



RESEARCH ARTICLE

Influence of Amino acids on the Economic Characters of Silkworm, *Bombyx mori* L.

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ABSTRACT

Studies were undertaken to assess the influence of amino acids on the larval and cocoon characters of silkworm, *Bombyx mori* L. For the experiments, three amino acids namely glycine, alanine and serine were used at various concentrations viz., 10, 20, 50, 100 and 200 ppm. Supplementation of amino acids to silkworm larvae was done once daily from the first day of the fifth instar to spinning. The results revealed that all the concentrations positively influenced the mature larval weight, effective rate of rearing, effective rate of rearing, cocoon weight, shell weight, shell ratio and filament length. However, the improvement in all the parameters studied was highest at the concentration of 10 ppm, 100 ppm and 100 ppm, respectively in glycine, alanine and serine, over all other concentrations tested. *Per os* application of amino acids at specific concentrations also significantly reduced the fifth instar larval duration, a desirable character.

Key words: *Bombyx mori*; Cocoon weight; Shell weight; ERR; Amino acids

INTRODUCTION

Mulberry silkworm, *Bombyx mori* L. emits the lustrous raw material for the production of 'Queen of the Textiles', the silk, which could not be substantiated even by the advanced scientific innovations. *B. mori* is a domesticated oligophagus sericigenous insect that feeds only on mulberry, *Morus* spp. Mulberry leaves supply all the essential nutrients needed for silk protein synthesis, such as amino acids, minerals, sugars, proteins, vitamins, etc. However, the nutritional status of the mulberry varies based on the prevailing environmental conditions and crop management practices. Many-a-times, due to the poor nutrient status of mulberry, crop failure occurs, leading to substantial economic loss to the silkworm growers. This could be avoided by supplying the essential nutrients required for the normal growth and development of silkworm larvae in sufficient quantities through the fortification of mulberry leaves.

Among different supplementation experiments with vitamins, antibiotics, amino acids, sugars, trace elements, etc., the fortification of mulberry leaves with amino acids was found to be more beneficial since it improved the leaf quality and the silk yield by 40-60 per cent (Senguptha *et al.*, 1972).

The amino acids play an essential role in different phases of insect life, and their overall metabolic pathways are the same in insects as in other animal classes. The amino acids fulfill additional metabolic

functions such as osmoregulation in aquatic insects (Beadle and Shaw, 1950), neural transmitters (Chen and Widmer, 1968), detoxification of specific metabolites, synthesis of phospholipids, energy source and morphogenetic processes. The major function of intracellular amino acid is assumed to be an obligatory precursor for protein synthesis. High levels of free amino acids characterize insects, their total haemolymph quantity is about 100 to 300 times higher than that present in the human blood (Chen and Widmer, 1968).

Studies undertaken by various researchers over the period of time on supplementation with amino acids showed significant enhancement in the production of raw silk. Enrichment of mulberry leaves with different amino acids viz., glycine (Sharadha and Bhat, 1956; Ravi *et al.*, 1994 and Saad *et al.*, 2019), essential amino acids (Arai and Ito, 1964; Ito, 1967 and Inokuchi *et al.*, 1967), tyrosine, phenylalanine and alanine (Nagarajan and Radha, 1990), glycine, serine and alanine (Mustafa and Elkaraksy, 1990), aspartic acid (Kabala *et al.*, 1994), glycine, phenylalanine, serine and aspartic acid (Vadivel, 1995), alanine and asparagine (Radjabin *et al.*, 2010), arginine and histidine (Chakrabarthy and Kaliwal, 2012) and serine (Gokul, 2015) has increased the larval, cocoon, reeling and silk productivity-related parameters.

The findings of different workers motivated the authors to undertake investigations to ascertain

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the exact minimal concentration of selected amino acids required for increasing the larval and cocoon characters. Hence, an attempt has been made in this direction with the amino acids namely glycine, alanine and serine.

