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Application of Tryptophan as Supplementary Nutrient on Dehydrogenase Activities and Economic Parameters in *Bombyx Mori* L.

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ABSTRACT

The important growth regulating factor in silkworm or in any organism is nutrition. Dietary nutrients not only determine growth and development of silkworm larvae but also cocoon yield. An attempt has been made in the present investigation to record the influence of mulberry leaf supplemented with tryptophan at varied concentrations *viz.*, 0.5, 1.0 and 1.5 % on succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) activities as well as economic parameters in bivoltine double hybrids FC1 x FC2 and FC2 x FC1. The study revealed that both the hybrids recorded highest enzyme activities at 1.5 % concentration of tryptophan supplementation over remaining concentrations and elevated the cocoon weight, shell weight, shell percentage, filament length, filament weight, denier and renditta.

KEYWORDS: *Bombyx mori*; tryptophan; economic parameter; bivoltine hybrids; dehydrogenase activities

INTRODUCTION

The growth, reproductive potentiality, quantum and quality of cocoon produced by the silkworm (*Bombyx mori* L) depends on nutrients composition of diet [1]. Silkworm utilizes mulberry nutrients and convert it into silk protein *viz.*, fibroin and sericin in the form of cocoons. The quality of mulberry leaf is dependent on several factors which includes soil type, mulberry variety, irrigation schedule, fertilizer input, spacing, maturity and eco-climatic variations [2, 3, 4]. As the quality on mulberry leaf has an intimate relation to the healthy growth of larvae and quality of their

cocoons, chemical composition of the leaves has a great scope in determining the food value.

In addition to the chemical constitution of mulberry leaves, the quantity of leaf provided to the silkworm will also have effect on the physiology and economic characters. Moreover, there is a correlation between the amount of mulberry leaves eaten by the silkworm and silk protein in the silk gland [5, 6]. The relationship between the amount of food intake and cocoon productivity [7]. These reports have confirmed some negative effects of nutritional restrictions on the larval growth and cocoon characters. However, utilization of these nutrients varies among the silkworm breeds.

The dietary protein and amino acids have positive correlation in respect of silk protein synthesis. The optimal levels of dietary protein for good growth and silk production in those in the range 22 to 26%, which is in agreement with protein content of mulberry. The nutritive value of various kinds of protein is completely dependent on their compositions of amino acid. The effects of graded levels of protein in the diet on growth, silk production and nitrogen metabolism have been demonstrated by several researchers [8, 9, 10, 11, 12]. The various levels of protein in the diet not only hampered the growth and silk production, but also the digestibility and protein concentration in the haemolymph [13]. Silkworms obtain 72 to 86 % of their amino acids requirements from mulberry leaves and more than 60% of the absorbed amino acids are used for silk production [14]. The amino acids play an important role in glucose, tryptophan and organic acid metabolism. However, the tryptophan is an essential amino acid and exhibit antioxidant property in *B.mori* [15]. The catabolism of tryptophan involves various biochemical pathways which includes kynurenine-anthranilate, serotonin and indolacetic acid. In addition to this it helps to synthesis nicotinic acid (NAD or NADP) with the aid of various enzymes.

Further, the succinate dehydrogenase (SDH) is found in the inner membrane of mitochondria oftenly referred as mitochondrial index enzyme. The SDH catalyses the reaction in the Krebs cycle and help form fumarate from succinate. The activity levels of SDH has been studied in a wide range of insects by many workers who have defined the enzyme in egg, larva, pupa and adult stages [16, 17, 18, 19].

In some selected silkworm breeds, the activity levels of SDH has positive correlation with most of the economic characters except renditta [20]. Further more, gel electrophoresis study revealed that the enzyme exhibit variations among selected breeds. In silkworm, *B. mori* the activity levels of SDH varies among different breeds and hybrids [21]. It has been observed that increase in the activity levels of succinate dehydrogenase and amylase in F₁ progeny batches raised from EMS treatment at varied concentrations [22].

