.0% (2.68) and 1.5% (2.74).

Among the 9 treatment combinations, the interaction effect of amino acid supplemented mulberry leaf with respect to control treatments showed the lowest filament size (denier) of 2.60 in silkworm batches fed with Methionine at 0.5% concentration, as against control-I (2.91) and control-II (2.98) which showed the highest filament size (denier). (Table-14)

Table 12: Effect of amino acid supplemented mulberry leaf on shell percentage of silkworm, *Bombyx mori* L.

Treatment \ Concentration	C1: 0.5%	C2: 1.0%	C3:1.5%	Mean
A1: Methionine	24.89	23.43	22.54	**23.66**^{a}
A2: Tryptophan	22.56	21.52	21.18	**21.76**^{c}
A3: Lysine	23.50	22.84	21.71	**22.68**^{b}
Control-I	-	-	-	**20.31**
Control-II	-	-	-	**20.13**
Mean	**23.69**^{a}	**22.60**^{b}	**21.81**^{c}	

*Means with different superscripts in column and rows are significantly different from each other

C.D ($p \leq 0.05$)

Amino Acid (A)	:	0.282
Concentration (C)	:	0.282
A×C	:	0.490
Control Vs. Rest	:	0.488

Table 13: Effect of amino acid supplemented mulberry leaf on average filament length (m) of silkworm, *Bombyx mori* L.

Treatment \ Concentration	C1: 0.5%	C2: 1.0%	C3:1.5%	Mean
A1: Methionine	1110	1044	1026	**1060[a]**
A2: Tryptophan	933	917	900	**916[c]**
A3: Lysine	1079	1037	970	**1028[b]**
Control-I	-	-	-	**833**
Control-II	-	-	-	**831**
Mean	**1040[a]**	**999[b]**	**965[c]**	

*Means with different superscripts in column and rows are significantly different from each other

C.D (p≤0.05)

Amino Acid (A)	:	17.300
Concentration (C)	:	17.300
A×C	:	29.964
Control Vs. Rest	:	26.799

Table 14: Effect of amino acid supplemented mulberry leaf on filament size (denier) of silkworm, *Bombyx mori* L.

Treatment \ Concentration	C1: 0.5%	C2: 1.0%	C3:1.5%	Mean
A1: Methionine	2.60	2.60	2.70	2.63[a]
A2: Tryptophan	2.74	2.81	2.82	2.79[b]
A3: Lysine	2.63	2.64	2.71	2.66[a]
Control-I	-	-	-	2.91
Control-II	-	-	-	2.98
Mean	2.65	2.68	2.74	

*Means with different superscripts in column are significantly different from each other

C.D ($p \leq 0.05$)

Amino Acid (A)	:	0.124
Concentration (C)	:	NS
A×C	:	NS
Control Vs. Rest	:	0.208

4.15 Raw silk percentage:

Among the treatments and concentration a significant difference (at $p \leq 0.05$) was observed in raw silk percentage**.** The significantly higher raw silk percentage of 16.72 per cent was recorded in silkworm batches fed with Methionine supplemented leaves, which was statistically at par with Lysine (16.58 per cent) and it was immediately followed by Tryptophan (15.76 per cent). However, among the concentrations of amino acids 0.5% concentration showed relatively higher raw silk percentage of 16.95 per cent which was statistically at par with 1.0% (16.37 per cent) and it was followed by 1.5% (15.74 per cent).

The statistical analysis of the data represented that among the 9 treatment combinations, the interaction effect of amino acid supplemented mulberry leaf with respect to control treatments showed a significantly higher raw silk percentage of 17.37 per cent in silkworm batches fed with Methionine at 0.5% concentration, followed by 17.03 per cent at 0.5% Lysine and lowest raw silk percentage of 15.04 per cent was recorded in Tryptophan at 1.5% concentration which was again significantly superior than that of control-I (14.62 per cent) and control-II (14.57 per cent). (Table-15)

4.16 Fecundity:

A significant difference (at $p \leq 0.05$) was observed in fecundity among different treatments and concentrations, where in silkworm batches fed with Tryptophan supplemented leaves showed significantly higher fecundity of 562, which was followed by Methionine (507) and Lysine (493). However, among the concentrations of amino acids 0.5% concentration showed significantly higher fecundity of 556 eggs per layings which was followed by concentrations, 1.0% (513) and 1.5% (494).

The statistical analysis of the data represented that among the 9 treatment combinations, the interaction effect of amino acid supplemented mulberry leaf with respect to control treatments showed a significantly higher fecundity in silkworm batches fed with Tryptophan at 0.5% concentration with 590 eggs per layings, which was followed by 560 eggs per layings at 1.0% Tryptophan and lowest fecundity of 464 was recorded in Lysine at 1.5% concentration, which was again significantly superior than that of control-I (437) and control-II (436). (Table-16)

4.17 Total protein content in haemolymph (mg/ml):

The significantly higher total protein content of 66.99 mg/ml was recorded in haemolymph collected from silkworm batches fed with Methionine supplemented leaves which was statistically significant (at $p \leq 0.05$) over the total protein content recorded by all other treatments. It was followed by Lysine (63.51 mg/ml) and Tryptophan (59.48 mg/ml). However, among the concentrations of amino acids 0.5% concentration showed significantly higher total protein content of 68.22 mg/ml in haemolymph followed by concentrations, 1.0% (62.42 mg/ml) and 1.5% (59.34 mg/ml).