MATERIALS AND METHODS

The experiments were conducted by fortifying the mulberry leaves with amino acids such as glycine, alanine and serine individually to ascertain the influences of amino acids in the economic characters of silkworm. The methodology adopted and materials used for the experiments are detailed below.

i) Disinfection

Silkworm rearing house and rearing equipment were thoroughly disinfected with 2.5 per cent Serichlor in 0.5 per cent slaked lime solution before commencement of silkworm rearing. Subsequently, the rearing house was closed in airtight condition for one day to ensure 100 per cent disinfection and then opened to remove the chlorine smell (Dandin and Giridhar, 2014).

ii) Silkworm rearing

The silkworm, Double Hybrid (DH), was used for experiments and the rearing was conducted during the period favorable for bivoltine silkworm i.e., August to February. The leaves from well grown mulberry plants (Variety V1) were utilized for silkworm rearing. The feeding for larvae was provided three times per day without starvation. The bed cleaning was done using nylon net and the sufficient spacing was provided as age of the larvae increased. The bed disinfectant was dusted at 4-5 g/sq. ft area to prevent diseases immediately after moulting and the leaves were provided 45 – 60 minutes later (Dandin and Giridhar, 2014). The ripened larvae were mounted with Rotary mountage and well-built cocoons were harvested six days after the spinning.

iii) Administration of amino acids

The stock solution of 500 ppm was prepared by completely dissolving 500 mg of amino acid in one litre of distilled water. Various required concentrations viz., 10, 20, 50, 100 and 200 ppm were prepared carefully by serial dilution technique from the stock solution.

The fresh mulberry leaves were harvested on 60 days after pruning and weighed with an electronic balance. The weighed quantity of leaves was sprayed with an aqueous solution of the respective amino acid by using a glass atomizer. Two larval groups, one fed with mulberry leaves sprayed with distilled water and another fed with untreated mulberry leaves (Control), was maintained for comparison.

The treated leaves were shade dried for 15 to 30 minutes and fed to silkworm larvae once daily in the morning from the first day of the fifth instar to spinning.

iv) Observations recorded

Various economic characters viz., mature larval weight, fifth instar larval duration, effective rate of rearing (ERR), cocoon weight, shell weight, shell ratio, and filament length were observed. For recording the data, randomly selected 10 samples (larvae, cocoon and shell) were used from the experiments.

Statistical analysis

The experiments were conducted by taking up silkworm rearing two times in Completely Randomized Design. Each treatment was replicated three times with 50 larvae per replication. Statistical analysis of data was done using the method suggested by Panse and Sukhatme (1957). The results of the experiments are presented below.

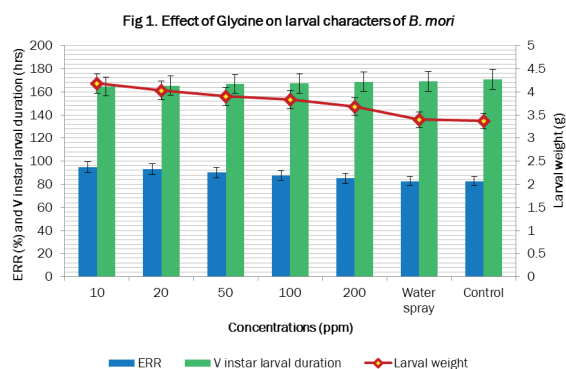
RESULTS AND DISCUSSION

Per os administration of amino acids such as glycine, alanine and serine in various concentrations viz., 10, 20, 50, 100 and 200 ppm to *B. mori* larvae once daily in the morning starting from first day of fifth instar to spinning significantly increased the larval as well as cocoon economic characters. The results of the experimental studies undertaken are given below elaborately.

I. Influence of Glycine on larval and cocoon characters

a. Larval characters

The studies showed that the enrichment of mulberry leaves with glycine in varied concentrations elucidated positive effects on the biological characters of silkworm larvae (Fig. 1). *Per os* administration of glycine @ 10 ppm significantly improved the mature larval weight to 4.18 g from 3.37 g which was statistically superior over all other concentrations followed by 20 ppm (4.03 g). The concentrations such as 50 and 100 ppm were found to be statistically on par with each other. Among the different concentrations tested, 200 ppm recorded minimum mature larval weight of 3.68 g; however, this was found to be statistically higher than the control. This result is in accordance with the findings of Sridhar and Radha (1986), who reported the highest larval weight gain at 10 ppm level of glycine. Saad *et al.* (2019) observed that the feeding the *B. mori* larvae with mulberry leaves immersed in 1 per cent glycine during fifth instar once resulted in significant increase in mature larval weight (5.12 g) over the control (4.74 g). This finding also falls in line with the present observation.