Similarly, lactate dehydrogenase (LDH) abundantly found in fat bodies of insects. It is a glycolactic enzyme involved in conversion of

lactic acid into pyruvic acid and vice-versa by the help of co-enzyme NAD. In *B.mori*, organophosphorus insecticides decrease the LDH activity which reduces mobilization of pyruvate resulting in decrease in respiration rate at the tissue level [23]. Similarly, exposure of phoxim insecticides decrease the growth and survival of silkworm with decrease in LDH [24]. It has been observed that topical application of prostaglandin to silkworm larvae increases LDH activity with increased larval weight [25]. However, effect of supplementation of tryptophan on dehydrogenase enzymes and economic parameters in the newly evolved bivoltine silkworm hybrid is fragmentary. Hence the present investigation has been undertaken.

MATERIALS AND METHODS

The bivoltine silkworm double hybrids namely FC1 x FC2 and FC2 x FC1 were selected and larvae were reared by employing standard rearing techniques [26].

Supplementation of Tryptophan:

Tryptophan at varied concentrations *viz.*, 0.5, 1.0 and 1.5 % were prepared by using distilled water and sprayed on ventral surface of the mulberry leaf and surface dried under shade and fed to the silkworms. The larvae divided into five batches *viz.*, batch I (T1), batch II (T2) and batch III (T3) and were reared on mulberry leaf supplemented with tryptophan at 0.5, 1.0 and 1.5 %, respectively along with control batch IV (T4), where the larvae reared on mulberry leaf supplemented with distilled water and batch V (T5) reared on natural diet (absolute control). The treated leaves were fed to silkworm once a day during fourth and fifth instars. In each treatment three replications were maintained. A minimum of ten larvae were used from each treatment to record the enzyme activity as well as economic parameters namely: larval weight, cocoon weight, shell weight, shell percentage, filament length, denier and renditta.

Estimation of SDH and LDH in selected bivoltine double hybrids:

The succinate and lactate dehydrogenase activities were estimated in 5th instar 1st day, 3rd day and 5th day larvae of bivoltine hybrids FC1 x FC2 and FC2 x FC1. The fat body tissue homogenate of 1% (w/v) was prepared by using distilled water and

centrifuged at 3,000 rpm for 10 minutes, the crude extract was collected and used as enzyme source.

The SDH and LDH activities were estimated [27]. One ml of tissue extract was incubated with 1ml of sodium succinate, 1ml of phosphate buffer and 1ml of INT at 37° C for 1 hr by using water bath. For LDH lithium lactate was used as a substrate. Then enzyme activities were stopped by adding 6ml of glacial acetic acid followed by 6ml of toluene. The reaction mixture was kept overnight at refrigerator. The colour intensity was measured at 495nm by using spectrophotometer. The standard curve was used for calculation. The enzyme activity was expressed in terms of μ moles of formazone (product generated) /g protein/h.

The parameters such as shell percentage, filament length, denier and renditta were calculated by using the following formulae.

$$\text{Shell ratio (\%)} = \frac{\text{shell weight (g)}}{\text{Coccon weight (g)}} \times 100$$

$$\text{Filament length (L)} = R \times 1.125$$

Where, R = Number of revolutions recorded by an epprouvette and

1.125 = Circumference of epprouvette in meter.

$$\text{Denier} = \frac{\text{Weight of the filament}}{\text{Length of the filament}} \times 9000$$

It denotes thickness of the silk filament.

$$\text{Renditta} = \frac{\text{Weight of cocoons reeled}}{\text{Weight of raw silk obtained}}$$

Unit quantity of cocoons required to produce one unit of raw silk.

The obtained data was compiled and statistically analysed by using standard methods.

RESULTS AND DISCUSSION

The nitrogen is the main component of amino acids and protein which is one of the essential elements for the growth and development of insects. Although some insects are able to maintain their need in some other processes, silkworm use 65% of absorbed nitrogen during 5th instar larvae for silk production. Therefore, nitrogen sources present in the diet could have high effects on larval growth and cocoon production [28, 29].

Amino acids have multiple metabolic functions in the living cells. Diversity in the amount of free amino acids of haemolymph in generally affected by diet. Variation in the type of amino acids occurs due to different reasons. Silkworm

absorbs 72-86% of amino acids from the mulberry leaves and in the females more than 60% of absorbed amount is consumed for silk production [14].

