Biological Forum
an International Journal 17(9): 83-88(2025)
ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

The Variation in Aminotransferase Activities and Economic Traits in Silkworm as Influenced by Mulberry Leaf Enriched with Spirulina

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(Received: 19 June 2025; Revised: 26 July 2025; Accepted: 23 August 2025; Published online: 14 September 2025)
(Published by Research Trend)

ABSTRACT: The current study was carried out to record the influence of mulberry leaves enriched with spirulina at different concentrations *viz.*, 600, 800 and 1000 ppm on aminotransferase enzymes and economic traits of silkworm hybrid FC1×FC2. The larvae supplemented with spirulina at 1000ppm registered maximum aspartate and alanine aminotransferase activity levels in the fat body and midgut tissues over control batch. Further, larvae treated with higher concentration (1000ppm) of spirulina exerted increase in larval weight, cocoon weight, shell weight, shell ratio, filament length, filament weight and raw silk percentage when compared to other two concentrations. However, both enzyme activities were maximum during 5th instar 6th day old larvae but it was minimum in 1st day old larvae.

Keywords: *Bombyx mori*, Spirulina, Aminotransferase enzymes, Economic traits.

INTRODUCTION

The silkworm, *Bombyx mori* L. is a monophagous insect and exclusively feed upon mulberry leaves during larval stage in order to restore energy for metamorphosis. The mulberry silk contributes over 90% of global silk production and India ranks second position next only to China. The cocoon yield is strongly influenced by leaf quality and rearing of productive breeds. The nutritional study of silkworm has greater importance as it involves conversion of leaf nutrients into body elements. It is the dietary nutrients of mulberry play a significant role in determining cocoon production and yield. There is a correlation between utilization of mulberry nutrients with silk production (Fakuda *et al.*, 1963). The utilization of these nutrients differs among silkworm breeds. Any short supply of these nutrients will affect silkworm growth and cocoon parameters. Hence, silkworms require nutrients at optimum level and below this level it is essential to supplement the nutrients through the diet. In the recent year, different types of fortifying agents were used so as to improve larval and economic parameters of the silkworm which includes proteins, carbohydrates, vitamins, amino acids, sterols, hormones, antibiotics, salts and other chemicals (Chowdhry *et al.*, 1996).

A quite a good number of reports are available on botanical extracts used as fortifying agent, which influence the larval and cocoon parameters of the silkworm. For instance, mulberry leaves fortified with ragi, rice, wheat, sorghum, soyabean, horse gram, green gram and cow pea flour found to be effective in enhancing larval and cocoon parameters as well as silk proteins *viz.*, fibroin and sericin (Rekha, 2004; Anil Kumar and Prashanth 2018; Manjula *et al.*, 2010 and 2011; Sundar Raj *et al.*, 2000a). Similarly, supplementation of plant extracts like *Lantana camara, Clerodendrum inerme* increases silk filament length and weight (Mamadapur, 1994). Furthermore, the larvae supplemented with *Curcuma longa* (Turmeric) has resulted increased synthesis of biomolecules such as total protein and free amino acid contents (Harihara Raju *et al.*, 2012) and with *Dolicos lablab* flour also enhance enzyme activities *viz.*, amylase, sucrose, protease, urease and trehalase in the haemolymph and midgut (Manjula *et al.*, 2010b).

The spirulina (Blue-Green algae) which has high nutritive value and is composed of 18 amino acids, 50 different types of mineral elements, several vitamins, carbohydrates such as mucopolysaccharides, rhamnose, glycogen and with omega-3 fatty acids (Venkatesh Kumar *et al.*, 2009). Because of strong antioxidant and immune boosting properties, spirulina widely used as fortifying agent in order to improve biological performance of insects including silkworm (Dyana Selin *et al.*, 2018). The silkworm reared on mulberry leaves fortified with spirulina showed marked improvement in larval weight, cocoon weight, shell weight, shell ratio and cocoon yield (Sameena Maqbool *et al.*, 2023). This type of trend was true for morphometric characters such as larval length, width and weight (Ganesh Prabhu *et al.*, 2011; Chand Asaf and Mahavishnu, 2018a). Moreover, reproductive traits like testis and ovary weight and biomolecules *viz.*,

carbohydrate, glucose, glycogen, lactic acid and pyruvic acid were significantly higher in spirulina treated groups (Kumar and Bakiya Lakshmi, 2023). Similarly, worms treated with combination supplement *Serrigrow++* (spirulina, yeast and probiotic bacteria) enhance the cocoon parameters, silk protein contents, amylase and protease enzymes (Nalawade and Momin 2023). The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are the key enzymes involved in amino acid metabolism and also help to maintain amino acid concentration in different tissues which in turn reflects on biological performance of silkworm. Lot of work has been undertaken spirulina as supplementing agent for enhancing economic parameters of the silkworm. However, information available on supplementation of spirulina on aminotransferase enzymes in silkworm is meager. Keeping this in view current investigation was initiated.