The effective rate of rearing (ERR) is the main indicator for assessing successful silkworm rearing. The present experiments proved that glycine @ 10 ppm was the best treatment in ERR (95.00 %), among the various concentrations studied, followed by 20 ppm (92.95 %). The concentrations namely 50 and 100 ppm registered ERR of 90.10 and 87.50 per cent, respectively and were found to be statistically on par with each other. The minimum ERR of 82.70 per cent was recorded in the control. Here, the enhancement in ERR ranged from 2.50 to 9.38 per cent. The present result is in parallel with the findings of Ito and Arai (1965) and Chakrabarthy and Kaliwal, (2012), who reported that higher ERR indirectly indicates the lesser susceptibility of the silkworm larvae to dreaded diseases and this resistance was due to conversion of amino acids into antibodies.

The reduction in larval duration is beneficial since it reduces the labour requirement for feeding the silkworm. In the present experiment, the fifth instar larval duration was significantly brought down to 164.50 hrs from 170.60 hrs due to the application of glycine at 10 ppm concentration. This was on par with 200 ppm and significantly superior over all other concentrations tested. The larval batch fed with 50, 100 and 200 ppm treated mulberry leaves did not differ statistically with each other. This result fall in line with Ito and Arai, (1965), Mustafa and Elkaraksy (1990), Vadivel (1995) and Saad *et al.* (2019) who reported that supplementation of glycine significantly decreased the fifth instar larval duration.

b. Cocoon characters

Investigations obviously proved that fortification of mulberry at varied concentrations of glycine and feeding the silkworm larvae had significant impact on the cocoon characters (Table 1). Maximum cocoon weight of 2.10 g was registered in the larval batch fed with 10 ppm of glycine sprayed mulberry leaves, which was followed by 20 ppm (2.03 g). The treatments, 50 and 100 ppm, were statistically on par by registering the cocoon weight of 1.96 and 1.93 g, respectively. Among the different concentrations of glycine, the lower cocoon weight

of 1.85 g was registered in 200 ppm, however, this treatment showed improvement statistically over the control (1.77 g). The present observation is in parity with the findings of Murthy and Srinivasaya (1953), who reported that supplementation of glycine, increased the cocoon weight by enhancing the sericin and fibroin synthesis in the posterior and middle silk gland respectively. The report by Saad *et al.* (2019) revealed that feeding the silkworm during fifth instar once with mulberry leaves treated with 1 per cent glycine significantly enhanced cocoon weight to 1.64 g from 0.956 g in control, also corroborates the present experimental results.

Shell weight and shell ratio are important characters that denote the available silk filament in a cocoon, based on which the floor price of the cocoon is arrived. In the present study, the shell weight and shell ratio of 0.46 g and 21.90 per cent, respectively, were recorded in the larval batch treated with glycine @ 10 ppm. The treatment with 50 ppm concentration was found to be statistically on par with 20 and 100 ppm in the case of shell weight and shell ratio. The lowest shell weight and shell ratio of 0.29 and 16.38 per cent, respectively, were registered in the control. This result corroborates with the previous findings of Krishnappa (1987), Kabila *et al.* (1994), Ravi *et al.* (1994) and Vadivel (1995), who have reported that application of glycine at 10 ppm either individually or in combination increased the shell weight and shell ratio.

Table 1. Effect of glycine on cocoon characters of *B. mori*

Concentration (ppm)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)	Filament length (m)
10	2.10	0.46	21.90	1096
20	2.03	0.42	20.68	1075
50	1.96	0.38	19.38	1052
100	1.93	0.36	18.65	1033
200	1.85	0.32	17.30	1027
Water spray	1.77	0.29	16.38	1006
Control	1.77	0.29	16.38	1002
SEd	0.03	0.01	0.60	8.60
CD (P=0.05)	0.06	0.03	1.20	17.00

Values are mean of three replications and pooled mean of two rearing.

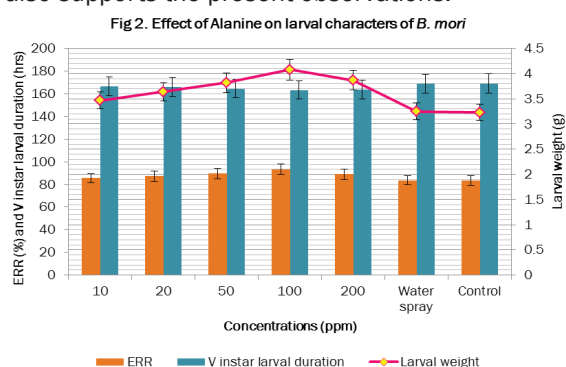
The application of glycine to silkworm larvae enhanced the length of silk filament. Significantly highest filament length of 1096 m was recorded in the larval batch fed with mulberry leaves having 10 ppm glycine. The next better concentrations were in the order of 20, 50, 100 and 200 ppm. The lowest filament length of 1002 m was observed in the control. Here, the improvement in the filament length ranged from 2.50 to 9.38 per cent. Fibroin content directly affects filament length, which might be largely influenced by lower concentration of glycine. The present observation is in line with the findings of Kodie *et al.* (1965), who observed that incorporating glycine residue into the fibroin leads to an increase

