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RESEARCH ARTICLE

Effects of Amino Acids on Food Intake, Silk Gland and Protein Content of Silkworm, *Bombyx mori* L

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ABSTRACT

Studies were undertaken to assess the effects of amino acids on the food intake, silk gland, and protein content of silkworm, *Bombyx mori* L. Three amino acids, namely Glycine, Alanine and Serine at various concentrations *viz.,* 10, 20, 50, 100 and 200 ppm were used for the investigations. The silkworm larvae were supplemented with amino acids, once daily in the morning from first day of fifth instar to spinning. Experimental results showed that irrespective of the concentrations studied, the amino acids had positive influence on the food intake, silk gland, and protein content of silkworms. Though all the treatments were found to significantly increase different parameters namely co-efficient of food utilization, weight and length of silk gland, protein content in silk gland, haemolymph and cocoon the enhancement was highest at concentration of 10 ppm, 100 ppm and 100 ppm, respectively in Glycine, Alanine and Serine.

Keywords: *Bombyx mori; Food intake; Protein; Haemolymph; Silk gland; Amino acids.*

INTRODUCTION

Mulberry silkworm, *Bombyx mori* L., a sericigenous insect, was domesticated for more than five thousand years due to continuous rearing. It secretes a long, lustrous shining silk filament, called *"Queen of Textiles"*. The silkworm is a monophagous insect feeds exclusively on mulberry (*Morus* spp.) leaves. Due to continuous biological as well as metabolic reactions in the larvae, the mulberry protein gets converted into silk proteins such as fibroin and sericin. The nutritional quality of mulberry leaves plays an important and indispensable role in enhancing the cocoon productivity and raw silk quality. Koul (1989) reported that the mulberry leaves suitable as food for silkworm should contain various biochemical components such as water (80%), proteins (27%), carbohydrates (11%), minerals, vitamins, etc. Many-a-times, the poor management of mulberry gardens results in the production of nutrient deficient leaves, ultimately leading to cocoon crop loss.

Fortification of mulberry leaves with various nutrients is one of the strategies by which cocoon productivity can be increased and the silk quality can be improved. Better growth and development of silkworms and the production of good quality cocoons could be achieved by feeding the silkworm larvae on nutritionally enriched mulberry leaves (Seki and Oshikane, 1959). The studies by different workers on fortification of mulberry leaves with essential nutrients like vitamins (Etebari *et al.,* 2004, Rahmathulla *et al.,* 2007); hormones (Magadum *et al.,* 1992; Saha and Khan, 1997), amino acids (Qadar *et al.,* 1994; Saha *et al.,* 1994; Saha and Khan, 1997) and minerals (Magadum *et al.,* 1992; Khan and Saha, 1995) were undertaken in different period of time to increase the larval growth and development of silkworm.

In addition to the above investigations, the supplementation of mulberry with different amino acids *viz.,* glycine, alanine and serine (Mustafa and Elkaraksy, 1990), tyrosine, phenylalanine and alanine (Nagarajan and Radha, 1990), glycine (Ravi *et al.,* 1994), glycine, phenylalanine, serine and aspartic acid (Vadivel, 1995), asparginine (Radjabi *et al.*, 2010) and arginine and histidine (Chakrabarthy and Kaliwal, 2012) showed significant enhancement in larval and cocoon characters as well as raw silk productivity.

Perusal of reports by earlier experiments indicates that among the various fortification studies with vitamins, hormones, amino acids, and minerals, the enrichment of mulberry leaves with amino acids was found to be more promising since it greatly influenced the mulberry leaf quality and the cocoon yield. However, the findings with respect to the influence of amino acids on food consumption and protein content are very limited. Hence, an earnest attempt was made to investigate the effects of amino acids on food intake, silk gland traits, and protein content in mulberry silkworm.

Materials and Methods

Experiments were undertaken to assess the effects of amino acids *viz.,* Glycine, Alanine and Serine in food consumption, silk gland parameters and protein content in silkworm, *B.mori*. The methodology adopted and materials used for the experiments are given below in detail.

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i) Rearing of silkworm

The rearing of Double Hybrid silkworm was conducted using mulberry leaves (variety: G4) from a well-maintained three years old plantation by adopting standard procedures including disinfection of rearing house and appliances as described by Dandin and Giridhar (2014). The rearing bed was applied with disinfectant at 5 g/sq. ft., immediately after every moult, to prevent infectious diseases as precaution (Baig and Pradip Kumar, 1987). The ripened larvae were allowed to spin the cocoon in Netrika and well-built cocoons were harvest on sixth separately replication-wise.

ii) Application of amino acids

The amino acids *viz.,* Glycine, Alanine and Serine with the quantity of 500 mg were dissolved in one litre of distilled water separately, serving as a stock solution (500 ppm). From the stock solution, the needed concentrations *viz.,* 10, 20, 50, 100 and 200 ppm were arrived by serial dilution.