MATERIALS AND METHODS

The present experimental study was conducted at Department of Studies in Sericulture Science, University of Mysore, Mysuru during 2024-25. The bivoltine double hybrid FC_1xFC_2 larvae were reared under standard environmental conditions followed by the methods of Krishnaswami (1986). After third moult larvae were divided into four groups of 100 worms each in three replications. The first three groups namely group I, group II and group III were reared on mulberry leaves sprayed with spirulina solutions at the concentrations of 600, 800 and 1000ppm, respectively. After spraying spirulina solution, the leaves were dried under room temperature and fed to silkworm daily once as first feed during 4^{th} and 5^{th} instars. The worm fed with mulberry leaves treated with distilled water (control) was maintained for comparisons. Ten larvae were used from each treatment in three replications to score enzyme activity and economic traits such as larval weight, cocoon weight, shell weight, shell ratio, filament length, filament weight, denier, renditta and raw silk percentage.

Estimation of aspartate and alanine aminotransferase enzymes

The aspartate aminotransferase activity in fat body and midgut tissue were estimated by adopting the method of Reitman and Frankel (1957). 100mg of tissue was homogenized with 10ml of distilled water and centrifuged at 3000rpm for 10 minutes. For 1ml of supernatant, 0.5 ml of glutamic oxaloacetate solution was added and incubated at 37°C for 1hour. Then 0.5ml of 2,4-D was added and incubated for about 15 minutes at room temperature. To this 5ml of 0.4N NaOH was added and after 15 minutes OD was measured at 510nm against blank. For alanine aminotransferase, glutamic pyruvate solution was used as a substrate. The standard curve is calibrated and activities was measured by using following formula.

$$\text{Amount of AST/ALT} = \frac{\text{standard graph value x 60}}{\text{mg of tissue taken x incubation time}} \text{ x 1000}$$

The parameters, namely shell ratio, filament length, denier, renditta and raw silk percentage were computed using the following formulae.

$$\text{Shell ratio (\%)} = \frac{\text{shell weight}}{\text{cocoon weight}} \text{ x 100}$$

Filament length (m): L= R×1.125

R = Number of revolutions recorded by an epprouvette.

1.125 = Circumference of epprouvette in meter.

$$\text{Denier} = \frac{\text{filament weight}}{\text{filament length}} \text{ x 9000}$$

It indicates 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.

$$\text{Raw silk percentage(\%)} = \frac{\text{reeled silk weight}}{\text{green cocoon weight}} \text{ x 100}$$

The obtained data was statistically analysed by standard deviation method.

RESULTS AND DISCUSSIONS

A. Influence of mulberry leaves supplemented with spirulina on aspartate and alanine aminotransferase enzymes

The data pertaining to aminotransferase enzymes depicts that silkworm hybrid FC_1xFC_2 larvae nourished with mulberry leaves treated with spirulina at 1000ppm concentration registered higher activity levels of AST with 153.08µ moles of oxaloacetate/g protein/hour in the fat body and 118.57µ moles in midgut tissues over respective control (142.73 and 104.21µ moles) during 5^{th} instar 6^{th} day larvae and among treatments it was least in 5^{th} instar 1^{st} day old larvae (39.21 and 56.21 µ moles) at 600ppm concentration in midgut and fat body tissues, respectively (Fig. 1). It is also evident from the data is that larvae treated with spirulina at 1000ppm concentration recorded maximum activity level of ALT with 121.09 µ moles of glutamate pyruvate/g protein/hour in the fat body and 97.38 µ moles in midgut tissue when compared to their control (106.82 and 85.32 µ moles) during 5^{th} instar 6^{th} day larvae. On the other hand, it was minimum in 5^{th} instar 1^{st} day old larvae (19.28 and 31.44 µ moles) at 600 ppm concentration in midgut and fat body tissues among treatments, respectively (Fig. 2).