in filament length. Further, the reports over the period of time by various researchers (Sengupta *et al.*, 1972; Sridhar and Radha, 1986 and Krishnapa, 1987) showing enhanced filament length due to the application of glycine also confirm the present observations. In addition to this, the finding by Saad *et al.* (2019), who reported that feeding the fifth instar worms with mulberry leaves treated with 1 per cent glycine, elucidated a significant increase in the length of silk filament from 1226.25 m (control) to 1466.44 m, also falls in line with the experimental results.

II. Influence of alanine on larval and cocoon characters

a. Larval characters

Supplementation of amino acid with mulberry leaves invariably increased the larval characters of silkworm over the control (Fig. 2). The application of alanine @ 100 ppm significantly increased the larval weight (4.08 g) than all other concentrations. The treatments having 200 and 50 ppm were found to be on par with each other with the cocoon weight of 3.87 and 3.82 g, respectively. These concentrations were followed by 20 and 10 ppm. The lowest mature larval weight of 3.23 g was registered in the control. This result is very well in line with the findings of Sengupta *et al.* (1972), Mustafa and Elkarasky (1990) and Ravi *et al.* (1994), who reported an increase in mature larval weight by supplementation of amino acid mixture. Radjabi (2010) observed that fortification of mulberry leaves with 0.1 per cent alanine and feeding the silkworm larvae from the beginning of the fourth instar to the end of the fifth instar, once in a day significantly improved the larval weight to 2.91 g from 2.58 g in control. This finding also supports the present observations.



Significantly superior ERR of 93.40 per cent was recorded in alanine @ 100 ppm over the control. The next better treatments, 200 and 50 ppm were found to be on par with each other, which were followed by 20 and 10 ppm. The least ERR of 83.62 per cent was observed in the control. The ERR was increased from 2.22 to 11.70 per cent over the control due to application of alanine. The present result is in

parallel with the findings of Ito and Arai (1965) and Chakrabarthy and Kaliwal (2012), who observed enhanced resistance and lesser susceptibility of the larvae to deadly infectious diseases, increased ERR and cocoon formation.

Irrespective of the concentrations, the application of alanine significantly reduced the fifth instar larval duration. The treatment with 100 ppm registered minimum larval duration of 163.40 hrs, which was statistically on par with 200 ppm (163.75 hrs) and 50 ppm (164.50 hrs). These were followed by 20 and 10 ppm concentrations. The fifth instar larval duration of larval batch fed with untreated mulberry leaves lasted for 170.20 hrs. These observations can be corroborated with the findings of Mustafa and Elkarasky (1990), who found that supplementation of glycine, alanine and serine to silkworm significantly decreased the fifth instar larval duration.

b. Cocoon characters

The oral application of alanine resulted in improvement in cocoon related characters (Table 2). Among different concentrations studied, alanine @ 100 ppm registered highest cocoon weight of 2.02 g which showed statistical superiority over all other treatments. This was followed by 200 ppm (1.95 g) and 50 ppm (1.90 g) which were statistically on par. The least cocoon weight of 1.61 g was observed in the larval batch fed with untreated mulberry leaves. Here, the gain in cocoon weight ranged from 6.21 to 25.47 per cent over the control. This result got strengthened with the findings of Chakrabarthy and Kaliwal (2012), who reported that supplementation of amino acids to silkworm increased the cocoon parameters. Further, the present observations are strengthened by findings of Radjabi (2010), who reported that feeding the silkworm with alanine (0.2 %) fortified mulberry leaves during fourth and fifth instars of silkworm, once in a day led to significant increase in cocoon weight (1.51 g) over the control (1.35 g).