The mulberry leaves supplementation with nitrogenous compounds and amino acids was evaluated for their effects on silkworm rearing by several workers.

The fat body has a storage function of reserve materials such as glycogen, proteins etc, and it is a site of various intermediary metabolic pathways [15]. Its synthesis various metabolites and it has intimate contact with the haemolymph. Thus, it is reasonable to expect that the disruption of the fat body and correlated with biochemical parameters of the haemolymph in *B.mori*.

The data pertaining to enzymes study depicts that silkworm hybrid FC1 x FC2 fed on mulberry leaves supplemented with tryptophan recorded higher activities levels of LDH and SDH in the fat body at 1.5% concentration (4.29 μ moles of formazone / g protein / hour) and (4.12 μ moles of formazone / g protein / hour) in 5th instar 5th day larvae followed by 5th instar 3rd day and 5th instar 1st day, respectively. Similar trend was also noticed in FC2 x FC1 at 1.5% concentration (Fig.1 and Fig.2).

The SDH is a mitochondrial flavo protein, which removes hydrogen from succinate and transfers two electrons to cytochrome-b. The activity level of SDH varies not only in different insect species but also in the same species at different stages of metamorphosis. The various amino acids are degraded to products which ultimately enter into the kerbs cycle. The nature of these intermediates establishes the nature of amino acids such as glucogenic, ketogenic or both. The ketogenic amino acid like tryptophan is degraded into acetyl co-A which may be converted into fat as well as glycogen. In both the hybrids, SDH and LDH activities were maximum at higher concentration (1.5 %) than lower concentrations (0.5 and 1.0 %) of tryptophan supplementation. The elevation in SDH activity at higher concentration might be due additional supplementation of tryptophan that enhances the synthesis of acetyl co-A and same has been mobilized into kerb cycle indicating increased biological oxidation. Similarly, LDH belongs to oxido reductase group which converts lactate into pyruvate in the presence of NAD co-enzyme. The elevation in

LDH activity at higher concentration of tryptophan supplementation might be due increased in synthesis of nicotinamide adenine dinucleotide (NAD) which accelerate enzymatic reaction. These results are on par with the findings of researchers [30] who have reported

that increase in alanine and aspartate amino transferases activity at lower concentration (0.5%) of methionine supplementation over remaining concentrations in the silkworm breeds MU303and MU11.