The statistical analysis of the data represented that among 9 treatment combinations, the interaction effect of amino acid supplemented mulberry leaf with respect to control treatments showed a significantly higher total protein content of 70.59 mg/ml was recorded in haemolymph collected from silkworm batches fed with Methionine at 0.5% concentration, followed by 69.83 mg/ml at 0.5% Lysine and lowest total protein content of 55.45 mg/ml was recorded in Tryptophan at 1.5% concentration which was again significantly superior than that of control-I (50.14 mg/ml) and control-II (49.96 mg/ml). (Table-17)

Table 15: Effect of amino acid supplemented mulberry leaf on raw silk percentage (%) of silkworm, *Bombyx mori* L.

Concentration / Treatment	C1: 0.5%	C2: 1.0%	C3:1.5%	Mean
A1: Methionine	17.37	16.65	16.14	**16.72[a]**
A2: Tryptophan	16.44	15.80	15.04	**15.76[b]**
A3: Lysine	17.03	16.66	16.04	**16.58[a]**
Control-I	-	-	-	**14.62**
Control-II	-	-	-	**14.57**
Mean	**16.95[a]**	**16.37[a]**	**15.74[b]**	

*Means with different superscripts in column and rows are significantly different from each other

C.D ($p \leq 0.05$)

Amino Acid (A)	:	0.698
Concentration (C)	:	0.698
A×C	:	0.121
Control Vs. Rest	:	0.110

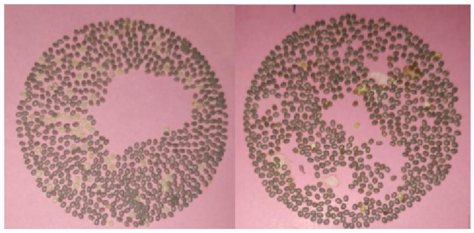
Table 16: Effect of amino acid supplemented mulberry leaf on fecundity of silkworm, *Bombyx mori* L.

Concentration / Treatment	C1: 0.5%	C2: 1.0%	C3:1.5%	Mean
A1: Methionine	545	495	482	**507**^{b}
A2: Tryptophan	590	560	537	**562**^{a}
A3: Lysine	533	483	464	**493**^{c}
Control-I	-	-	-	**437**
Control-II	-	-	-	**436**
Mean	**556**^{a}	**513**^{b}	**494**^{c}	

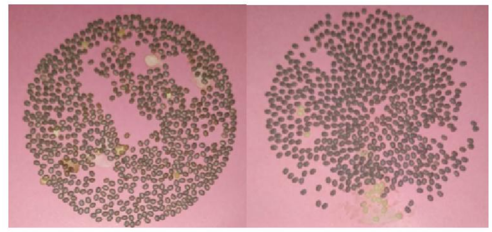
*Means with different superscripts in column and rows are significantly different from each other

C.D ($p \leq 0.05$)

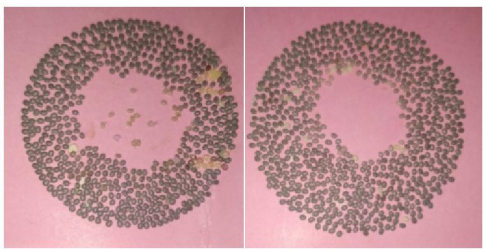
Amino Acid (A)	:	5.861
Concentration (C)	:	5.861
A×C	:	10.152
Control Vs. Rest	:	9.132



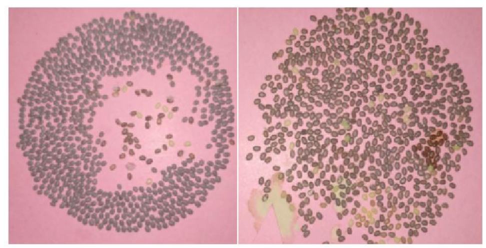
Eggs laid by silkworm batches treated with Methionine



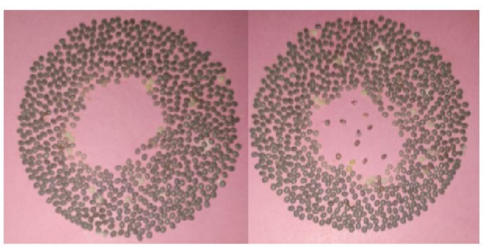
Eggs laid by silkworm batches treated with Control-I



Eggs laid by silkworm batches treated with Lysine



Eggs laid by silkworm batches treated with Control-II



Eggs laid by silkworm batches treated with Tryptophan

Plate 8: Egg layings from different treatments

Table 17: Effect of amino acid supplemented mulberry leaf on total protein content in haemolymph (mg/ml) of silkworm, *Bombyx mori* L.