Table 2. Effect of alanine on cocoon characters of *B. mori*

Concentration (ppm)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)	Filament length (m)
10	1.71	0.34	18.18	1030
20	1.80	0.37	20.56	1054
50	1.90	0.40	20.05	1076
100	2.02	0.45	22.28	1105
200	1.92	0.41	21.35	1082
Water spray	1.61	0.28	17.39	1005
Control	1.61	0.28	17.39	1000
SEd	0.04	0.01	0.30	9.50
CD (P=0.05)	0.08	0.02	0.60	19.00

Values are mean of three replications and pooled mean of two rearing.

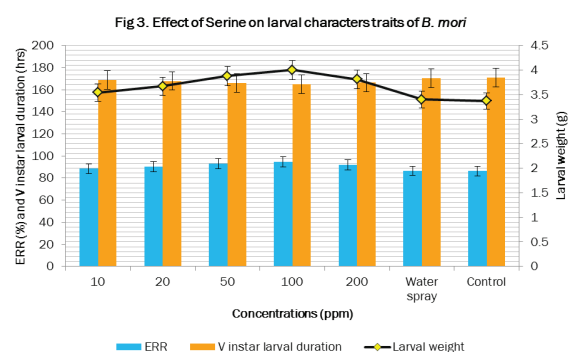
The highest shell weight and shell ratio of 0.45 g and 22.18 per cent, respectively, were recorded in 100 ppm of alanine, which was found to be statistically on par with 200 ppm (0.43 g and 22.18 %, respectively). Here, the increase in shell weight and shell ratio was 21.43 and 60.71 per cent, respectively. This treatment was followed by 50 ppm (0.40 g and 20.05 %, respectively) and 20 ppm (0.37 g and 20.56 %, respectively) which did not differ statistically. The lowest shell weight and shell ratio were recorded in the control. Nagarajan and Radha (1990) observed increased cocoon productivity, shell weight and shell ratio by feeding the silkworm with enriched mulberry leaves with tyrosine, phenylalanine and alanine. Significantly maximum shell weight and shell ratio of 0.335 g and 20.94 per cent, respectively, were observed, when the silkworm larvae were fed by mulberry leaves treated with alanine @ 1 per cent (Radjabi, 2010). This finding falls more or less in line with the present observations.

Filament length is a very important one among the various characters that contribute to higher silk yield. Silk filament length showed great deal of variation among different treatments. The longest filament with the length of 1105 m was registered in the larval batch treated with 100 ppm alanine followed by 200 and 50 ppm; however, both the concentrations did not have significant difference. The minimum filament length of 1000 m was observed in the control. The present observation falls well in line with the findings of Nagarajan and Radha (1990), who observed enhanced silk filament length by supplementing tyrosine, phenylalanine and alanine along with mulberry leaves.

III. Influence of serine on larval and cocoon characters

a. Larval characters

Feeding the silkworm larvae with mulberry leaves sprayed with serine at varied concentrations elucidated significant improvement on biological characters of larvae (Fig. 3). The highest mature larval weight of 4.00 g was observed in 100 ppm. Here, the weight gain in the mature larval is 18.69 per cent over the control. The next better concentrations were in the order of 50, 200, 20 and 10 ppm. The least mature larval weight of 3.37 g was registered in the larval batch fed with untreated mulberry leaves. The present observations can be corroborated with the studies of Vadivel (1995) and Gokul (2015) who reported that the supplementation of amino acids viz., glycine and serine resulted in increased fifth instar larval weight.



Highest ERR of 94.70 per cent was recorded in 100 ppm which was found to be statistically superior over all other concentrations. This was followed by 50 (92.95 %) and 200 (92.00 %) ppm, which were found to be on par among themselves. The least ERR of 86.30 per cent was observed in control. This observation falls more or less in line with the findings of Vadivel (1995) who reported that oral application of different amino acids had resulted in increased ERR and other economic traits of silkworm. Ramesh *et al.* (2018) reported that feeding the *B. mori* larvae with mulberry leaves treated with 0.25 per cent serine during third, fourth and fifth instars pronounced significant enhancement in ERR (88.95 g) over the control (82.27 g). This finding also supports the present observations.