Table 1: Influence of mulberry leaves fortified with spirulina at varied concentrations on larval andcocoon characters of $FC_1 \times FC_2$ silkworm hybrid.

Concentration (ppm)	Larval weight (g)	Larval weight (g)	Cocoon weight(g)	Shell weight(g)	Shell Ratio(%)	Filament length(m)	Filament weight(g)	Renditta	Denier	Raw silk percentage
600	5.30±0.04	5.30±0.04	2.172±0.007	0.483±0.002	22.23±0.08	1242.59±5.29	0.467±0.001	5.27±0.01	3.37±0.02	19.44±0.51
800	5.50±0.02	5.50±0.02	2.197±0.002	0.498±0.001	22.66±0.02	1277.85±2.64	0.470±0.001	5.23±0.01	3.30±0.01	19.51±0.45
1000	5.55±0.026	5.55±0.026	2.216±0.002	0.504±0.001	22.73±0.043	1339.94±9.069	0.474±0.002	5.18±0.02	3.18±0.02	19.57±0.51
Control (DW)	5.15±0.026	5.15±0.026	2.155±0.005	0.470±0.002	21.80±0.176	1224.13±4.008	0.448±0.003	5.31±0.02	3.29±0.01	18.98±0.21

All living organism are capable of synthesizing several amino acids in their body by transamination process with the aid of transferase enzymes. The alanine aminotransferase help to form pyruvate and glutamate from alanine and alpha-ketoglutarate. Similarly, aspartate aminotransferase converts aspartate and alpha ketoglutarate into glutamate and oxoloacetate (Jayaraju, 2022). The elevated levels of these enzymes indicates diseases associated with liver and cardiac muscle and also induced chemical toxicity. Moreover, these enzymes play significant role in intermediary metabolism of amino acids in which carbon skeleton is metabolized in different forms of amino acids. These enzyme activities differ sharply with different larval stages and silkworm breeds (Pezhman Nabizadeh, 2010).

It is evident from the result is that both AST and ALT activity were relatively high in the fifth instar 6th day old and it was least in fifth instar 1st day old larvae in all the treatments. It clearly depicts that silkworm required more amount of spirulina nutrients as age proceeds. Irrespective of the treatments, AST activity was relatively higher when compared to ALT indicating utilization of spirulina nutrients differ which leads to variation in enzyme activities in the silkworm. The results of the present investigation are in concurrence with the observations of Harihara Raju *et al.* (2012), wherein worms reared on mulberry leaves fortified with turmeric at 1.0% concentration has resulted increase in AST and ALT activities over control. Further, activity levels of these enzymes were maximum in the silk gland next best once were intestine, muscle and haemolymph. As per Anil Kumar and Prashanth (2018), larvae nourished with soya flour at 4.0% expressed maximum levels of ALT and AST activities and with *Dolichos lablab* and *Vigna unguiculata* flour at 7.5% concentration (Manjula *et al*., 2010b and 2011).

Impact of mulberry leaves supplemented with spirulina on commercial parameters.

1. Larval and cocoon weight

The silkworms nourished with mulberry leaves extra foliated with spirulina at 1000ppm concentration recorded higher larval weight of 5.55g and cocoon weight of 2.216g over control (5.15 and 2.155g. respectively) (Table 1). It is presumed that spirulina nutrients balances anabolic and catabolic process which helps to converts dietary nutrients into body elements resulting increased larval and cocoon weight. These results are in conformity with those of Dyana Selin *et al.* (2018) who have reported that supplementation of spirulina at 4.0% enhance larval and cocoon weight as compared to 5.0, 3.0, 2.0 and 1.0%. Bhuvaneshwari *et al.* (2023) have reported that fortification of mulberry leaves with spirulina at the rate of 2.0% not only increase the synthesis of biomolecules but also larval and cocoon weight. A similar effect was observed with potato leaf extract (Krishna Prasad *et al.*, 2001). This type of trend was noticed with plant extracts of *T. procumbens, P. niruri, A. vasica and T. bellirica* (Rajashekaragouda, 1991; Takhlique, 2012).