Treatment \ Concentration	C1: 0.5%	C2: 1.0%	C3:1.5%	Mean
A1: Methionine	70.59	66.25	64.14	**66.99[a]**
A2: Tryptophan	64.25	58.74	55.45	**59.48[c]**
A3: Lysine	69.83	62.26	58.42	**63.51[b]**
Control-I	-	-	-	**50.14**
Control-II	-	-	-	**49.96**
Mean	**68.22[a]**	**62.42[b]**	**59.34[c]**	

*Means with different superscripts in column and rows are significantly different from each other

C.D ($p \leq 0.05$)

Amino Acid (A)	:	0.595
Concentration (C)	:	0.595
A×C	:	0.103
Control Vs. Rest	:	0.188

4.18 Total protein content in silk gland (mg/g):

The significantly higher total protein content of 185.23 mg/g was recorded in silk gland collected from silkworm batches fed with Methionine supplemented leaves which was statistically significant (at $p \leq 0.05$) over the total protein content recorded by all other treatments. It was followed by Lysine (178.05 mg/g) and Tryptophan (168.52 mg/g). However, among the concentrations of amino acids 0.5% concentration showed significantly higher total protein content of 186.77 mg/g in silk gland followed by concentrations, 1.0% (175.89 mg/g) and 1.5% (169.14 mg/g).

The statistical analysis of the data represented that among 9 treatment combinations, the interaction effect of amino acid supplemented mulberry leaf with respect to control treatments showed a significantly higher total protein content of 195.89 mg/g was recorded in silk gland collected from silkworm batches fed with Methionine at 0.5% concentration, which was followed by 188.75 mg/g at 0.5% Lysine and lowest total protein content of 160.46 mg/g was recorded in Tryptophan at 1.5% concentration which was again significantly superior than that of control-I (146.78 mg/g) and control-II (146.33 mg/g). (Table-18)

4.19 Total free amino acid content in haemolymph (mg/ml):

The significantly higher total free amino acid content of 23.73 mg/ml was recorded in haemolymph collected from silkworm batches fed with Methionine supplemented leaves which was statistically significant (at $p \leq 0.05$) over the total free amino acid content recorded by all other treatments. It was followed by Lysine (22.56 mg/ml) and Tryptophan (21.06 mg/ml). However, among the concentrations of amino acids 0.5% concentration showed significantly higher total free amino acid content of 24.35 mg/ml in haemolymph followed by concentrations of 1.0% (22.46 mg/ml) and 1.5% (20.54 mg/ml).

The statistical analysis of the data represented that among 9 treatment combinations, the interaction effect of amino acid supplemented mulberry leaf with respect to control treatments showed a significantly higher total free amino acid content of 25.86 mg/ml was recorded in haemolymph collected from silkworm batches fed with Methionine at 0.5% concentration, followed by 24.53 mg/ml at 0.5% Lysine and lowest total free amino acid content of 19.23 mg/ml was recorded in Tryptophan at 1.5% concentration which was again significantly superior than that of control-I (16.03 mg/ml)and control-II (15.90 mg/ml). (Table-19)

4.20 Total free amino acid content in silk gland (mg/g):

The significantly higher total free amino acid content of 148.81 mg/g was recorded in silk gland collected from silkworm batches fed with Methionine supplemented leaves which was statistically significant (at $p \leq 0.05$) over the total free amino acid content recorded by all other treatments. It was followed by Lysine (143.38 mg/g) and Tryptophan (134.98 mg/g). However, among the concentrations of amino acids 0.5% amino acid supplementation showed significantly higher total free amino acid content of 151.80 mg/g in silk gland followed by concentrations of 1.0% (143.44 mg/g) and 1.5% (131.94 mg/g).

The statistical analysis of the data represented that among 9 treatment combinations, the interaction effect of amino acid supplemented mulberry leaf with respect to control treatments showed a significantly higher total free amino acid content of 157.76 mg/g was recorded in silk gland collected from silkworm batches fed with Methionine at 0.5% concentration, followed by 152.43 mg/g at 0.5% Lysine and lowest total free amino acid content of 124.23 mg/g was recorded in Tryptophan at 1.5% concentration which was again significantly superior than that of control-I (118.73 mg/g) and control-II (118.46 mg/g). (Table-20)

Table 18: Effect of amino acid supplemented mulberry leaf on total protein content in silk gland (mg/g) of silkworm, *Bombyx mori* L.