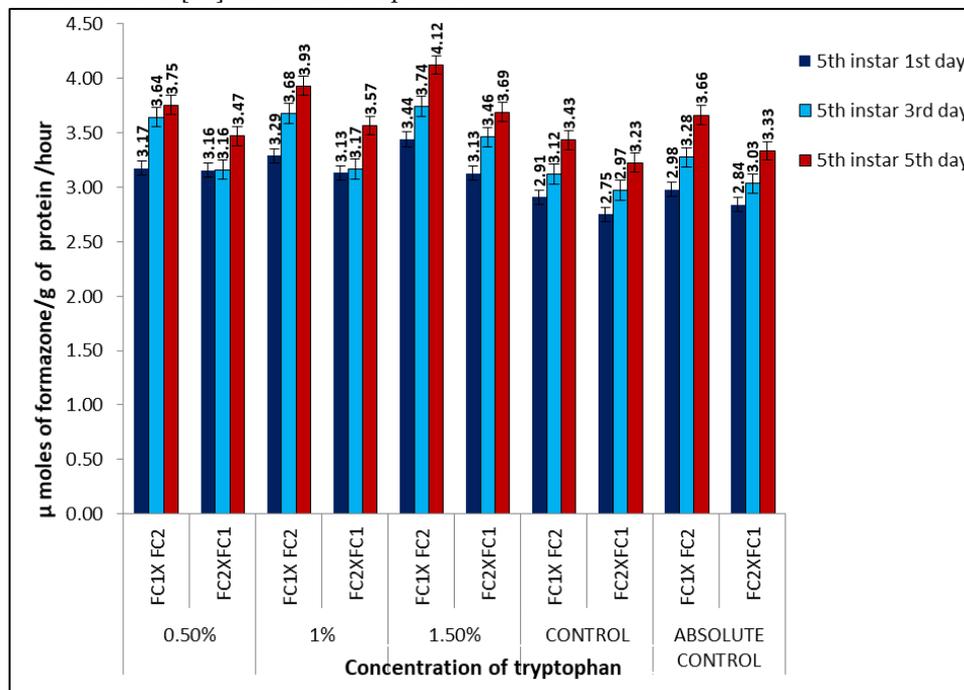


Fig. 1: Effect of mulberry leaves supplemented with tryptophan at varied concentrations on SDH activity in fat body of bivoltine double hybrids

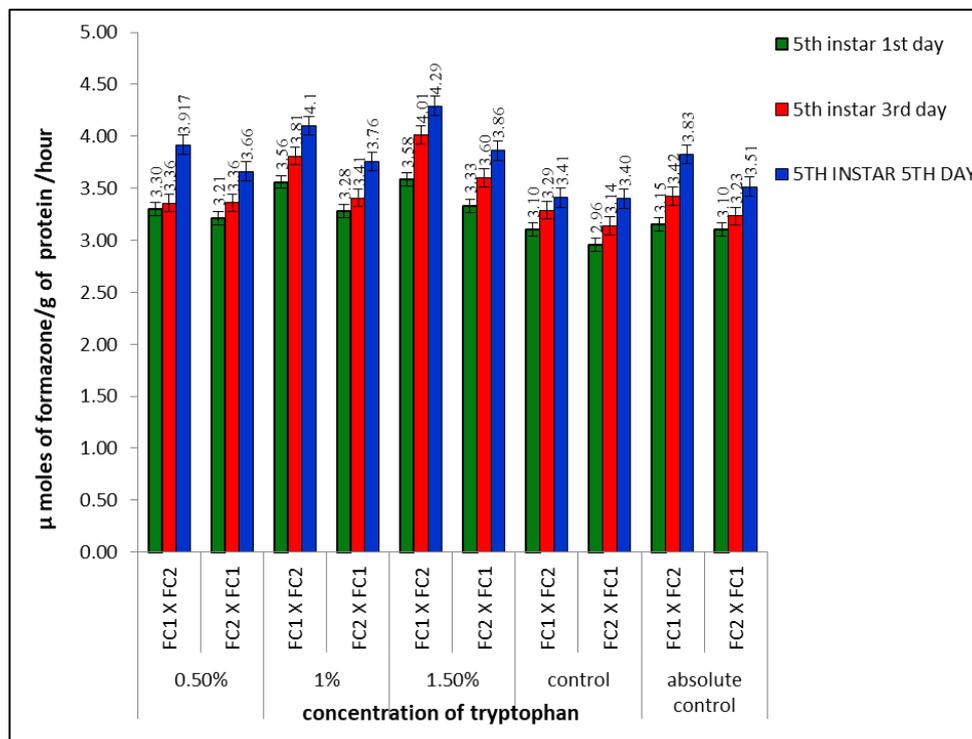


Fig. 2: Effect of mulberry leaves supplemented with tryptophan at varied concentrations on LDH activity in fat body of bivoltine double hybrids

Further, the hybrid FC1 x FC2 expresses higher activity level of SDH and LDH compared to FC2 x FC1. It clearly indicates that the utilization of tryptophan differed genetically among the hybrids. The present findings are in conformity with the findings of earlier workers [31] who have reported that utilization of pyridoxine genetically differ among the silkworm hybrid FC1 and FC2.

In both hybrids, SDH and LDH activities were highest in 5th instar 5th day, followed by 3rd and 1st day old larvae over control groups. It clearly shows that increase in absorption and utilization of tryptophan with advancement of age. These results are in line with the earlier researchers [30] who have opined that increase in aminotransferase activities with the advancement of age in the silkworm during the supplementation of methionine.