2. Shell weight and shell ratio

The worms supplemented with spirulina at 1000ppm concentration registered higher shell weight and shell ratio of 0.504g and 22.73%, respectively. However, control batch recorded 0.470g and 21.80%, respectively (Table 1). The increased trend for the said parameters due to better conversion of additional nutrients of spirulina for the synthesis of silk proteins which might have enhance shell weight and shell ratio. These results are in agreement with the earlier findings of Neha Pande and Sharma (2023) who have observed that larvae treated with spirulina at 1.0 and 2.0% exerted higher shell weight and shell ratio over control and the effects were same with 500 ppm (Sameena Maqbool *et al.*, 2023). Further, higher shell ratio was observed with

potato leaf, *C. inermae* and *L. camara* plant extracts (Krishna Prasad *et al.* 2001; Mamadapur, 1994). Similar results were found with aloe vera (Vitthalrao Khyade and Anil Shendage, 2012).

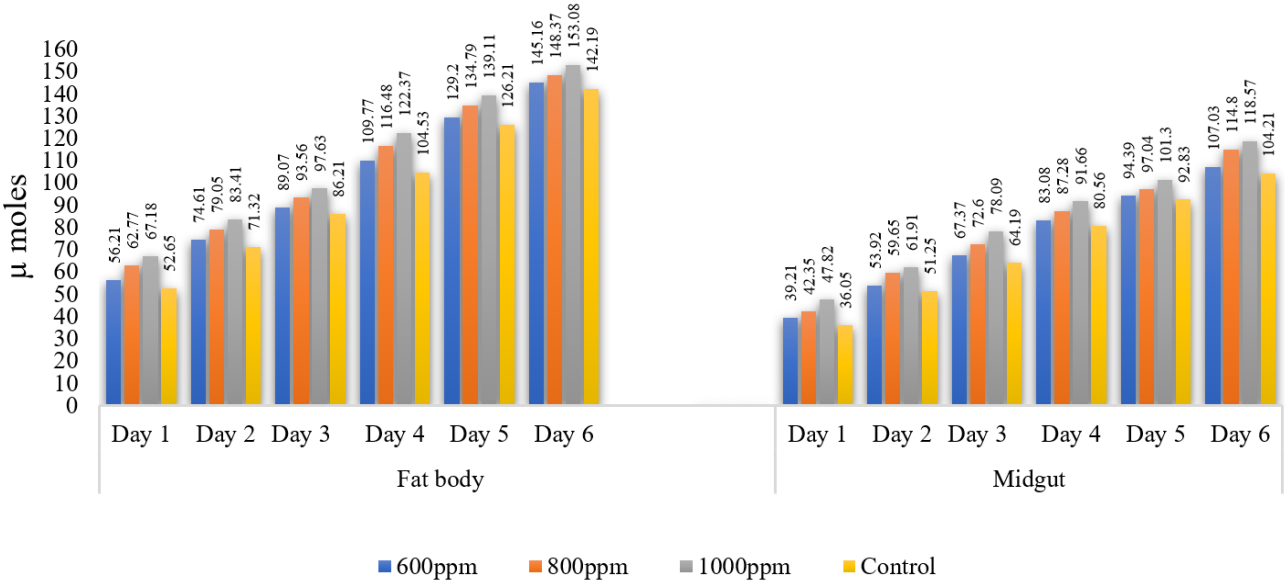

Fig. 1. Effect of fortified mulberry leaves with spirulina on aspartate aminotransferase enzyme in 5th instar larva of FC1xFC2 silkworm hybrid.