Treatment \ Concentration	C1: 0.5%	C2: 1.0%	C3:1.5%	Mean
A1: Methionine	195.89	180.47	179.32	**185.23[a]**
A2: Tryptophan	175.67	169.44	160.46	**168.52[c]**
A3: Lysine	188.75	177.76	167.65	**178.05[b]**
Control-I	-	-	-	**146.78**
Control-II	-	-	-	**146.33**
Mean	**186.77[a]**	**175.89[b]**	**169.14[c]**	

*Means with different superscripts in column and rows are significantly different from each other

C.D ($p \leq 0.05$)

Amino Acid (A)	:	0.116
Concentration (C)	:	0.116
A×C	:	0.202
Control Vs. Rest	:	0.337

Table 19: Effect of amino acid supplemented mulberry leaf on total free amino acid content in haemolymph (mg/ml) of silkworm, *Bombyx mori* L.

Treatment \ Concentration	C1: 0.5%	C2: 1.0%	C3:1.5%	Mean
A1: Methionine	25.86	23.70	21.63	**23.73^{a}**
A2: Tryptophan	22.66	21.30	19.23	**21.06^{c}**
A3: Lysine	24.53	22.40	20.76	**22.56^{b}**
Control-I	-	-	-	**16.03**
Control-II	-	-	-	**15.90**
Mean	**24.35^{a}**	**22.46^{b}**	**20.54^{c}**	

*Means with different superscripts in column and rows are significantly different from each other

C.D ($p \leq 0.05$)

Amino Acid (A)	:	0.259
Concentration (C)	:	0.259
A×C	:	0.449
Control Vs. Rest	:	0.405

Table 20: Effect of amino acid supplemented mulberry leaf on total free amino acid content in silk gland (mg/g) of silkworm, *Bombyx mori* L.

Treatment \ Concentration	C1: 0.5%	C2: 1.0%	C3:1.5%	Mean
A1: Methionine	157.76	150.53	138.13	**148.81[a]**
A2: Tryptophan	145.20	135.53	124.23	**134.98[c]**
A3: Lysine	152.43	144.26	133.46	**143.38[b]**
Control-I	-	-	-	**118.73**
Control-II	-	-	-	**118.46**
Mean	**151.80[a]**	**143.44[b]**	**131.94[c]**	

*Means with different superscripts in column and rows are significantly different from each other

C.D ($p \leq 0.05$)

Amino Acid (A)	:	1.436
Concentration (C)	:	1.436
A×C	:	1.231
Control Vs. Rest	:	2.241

Chapter-5

DISCUSSION

A well-nourished silkworm is better equipped to face challenges of unfavourable environment and disease which often are a drag on its growth functions and cocoon production. A number of new/ traditional silkworm breeds/ hybrids underperformed in field conditions mainly due to impaired and/or insufficient larval nutrition. The unprecedented yield drop in commercial silkworm rearing is disconcerting. Despite domestication, silkworm gastronomics has not lost the character of its cousins in the wild. A number of foliar volatiles draw the silkworm towards its food source and initiate the feeding response. But, sustained feeding response is created only when organic nutrients and leaf moisture are present in desired quantity and form. Leaf moisture level is an index of the leaf palatability and digestibility of its organic nutrients. Proteins (20%), carbohydrates (7.5%) and lipids (2.5%) are the major organic nutrients. Foliar proteins have all the essential amino acids for normal silkworm growth and development.

Mulberry nutrition plays an important role in improving the growth and development of *Bombyx mori* L. It is stated that silk production is dependent on the larval nutrition and nutritive value of mulberry leaves, which plays a very effective role in producing good cocoons (Legay, 1958). Miyashita (1986) observed that leaf quality alone contributes to about 38.2% of success of cocoon crop. Significant seasonal variations occur in the nutritional value and composition of mulberry leaves depending on factors *viz*., weather, pests and disease as well as silkworm race (Ito, 1978).

Mulberry leaf protein was observed to contribute to 70% of silk protein (Fukuda *et al*., 1959) and hence considered as vital nutrient. Silkworms obtain 72-86% of their amino acids from mulberry leaves and more than 60% of the absorbed amino acids are used for silk production (Lu and Jiang, 1988). The digestible foliar proteins contain 82% useful amino acids, 49% essential amino acids and 33% nonessential amino acids, in order to meet the growth and other metabolic requirement of silkworm (Reddy, 2011). Proteins are made up of several different combinations of approximately 20 different amino acids. During the process of digestion, proteins are broken down into individual amino acids that are absorbed into the bloodstream. The amino acids are then incorporated into new protein molecules. If a diet is inadequate in any essential amino acid, protein synthesis cannot proceed beyond the rate at which that amino acid is available.

In the present study, out of the ten essential amino acids, three limiting amino acids of mulberry *viz*. Methionine, Tryptophan and Lysine in three different concentrations i.e., 0.5%, 1.0% and 1.5% were utilized for fortifying the mulberry leaves, to find out their impact on economic traits like rearing parameters, cocoon and post cocoon parameters and biochemical constituents of the silkworm tissues.