Matured larval weight

Silkworm hybrids reared on fortified mulberry leaves with tryptophan at different concentrations exhibited notable influence on larval weight. The FC1 x FC2 and FC2 x FC1 exhibit gain in the larval weight of 4.36 and 4.27g when the worms supplemented with tryptophan at 1.5% over control batches (Table 1). The increase in larval weight might be due to additional supplementation of tryptophan along with mulberry leaves. The present findings are in conformity with the earlier finding [32] who has reported that in order to have best larval growth, insects need optimum level of amino acids, being used for structural purposes such as enzymes and transport receptors. In the present study, higher concentration of tryptophan supplementation at 1.5% was found promising than remaining concentrations. Earlier workers [33] have observed that supplementation of glutamic acids and glycine at varied concentration enhances larval weight in the bivoltine hybrid (107' 110). Similar results were also observed in some other amino acids [34, 35, 36, 37].

Cocoon weight

Supplementation of tryptophan at varied concentrations on silkworm hybrids registered

encouraging results on cocoon weight. The larvae reared on tryptophan at 1.5% expressed higher cocoon weight of 2.32 and 2.27g in FC1 x FC2 and FC2 x FC1, respectively (Table 1). The increase in cocoon weight in both the breeds might be due to increase in absorption of tryptophan by midgut epithelial cells followed by different body cells and transformation to cellular structure. These results are in agreement with those of earlier workers [38] who have noticed that silkworms reared on mulberry leaves supplemented with threonine at 2% recorded significantly higher cocoon weight (1.70 g) as against control batch. Similar results were also observed with aspergine and alanine supplementation [39], Proline and amino acid mixture supplementation [40].

Shell weight

Silkworms nourished with mulberry leaves fortified with tryptophan at different concentration registered notable influence on shell weight. The worms supplemented with tryptophan at 1.5% exerted higher shell weight (0.48g) in FC1 x FC2 and the other hand FC2 x FC1 recorded highest shell weight of 0.40g over control batches (Table 1). The increase in shell weight might be due to additional supplementation of tryptophan which enhances the biosynthesis of silk protein. These results are in conformity with the findings of earlier researchers [34] who have opined that supplementation of mulberry leaves with aspartic acid at 2% have enhanced shell weight. Similar trend was also noticed for some other amino acids [41, 42, 35, 36].

Shell percentage

Silkworms nourished with mulberry leaves extra foliated with tryptophan exerted marked influence on shell percentage. A concentration of tryptophan at 1.5% resulted in higher shell ratio of 21.20 and 17.40 % in FC1 x FC2 and FC2 x FC1, respectively compared to control batches (Table 1). Increase in the shell ratio might be due to enhanced silk productivity by additional supplementation of tryptophan. These results are also supported by the observations of earlier worker [43] who has opined that the

silkworms reared on mulberry leaf supplemented with glycine at a concentration of 1.5% enhance shell ratio. Similar trend was also noticed with aspartic acid supplementation at the rate of 2% [34].

Hybrid	Treatments	Larval weight (g)	Cocoon weight (g)	Shell weight (g)	Shell percentage (%)	Filament length (m)	Filament weight (g)	Denier	Renditta
FC1xFC2	0.50%	4.32	2.26	0.47	21	904	0.27	2.7	8.4
		±	±	±	±	±	±	±	±
		0.03	0.02	0.01	0.53	4.36	0.01	0.10	0.28
	1.0 %	4.36	2.27	0.48	21	910	0.29	2.8	7.9
		±	±	±	±	±	±	±	±
		0.01	0.01	0.01	0.27	1.00	0.01	0.05	0.19
	1.50%	4.36	2.32	0.48	21	917	0.29	2.9	7.9
		±	±	±	±	±	±	±	±
		0.01	0.02	0.01	0.18	1.15	0.01	0.05	0.19
	Control	4.30	2.17	0.41	19	898	0.27	2.7	8.1
		±	±	±	±	±	±	±	±
		0.01	0.04	0.01	0.12	0.26	0.01	0.06	0.02
Absolute control	4.32	2.21	0.42	19	902	0.27	2.7	8.1	
	±	±	±	±	±	±	±	±	
	0.01	0.01	0.01	0.46	3.61	0.01	0.11	0.36	
FC2xFC1	0.50%	4.25	2.21	0.37	17	897	0.26	2.6	8.4
		±	±	±	±	±	±	±	±
		0.01	0.01	0.01	0.45	2.08	0.006	0.05	0.20
	1.0 %	4.26	2.26	0.39	17	897	0.27	2.7	8.5
		±	±	±	±	±	±	±	±
		0.01	0.02	0.01	0.30	1.73	0.006	0.06	0.20
	1.50%	4.27	2.27	0.40	17	898	0.28	2.8	8.0
		±	±	±	±	±	±	±	±
		0.01	0.01	0.01	0.19	1.53	0.006	0.05	0.17
	Control	4.20	2.04	0.36	17	888	0.26	2.6	8.0
		±	±	±	±	±	±	±	±
		0.01	0.02	0.03	1.27	2.65	0.006	0.06	0.14
Absolute control	4.23	2.10	0.38	18	893	0.26	2.7	8.0	
	±	±	±	±	±	±	±	±	
	0.03	0.01	0.02	0.70	4.93	0.006	0.07	0.14	