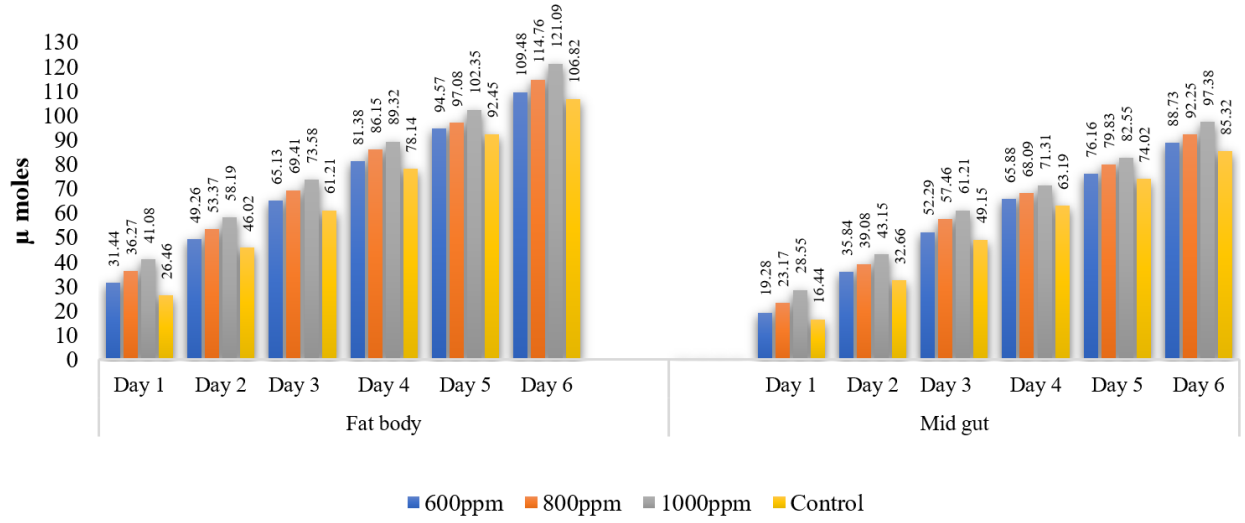

Fig. 2. Effect of fortified mulberry leaves with spirulina on alanine aminotransferase activity in 5th instar larva of FC1xFC2 silkworm hybrid.

3. Filament weight and length

The worms fed on mulberry leaves enriched with spirulina at 1000ppm concentration recorded highest filament weight of 0.474g and longest filament length of 1339.94m as against respective control batch (0.448g and 1224.13m) (Table 1). These results are also supported by the observations of Valantina Sangamithirai *et al.* (2014) wherein, worms treated with spirulina at 300ppm scored longer filament length over remaining concentrations *viz.*, 100 and 200ppm. Further, larvae administrated with bhumi amla (2.0%) and *C. inermae* extracts showed increase in filament length against control (Vitthal Khyade and Anil Shendage, 2012; Mamadapur, 1994).

4. Denier, renditta and raw silk percentage

Larvae reared on mulberry leaves fortified with spirulina at varied concentrations exhibited significant impact on denier, renditta and raw silk percentage. The worms treated with spirulina at 1000ppm concentration recorded lowest denier and renditta of 3.18 and 5.18 respectively, over control (3.29 and 5.31). On the other hand, highest raw silk percentage (19.57) was observed

at 1000ppm against control (18.98%) (Table 1). These results are in conformity in the findings of Saritha Kumari *et al.* (2011) who opined that, the lowest denier was recorded with supplementation of *P. niruri* and *A. vasica* extracts. Further, silkworm administrated with *W. somnifera*, *T. arjuna* and *T. cordifolia* extracts exhibited finer denier (Sridevi, 2003). Moreover, decreased renditta was noticed when the larvae treated with glycine at the rate of 10ppm (Sridhar and Radha, 1987). According to Sujatha and Purushotham Rao (2001), worms nourished with mulberry leaves treated with resepine and picrotoxin enhance raw silk percentage. Similar trend was observed with *E. prostrate, C. asiatica, W. somnifera* and *C. sativus* (Gayathri, 2005; Maribashetty *et al*., 2010 and Murari *et al.*, 2008).

CONCLUSIONS

The larvae reared on mulberry leaves fortified with spirulina significantly improves both biomolecules and economic traits of silkworm. The elevated levels of alanine and aspartate aminotransferase activities were observed in treated batches which indicate enhanced amino acid metabolism. Among the tested concentrations, 1000 ppm consistently registered maximum enzyme activities as well as economic traits.

FUTURE SCOPE

The spirulina can be used as fortifying agent after making formulation and used for commercial silkworm rearing by conducting extensive field trials.

Author Contribution. All authors are contributed equally during experiment and manuscript writing.

Acknowledgements. The authors express their sincere thanks to the Chairman, Department of Studies in Sericulture Science, University of Mysore, Manasagangotri, Mysuru, Karnataka, India, for providing laboratory facilities during experiments.

Conflict of Interest. Authors have declared that no conflict of interests exist.

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How to cite this article: Thippeswamy L., M. N. Anil Kumar, Sudeepthi S., Kiran Kumar D. and Tejashwini G. (2025). The Variation in Aminotransferase Activities and Economic Traits in Silkworm as Influenced by Mulberry Leaf Enriched with Spirulina. *Biological Forum, 17*(9): 83-88.