5.1 Impact of supplemented amino acids on commercial traits of silkworm, *Bombyx mori* L.

The present result revealed that there was a significant decrease in 5th instar and total larval duration in Methionine supplemented mulberry leaves (150, 630 hours) followed by Lysine (158, 638 hours) and Tryptophan (164, 644 hours) as against control-I (177, 657 hours) and control-II (177, 657 hours).The current results are in conformity with the findings of Laz (2010) who found that larval and pupal periods were significantly reduced in the parental and F_1 generations of silkworm by Methionine and Tryptophan supplemented leaves. Shivkumar *et al.* (2020) also reported that least larval duration of 25.01 days was recorded in Glutamic acid supplemented leaves at 0.1% concentration as against control 25.6 days. The duration of each larval stadium, though is predetermined, depends on the weight gain attained at the end of growth phase for the instar. Each instar is marked by a minimal and maximal limit of weight gain before passing into the next instar. The duration of the instar is prolonged if the minimal weight is not reached, and the duration is reduced when the maximal weight is reached sooner than completion of the duration of the instar. The changes in the duration of each instar occur within permissible limits of not exceeding a few hours and if mandatory minimal limits of larval growth are not reached at the end of the growth phase, supernumerary moults occur in some insects in order to provide for shortage of biomass accumulation before pupation occurs (Wigglesworth, 1972). Haniffa *et al*. (1994) observed extended larval duration in each instar when the number of feeds was reduced from 8 to 2. The difference in the larval duration with control and amino acid supplemented leaves could be attributed to the nutritional quality of the amino acid treated mulberry leaves. An increase in the larval duration suggests that the quality of the mulberry leaf is poor and does not support the required rate of growth during each instar of larval development. A number of investigators have reported different larval durations in same race in different seasons and with different mulberry varieties (Muthukrishnan and Delvi, 1974; Mathawan and Muthukrishnan, 1976; Haniffa and Periasamy, 1981).

It has also been found that amino acids supplemented mulberry leaf has significant effects on the larval weight with Methionine followed by Lysine and Tryptophan as compared to control-I and control-II. Among the concentrations, highest larval weight irrespective of amino acids was recorded at 0.5% followed by 1.0% and 1.5%, respectively. Similar results have been reported by Laz *et al*. (2010) in parental and F_1 generations of silkworm, *Bombyx mori* L. on Methionine and Tryptophan supplemented mulberry leaves. The present study clearly demonstrated that low concentration of Methionine, Lysine and Tryptophan supplemented food increased the performance of the economic characters in silkworm, *Bombyx mori* L. that are similar to the findings of Harpar *et al*. (1970) and Kade & Shepherd (1948) on other organisms. Kumar and Kumar (2018) also observed an increase in matured larval weight of silkworm breeds on Methionine fortified mulberry leaves. Chakraborty and Kaliwal (2012) also reported a significant increase in larval weight on amino acid treated groups.

Observations on the growth of silk gland revealed significantly higher silk gland weight of 1.81 g in silkworm batches fed with Methionine supplemented mulberry leaves, followed by Lysine (1.72 g) and Tryptophan (1.64 g) as against control-I (1.42 g) and control-II (1.39 g).This comparative increment in silk gland weight is in response to the elevated protein synthesis and enhancement of consumption of this amino acid resulting in this gain (Sarkar *et al*., 1995). Tazima (1978) reported that silk gland contributes 40% of the body mass of the larva. The high silk content of the bivoltine cocoon protects the pupae from very low ambient temperatures prevailing in temperate climates. Elkaraksy and Moustafa (1990) also found that addition of amino acids in low concentrations resulting an improvement in larval weight, silk gland weight, cocoon and shell weight.

The silk gland somatic index (SSI) represents the biomass of the silk gland in relation to total body weight. The values among all the treatments are relatively higher in Methionine than other two amino acids (Lysine and Tryptophan). The present result revealed maximum silk gland somatic index at 0.5% amino acid concentration resulted in higher growth rates for silk gland. The difference in the mean values of silk gland somatic index could be traced to the high silk gland somatic index values in all the three amino acid concentration of Methionine. The results of the silk gland somatic index which is the weight of the silk gland relative to body mass showed that the growth of the silk gland is increased by the stimulatory influence of amino acids to protein synthesis. Singh and Kumar (1996) reported 27.9% of silk gland somatic index in NB_{18}. The growth of silk gland is marked by the accumulation of organic compounds with particular reference to proteins (Ito and Arai, 1967; Tazima, 1978).

Cocoon yield/10,000 larvae by number and weight are correlated traits and in present study they were found higher among all the treatments irrespective of concentrations with significantly higher yield in silkworm batches fed with Methionine (9501, 17.29 kg), followed by Lysine (9305, 16.72 kg) and Tryptophan (9208, 15.95 kg) as compared to control-I (8679, 13.89 kg) and control-II (8673, 13.77 kg). Cocoon yield by weight in all the treatments also showed a significant difference with amino acid concentrations, the values being relatively higher at 0.5% in Methionine followed by Lysine and Tryptophan. The cocoon yield was significantly increased in the batches fed with Methionine supplemented mulberry leaves at 0.5%, 1.0% and 1.5% as against control batches, thus showing the influence of nutrition on the survivability and cocoon production. The role of nutrition in temperature tolerance is better manifested in bivoltine races and more significant in a high yielding and temperature sensitive race like CSR_2 with an ERR of 79.6% on M-5 and 90.77% and 92.26% on S-36 and V-1 mulberry varieties (Malik and Reddy, 2009). The cocoon yield and weight were higher in the batches fed with superior mulberry varieties like V-1 and S-36 compared to M-5, thus showing that both survivability and cocoon production greatly depend on the nutritional support (Reddy, 2011).