Filament length

Filament length has positive correlation with shell weight. The silkworms fed on mulberry leaf fortified with tryptophan at varied concentrations registered marked influence on filament length. The bivoltine hybrids FC1 x FC2 and FC2 x FC1 supplemented with tryptophan at 1.5% exerted longer filament length of 917 and 898m over control batches (Table 1). The increase in the filament length might be due to higher rate of silk protein synthesis by additional supplementation of tryptophan. These results are in conformity with the finding of researchers [35] who have noticed that supplementation of mulberry leaf with glycine and soya powder enhance filament length over unsupplemented batches. Similar results are also reported in bivoltine breed NB₄D₂ supplemented with soya protein [44].

Filament weight

Silkworm hybrids reared on fortified mulberry leaves with tryptophan at varied concentrations exhibited notable impact on filament weight. The hybrids FC1 x FC2 and FC2 x FC1 expressed gain in the filament weight of 0.29 and 0.28g, respectively with 1.5 % tryptophan supplementation as against control batches (Table 1). The increase in filament weight in both the hybrids might be due to higher rate of biosynthesis silk protein by additional supplementation of tryptophan through the diet. These results are in agreement with the findings of earlier researchers [42] who have observed that supplementation of mulberry leaf with glycine enhance filament weight over control batch.

Denier

Silkworms nourished with mulberry leaves extra foliated with tryptophan at lower concentration registered encouraging results than the higher. The larvae reared on tryptophan supplementation at 0.5% expressed lower denier of 2.7 in both the hybrids (Table 1). The results are in conformity in the findings of earlier workers [45] who have opined that fine denier (1.82 d) was recorded when the worms fed on mulberry leaves supplemented with soybean flour as against control (2.14 d) in Pure Mysore. Further, significantly fine denier (2.70 d) was registered in cocoons spun by the worms supplemented with soya bean flour over control batch (2.96 d) in PM×NB₄D₂ was also reported earlier workers [46].

Renditta

The silkworms reared on fortified mulberry leaves with tryptophan at different concentrations expressed encouraging results in respect of renditta. The lowest renditta of 7.9 and 8.0 was recorded in FC1 x FC2 and FC2 x FC1, respectively (Table 1). The improvement for this trait in both bivoltine hybrids at 1.5% concentration of tryptophan supplementation might be due to effective utilization of amino acid for the synthesis of silk protein in turn reflects on cocoon shell formation. These results are in agreement with the findings earlier researchers [42] who have observed that silkworm reared on mulberry leaf supplemented with 10ppm concentration of glycine exerted significant decrease in renditta. Further, silkworm hybrid PM × CSR₂ reared on mulberry leaves supplemented with proline and amino acid mixture at 1% improves the renditta than 2% was also reported earlier workers [47].

CONCLUSION

The results of the present study inferred that, fortification of mulberry leaves with tryptophan at 1.5% enhance economic parameters of mulberry silkworm. Hence farmer could be prescribed to use vitamin after suitable modification to get better cocoon yield.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest in this research article.

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