The fresh mulberry leaves were harvest on 60 days after pruning and weighed using electronic balance. These leaves were sprayed with an aqueous solution of respective amino acid and shade dried for 30 minutes. The treated leaves were fed to silkworm larvae, daily once in the morning starting from first day of fifth instar until spinning. One batch of larvae fed on mulberry leaves sprayed with distilled water was maintained along with untreated control *i.e.,* larval batch fed on normal mulberry leaves without any treatment / spray for comparison.

iii) Observations recorded

a) Food consumption

Weighed quantity of mulberry leaves were fed to the fifth instar larvae. Before each feeding, the larval litter and left over leaves were collected and weighed separately. The data recorded were used for computing the co-efficient of food utilization (El Garhy, 1974).

$$\text{Co-efficient of food utilization} = \frac{\text{Weight of food utilized}}{\text{Weight of food consumed}} \times 100$$

Where,

Weight of food consumed = Weight of leaves offered – weight of left over leaves

Weight of food utilized = Weight of food consumed–weight of silkworm faeces

b) Silk gland parameters

The silk gland was dissected out with sterilized surgical scissors from individual larva on fifth day of fifth instar and the weight was recorded with an electronic balance, and length was measured using scale.

c) Protein estimation

The silk gland was dissected out from the silkworm on fifth day under aseptic condition and macerated with pestle and mortar in phosphate buffer (pH 7.0). Then, the supernatant was collected after centrifuging the content at 5000 rpm at 4° C and kept at -20^{0}C for protein analysis. The haemolymph was collected in microtubes by cutting the prolegs of silkworm, which was centrifuged at 14000 rpm and after removing the supernatant kept at –20 °C for analysis (Etebari *et al.,* 2007). The estimation of protein content was carried out by adopting standard methodology described by Bradford (1976).

The proteins namely sericin and fibroin were analyzed by taking known quantity cocoon in a weighed crucible, to which 20 ml of 5 per cent sodium hydroxide was added and allowed to remain soaked for 12 hours. The sericin was completely removed by washing with boiling distilled water repeatedly, leaving behind the fibroin. Then the crucible containing fibroin was oven dried at 90° C for 24 hours. The percentage of fibroin and sericin was calculated using standard procedure (Radha and Muthukrishnan, 1981).

iv) Data analysis

The rearings of silkworm were taken up two times for each set of experiment in Completely Randomized Design. Three replications were maintained for each treatment having equal larvae per replication (50 Nos). The collected data were analyzed as Panse and Sukhatme (1957) suggested. The results of experiments are presented below.

RESULTS AND DISCUSSION

The *per os* administration of amino acids namely Glycine, Alanine and Serine at different concentrations *viz.,* 10, 20, 50, 100 and 200 ppm to silkworm larvae during fifth instar significantly increased the food intake, silk gland traits and protein content in *B. mori*.

A. Effects of amino acids on food intake and silk gland traits

Glycine

Enrichment of mulberry leaves with Glycine and feeding the silkworm showed improvement on food intake efficiency of the larvae (Fig. 1). Among the different concentrations tested, the CFU was maximum in Glycine @ 10 ppm (82.45) which was found to be statistically superior over all other concentrations. The next better treatments were 20 and 50 ppm which registered CFU of 79.80 and 77.28, respectively, and were found to be statistically on par with each other. These concentrations were followed by 100 and 200 ppm. The minimum CFU of 68.05 was recorded in the control. The enhancement in CFU ranged from 5.70 to 2.16 per cent. The present observations fall in line with the Vadivel (1995), who reported significantly higher CFU of

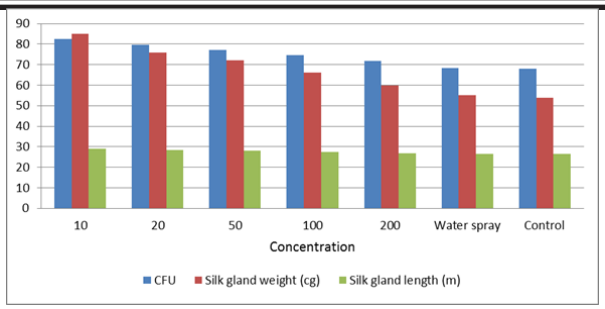

Fig. 1 Effect of Glycine on Coefficient Food Utilization (CFU) and silk gland traits

73.16 when the fifth instar silkworm larvae were fed on mulberry leaves sprayed with Glycine @ 10 ppm than the control (69.09). The present results also got strengthened with the findings of Chakravorthy *et al.* (2003) who observed the highest CFU when larvae were fed on amino acids treated mulberry leaves.

The application of amino acid to silkworm also improved the silk gland related parameters. The silk gland weight decreased as the concentration of Glycine increased. The weight and length of silk gland, the second largest organ of silkworm was highly enhanced by the administration of Glycine. Significantly highest silk gland weight and silk gland length of 85 cg and 29.00 m, respectively were observed in Glycine @ 10 ppm. Here, the enhancement in silk gland weight and silk gland length were 57.40 and 9.77 per cent, respectively over the control. The next better treatments were 20 and 50 ppm which showed statistical parity with each other. This was followed by 100 and 200 ppm concentrations. The silk gland weight is directly related to the quantity of fibroin present in the posterior region and sericin in the middle region of the silk gland. Corroborative evidences were shown by previous workers (Krishnappa, 1987; Ravi *et al.,* 1994 and Saad *et al.,* 2019), who have registered that glycine application at minimum concentration either individually or in combination increased the silk gland weight and length.