Silk conversion index is ratio of shell weight to silk gland weight (SCI). The silk conversion index is the percentage of the amount of silk fiber to the amount of silk in the lumen of the silk gland. The SCI values were significantly higher in Methionine and Lysine (23.81%, 23.45%) than control-I (22.86%), control-II (22.83%) and Tryptophan (22.62%). A qualitative difference in the silk stored in the lumen of the silk gland of all the amino acid treatment is apparent. The silk is biosynthesized and stored. Tryptophan and control batches appear to be associated with relatively more quantity of water which is expelled and dried when the silk squirts through the spinneret. Malik and Reddy (2009) reported that silk conversion index (SCI) values were lower for CSR_2 than NB_4D_2 races (Malik and Reddy, 2009). They further reported that the silk gland of NB_4D_2 was having relatively higher silk content in the lumen of the silk gland spins less fiber in weight than CSR_2..

Silk productivity was relatively better in Methionine followed by Lysine and Tryptophan as compared to control-I and control-II. Horie and Inokuchi (1978) reported the utilization of amino acids after deamination for fatty acid biosynthesis in different races of the mulberry silkworm, *Bombyx mori*.

The observations on cocoon weight and shell weight revealed that Methionine expressed significantly higher cocoon weight and shell weight followed by Lysine and Tryptophan. The increase in cocoon weight and shell weight might be due to the increase in absorption of supplemented amino acids. These results are in agreement with those of Nicodemo and Olivera (2014) who noticed that significantly higher cocoon weight and shell weight in silkworm batches fed with amino acid treated leaves, as against Control batches. Similar results were also observed with Aspergine and Alanine supplementation by Radjabi (2010); Proline and amino acid mixture supplementary with mulberry leaves by Bhojnae *et al*. (2014). Improvement in the shell percentage was also recorded in Methionine followed by Lysine and Tryptophan. Methionine, Lysine and Tryptophan showed an improvement of 24.2%, 16.7% and 12.1% at 0.5% concentration over control-I. These findings were supported by Chakraborty and Kaliwal (2012) who observed that oral supplementation of Arginine resulted in a significant increase in cocoon weight, shell weight and shell percentage. These results were also in conformity with the observations of Kumar and Kumar (2018) who also reported a significant improvement in cocoon weight, shell weight and shell percentage on Methionine supplemented mulberry leaves at 0.5% concentration.

Filament length has a positive correlation with shell weight. The silkworms fed on mulberry leaf fortified with amino acids at varied concentrations registered marked influence on filament length. The Methionine and Lysine supplemented leaf showed significantly longer filament length of 1110 meters and 1079 meters at 0.5% concentration over control-I (833 meters) and control-II (831 meters). The increase in the filament length might be due to higher rate of silk protein synthesis by additional supplementation of Methionine. Similar results have been reported by Kumar and Kumar (2018) on Methionine fortified mulberry leaf. These results are also in agreement with the findings of Raj *et al.* (2000) with soya protein supplemented mulberry leaf. The average filament size is significantly lower in Methionine (2.63) and Lysine (2.66) than Tryptophan (2.79) as against control-I (2.91) and control-II (2.98). Similar results were also observed by Raj *et al*. (2001) who recorded fine denier (1.82) in Pure Mysore race when fed on mulberry leaves supplemented with soyabean flour as against control (2.14). Kumar and Kumar (2018) also reported lower denier on Methionine supplemented mulberry leaves of 1.650 in MU_{11} and 1.70 in MU_{303} silkworm breeds.

There was an improvement of 19.24%, 16.92% and 12.85% in raw silk percentage when the larvae were fed on mulberry leaves supplemented with 0.5% of Methionine, Lysine and Tryptophan as against control-II. These findings fall in the line with the findings of Laz *et al*. (2006) who has reported significant increase in raw silk percentage on Methionine and Tryptophan supplemented mulberry leaves. Radjabi (2010) also reported that enrichment of mulberry leaf with Asperginine and Alanine amino acids could improve silk production in sericulture.

The results of present study showed that among the treatments there was a significant increase in fecundity in Tryptophan supplemented mulberry leaves followed by Methionine and Lysine. There was an improvement of 35.29% in fecundity when the larvae were supplemented with Tryptophan followed by Methionine (24.98%) and Lysine (22.23%) at 0.5% concentration as against control-II. Krishnappa (1987) reported that fecundity and fertility were well pronounced due to amino acid supplementation. Similar results were observed by Saha *et al*. (1994) who observed that Proline and Leucine enhanced the reproductive potentiality. Similarly, supplementation of Methionine and Tryptophan also increased fecundity of multivoltine silkworms (Laz, 2010). Radjabi (2010) also reported that bivoltine hybrid silkworms supplemented with Aspargine and Alanine at varied concentrations enhanced fecundity and hatching percentage due to increase in secretions of corpora allata which leads to vittelogenesis.