The least fifth instar larval duration of 165.00 hrs was noted in the larval batch fed by mulberry leaves fortified with 100 ppm serine, which was found to be on par with 50 and 200 ppm. Here, the reduction in fifth larval duration ranged from 1.81 to 3.51 per cent. This result falls in line with Mustafa and Elkaraksy (1990), who reported that supplementation of glycine, alanine and serine significantly decreased the fifth instar larval duration.

b. Cocoon characters

Studies clearly proved that enrichment of mulberry leaves with Serine in different concentrations showed significant improvement on the cocoon characters of silkworm (Table 3). Highest cocoon weight of 2.05 g was observed in the larval batch fed by mulberry leaves sprayed with 100 ppm of serine, which showed statistical superiority over all other concentrations. The cocoon weight was increased by 19.88 per cent over the control due to application of amino acid. This treatment was followed by 50 (1.98 g) and 200 (1.95 g) ppm, which were on par with each other. The minimum cocoon weight of 1.71 g was observed in control. The present observations are in agreement with Gokul (2015), who revealed that oral application of serine @ 100 ppm from first day of fifth instar to spinning; daily once in the morning significantly enhanced the cocoon weight to

2.04 g from 2.00 g in control. Ramesh *et al.* (2018) also observed that supplementation of serine @ 0.25 per cent to late-age silkworm significantly increased the cocoon weight by 66.12 per cent. This finding also supports the present observations.

Table 3. Effect of serine on cocoon characters of *B. mori*

Concentration (ppm)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)	Filament length (m)
10	1.80	0.31	17.22	1026
20	1.87	0.35	18.72	1047
50	1.98	0.40	20.20	1075
100	2.05	0.45	21.95	1100
200	1.95	0.39	20.00	1068
Water spray	1.71	0.27	15.79	1007
Control	1.71	0.27	15.79	1003
SEd	0.03	0.02	0.49	9.10
CD (P=0.05)	0.06	0.03	1.00	18.00

Values are mean of three replications and pooled mean of two rearing.

The administration of serine also improved the shell weight and shell ratio of the cocoon, irrespective of the concentrations. However, maximum shell weight and shell ratio of 0.45 g and 21.95 per cent, respectively were recorded in 100 ppm. Here, the enhancement in shell weight and shell ratio was 29.63 and 12.69 per cent, respectively over the control. The next better treatments were in the order of 50 and 200 ppm, however, both of which did not differ statistically from each other. The least shell weight and shell ratio of 0.27 g and 15.79 per cent, respectively, were observed in control. Enrichment of mulberry leaves with serine @ 0.25 per cent and feeding during late instars of silkworm significantly improved the shell weight to 0.80 g from 0.60 g in control. Likewise, the same concentration also enhanced the shell ratio to 20.02 from 18.07 per cent in control (Ramesh *et al.*, 2018). Further, Gokul (2015) found that fortification of mulberry leaves with 100 ppm serine and feeding the larvae from the beginning of the fifth instar until spinning, once in a day showed superior shell weight (0.47 g) and shell ratio (22.08 %) over the control. The present observations got strengthened by the above findings.

The filament length of the cocoon was increased due to *per os* application of alanine to silkworm larvae. The improvement ranged from 2.29 to 9.67 per cent with maximum silk filament length of 1100 m. The minimum filament length of 1003 m was registered in the control. Shyamala and Gowda (1980) and, Chakrabarthy and Kaliwal (2012) reported that supplementation of amino acids individually or in combinations increased the silk filament length and raw silk productivity. The supplementation of glycine and serine increased filament length and weight by increasing fibroin and sericin, respectively (Sridhar and Radha, 1986; Krishnapa, 1987; Dai *et*

al., 1987; Asakura *et al.*, 1991). The filament length was significantly enhanced to 1193 m from 1031 m (control) due to the application of serine @ 100 ppm to silkworm (Gokul, 2015). Ramesh *et al.* (2018) found that feeding the late-age silkworm with mulberry leaves sprayed with 0.25 per cent serine elucidated a significant increase in the length of silk filament from 810 m (control) to 964 m. The above findings are in parity with the present observations.

CONCLUSION

It is clear from the studies that supplementation of glycine, alanine and serine at the concentration of 10, 100 and 100 ppm, respectively to silkworm larvae once daily in the morning from the first day of the fifth instar to spinning improved the larval growth as well as cocoon characters. *Per os* administration of amino acids at specific concentrations also significantly decreased the fifth instar larval duration.

Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There was no conflict of interest in the publication of this content

Data availability

All the data of this manuscript are included in the M.S. No separate external data source is required. If anything is required from the M.S., certainly, this will be extended by communicating with the corresponding author through corresponding official mail : murugehka2002@yahoo.co.in

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