5.2 Impact of supplemented amino acids on biochemical constituents in haemolymph and silk gland of silkworm, *Bombyx mori* L.

Proteins are important biological macromolecules that are required for growth and development of the silkworm as well as biosynthesis of silk. They are major source of nitrogen and essential amino acids, which are to be obtained only through mulberry leaves in the monophagous silkworm, *Bombyx mori* L. Any deficiency in essential amino acids or the total requirement of nitrogen is reflected in growth, development and silk biosynthesis. The deficiency could arise either through the absence of specific nutrient in required quantity or failure of utilization of the nutrient present in the food by the silkworm.

The present study showed that the mean levels of protein in both haemolymph and silk gland irrespective of amino acid concentrations were relatively higher in larvae of Methionine supplemented mulberry leaf followed by Lysine and Tryptophan as against control-I and control-II. The results obtained from the present study showed greater quantities of protein/ml/gm of both the tissues in Methionine supplemented mulberry leaves followed by Lysine and Tryptophan at 0.5% concentration. Accordingly, a progressive increase in the protein content of the larval haemolymph and silk gland protein content was observed in batches fed with respective concentration of amino acids (Methionine and Lysine). The high protein content in the larvae fed with Methionine and Lysine could be attributed to the nutritional superiority of the leaf and increased consumption, utilization and absorption of amino acids by the larvae. The increases in protein levels of haemolymph and silk gland could be due to synthesis of new proteins by the tissues. The high moisture content with optimal levels of protein fulfills the nutritional requirement of the larvae, thereby providing essential materials for the synthesis of new membranes and other sub-cellular structures required for cell architecture (Reddy, 2011). Utilization of exogenous proteins is an important factor for growth and development of the larva in insects (Chen and Levenbook, 1966). Ito (1978) has reported that about 60% of the total nitrogen content of the mulberry leaf is used for silk biosynthesis.

Protein digestion yields amino acids and oligopeptides. The high haemolymph amino acid concentration impedes their downhill movement from the midgut lumen. But, the preceding water absorption transports amino acids by solvent drag effect. Also, specific transport mechanisms for individual and groups of amino acids were shown to be present in *B. mori* (Reddy, 2011). Insects distinguish themselves from other animals by their high contents of free amino acids whose metabolism has been the subject of numerous investigations (Chen and Levenbook, 1966; Chen, 1971). The composition of free amino acids in the haemolymph and the influence of dietary amino acids on the haemolymph amino acids have been studied by many workers (Inokuchi, 1971; Watanabe, 1980). It varies according to the larval development. Free amino acids in plant tissues are sensitive indicators of the nutritional status. The content of free amino acids in mulberry leaves affects the cocoon shell of the silkworm (Ito, 1976). It is reported that omission of any of the 10 essential amino acids from the artificial diet leads to block the protein synthesis and enhance the uric acid excretion (Horie *et al.*, 1970). Again, the omission of both glutamic acid and aspartic acid from the artifical diet results in marked retardation of larval growth (Horie and Watanabe, 1983). Generally when one of the essential amino acids tend to increase in the haemolymph, those amino acids considered to be metabolically related to the amino acid show a tendency to decrease. A marked difference in the amino acid content of silk protein produced by the silk gland and the nutritional value of mulberry leaf has been observed (Kirimura, 1962).

The mean free amino acid levels during the 5th instar larval development were relatively higher in haemolymph and silk gland in Methionine followed by Lysine and Tryptophan. The increase in free amino acids was found to be higher at 0.5% than at 1.0% and 1.5% concentrations, which might be due to its better utilization at lower doses of amino acid supplementation than at higher doses.

Also, the levels of increase in the percentage of free amino acid level in haemolymph and silk gland were found to be higher in Methionine (62.6% & 33.17%) followed by Lysine (54.2% & 28.6%) and Tryptophan (42.5% & 22.5%) at 0.5% concentration, respectively. It has been demonstrated that supplementation of low nutritive proteins with their limiting amino acids accelerated growth of the newly hatched larvae of the silkworm (Ito and Arai, 1965). However, the effectiveness of amino acid supplementation was different for different tissues. It may therefore be suggested that the quantitative requirements for essential amino acids vary according to the stage of tissue growth. A similar acceleration was observed using 5th instar larvae in this experiment. Inokuchi (1971) has shown that the composition of free amino acids in the haemolymph varied according to the kinds of diet. The transaminases are the important components of amino acid metabolism which mainly involved in transferring an amino group from an amino acid to another keto acid, thus forming another amino acid. The aspartate and alanine aminotransferase which serve as strategic link between carbohydrate and protein metabolism (Martin *et al*., 1981). The free amino acid increase in the haemolymph and silk gland might be due to additional supplement of amino acids which in turn increase the activities of both AST (aspartate aminotransferase) and ALT (alanine transaminase) enzymes in both the tissues. These results are in agreement with the earlier observation of Kumar and Kumar (2018) who has reported that increase in the activity of AST and ALT in the fat body at 0.5% concentration. Similar results were also observed by Manjula *et al.* (2010) in haemolymph when the silkworms fed on mulberry leaves supplemented with *Dolchos lablab* flour 7.5% concentration.

Chapter-6

SUMMARY AND CONCLUSION

The silkworm rearing was conducted at Division of Sericulture Crop Improvement in College of Temperate Sericulture, Mirgund and biochemical analysis was carried out at Proteomics Laboratory, Division of Biotechnology, SKUAST-K, Shalimar, during spring (May-June) in the year 2020.

- The disease free layings of silkworm breed APS_4 were obtained from the Division of Sericulture Crop Improvement, College of Temperate Sericulture, Mirgund.
- Disease free layings of said race were incubated, brushed and reared on Ichinose mulberry leaves up to 3^{rd} instar by following standard rearing procedure.
- For this research programme mass rearing of three disease free layings of APS_4 bivoltine silkworm breed was done up to 3^{rd} instar by feeding Ichinose mulberry leaf without any treatment. Just after 3^{rd} moult, three replications containing 100 worms were maintained for each treatment as well as for control batches. After 3^{rd} instar till spinning, the worms were reared on Goshoerami leaves on which the selected amino acid solutions were sprayed daily once as first feed during 4^{th} and 5^{th} instar with the help of an automizer and the treated leaves were shade dried for 10-15 minutes before feeding the silkworms in order to study the influence of mulberry leaf supplemented with limiting amino acids on economic traits and biochemical constituents of silkworm, *Bombyx mori* L.
- Three amino acids *viz.*, Methionine (HiMedia Laboratories Pvt. Ltd. Mumbai, Maharashtra), Tryptophan (HiMedia Laboratories Pvt. Ltd. Mumbai, Maharashtra) and Lysine (HiMedia Laboratories Pvt. Ltd.

Mumbai, Maharashtra) and three different concentrations *viz*., 0.5%, 1.0% and 1.5% were prepared for the present study.

- The nutritive value of amino acids is known by its growth promoting effect when different quantities of it are supplemented with the silkworm natural diet. A dose dependent change in the nutritive and economic characters at 0.5%, 1.0% and 1.5% showed that Methionine, Tryptophan and Lysine, were absorbed and assimilated more at lower doses of 0.5% concentration by the silkworm. These results indicated that excess amino acids in a diet may cause deficiencies in other amino acids and induce toxicity.
- Result revealed that amino acid fortification significantly reduced 5^{th} instar and total larval duration in the silkworm batches fed with 0.5% concentration of Methionine as compared to that of control batches.
- Also, the result of the present study revealed that amino acid fortification significantly improved the weight of 10 mature larvae, silk gland weight, silk gland somatic index, cocoon yield per 10,000 larvae (by number and weight), silk conversion index, silk productivity, single cocoon weight, single shell weight, shell percentage, filament length and raw silk percentage in the silkworm batches fed with 0.5% concentration of Methionine as compared to that of control batches where no supplementation of amino acids was done.
- However, the reproductive trait i.e., fecundity was found significantly superior in silkworm batches fed with 0.5% concentration of Tryptophan.
- The biochemical constituents *viz*., total protein and total free amino acids in selected tissues also recorded significant increase at 0.5% concentration of Methionine as compared to the other treatments as well as control batches.

- However, no significant difference was recorded in filament size (denier) among the treatments and concentrations but relatively finest denier was recorded in silkworm batches fed with Methionine at 0.5% concentration.
- The dietary supplementation of limiting amino acids improved the economic as well as biochemical constituents of silkworm in general, however the supplementation of Methionine particularly at 0.5% concentration markedly improves the nutritive value of mulberry leaf and thereby increased commercial characters of silkworm, *Bombyx mori* L. and total protein and total free amino acid levels in haemolymph and silk gland.
- This study will help to improve the host health and performance traits of silkworm and open new vistas for pursuit of sericulture development in the country which is facing survival threats due to palatability and suitability of leaf quality in different seasons.

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Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
College of Temperate Sericulture, Mirgund

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner Dr. Nisar Ahmad, Scientist D, CSR&TI, Pampore during Viva-Voce examination held on 25-02-2021 have been incorporated in the manuscript entitled, **"Influence of Mulberry Leaf Fortified with Limiting Amino Acids on Biochemical Constituents and Economic Traits of Silkworm, *Bombyx mori* L."** submitted by **Ms. Kahkashan Qayoom (Regd. No. MSS/2018/63).**

(Dr. Firdose Ahmad Malik)
Chairman
Advisory Committee