Alanine

Per os administration of Alanine at all the concentrations produced positive response in silkworm larvae (Fig. 2). Significantly highest CFU of 81.80 was observed in Alanine @ 100 ppm concentration. This was followed by 200 ppm (79.05) which was found to be on par with 50 ppm (76.40). The next better concentrations were 20 and 10 ppm. The lowest CFU was registered in the control. Supplementation of mulberry leaves with Alanine @ 100 ppm significantly improved the CFU to 82.45 from 76.72 in the control (Gokul, 2015). This finding strengthens the present observations.

The silk gland related parameters were also positively altered due to application of Alanine. The maximum silk gland weight and silk gland length of 86 cg and 28.50 m, respectively were observed in the larvae treated with 100 ppm Alanine. The administration of Alanine @ 100 ppm improved the silk gland weight and length by 45.76 and 8.61 per cent, respectively over the control. The next better concentrations were 200 and 50 ppm which were found to be statistically on par with each other and superior over to 20 and 10 ppm. Chen *et al.* (1967) and Radjabi *et al.* (2010) observed that supplementation of amino acids to silkworm larvae

has increased the silk gland weight and length, and also brought an increase in cocoon weight over the control. These findings are in line with the present observations.

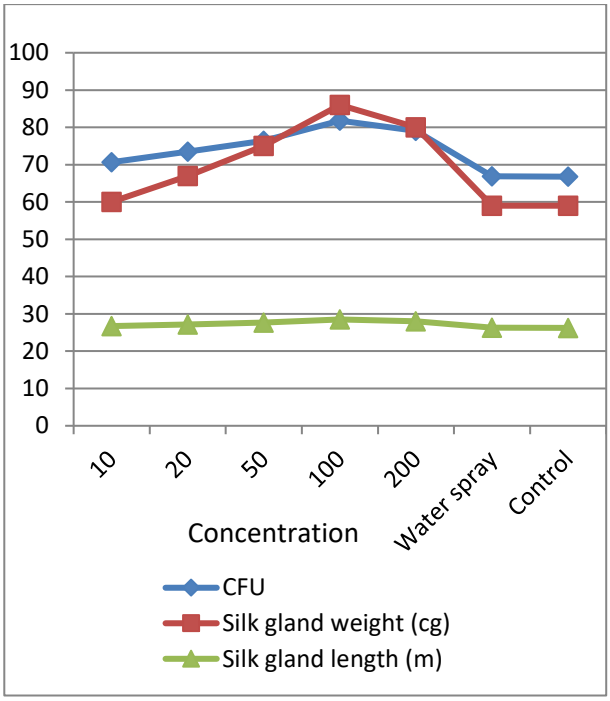

Fig. 2 Effect of Alanine on CFU and silk gland traits

Serine

The oral application of serine significantly influenced the food intake and silk gland related parameters of silkworm at all the concentrations (Fig. 3). However, maximum CFU of 81.25 was observed in Serine @ 100 ppm, which showed statistical superiority over all other treatments. Here, the enhancement is 21.67 per cent over the control. This was followed by 200 ppm which registered CFU of 78.60. The next better concentrations were 50 and 20 ppm which were found to be statistically on par with each other. The present results are in parity with the finding that fortification of mulberry leaves with serine and feeding to silkworm during fifth instar significantly enhanced the CFU to 74.12 from 68.86 in the control (Vadevel, 1995).

Highest silk gland weight and silk gland length of 85 cg and 29.20 m, respectively were observed in the larval batch fed on mulberry leaves sprayed with serine @ 100 ppm, which was found to be statistically superior over all other concentrations. This was followed by 200 ppm which registered the silk gland weight and silk gland length of 0.80 g and 28.80 m, respectively. Here, the concentrations *viz.,* 20 and 10 ppm were found to be statistically on par with each other. The least value of 0.59 g and 27.24 m, respectively for silk gland weight and silk gland length were observed in the control. Here, the improvement ranged from 6.78 to 44.07 per cent in case of silk gland weight and from 1.65 to 7.20 per cent in case of silk gland length over the control. The present observations can be corroborated with the findings of Gokul (2015), who recorded significantly heavier silk gland with the weight of 0.84 g and length of 29.50 cm, when the fifth instar silkworm larvae were fed on mulberry leaves sprayed with serine @ 100 ppm.

B. Effects of amino acids on protein content

Glycine

Feeding the silkworm larvae from first day of fifth instar to spinning stage with Glycine fortified mulberry leaves elucidated positive influence on protein content of silk gland, haemolymph and cocoon (Table 1). Silk gland protein is a parameter to scale the silk protein synthetic activity in silk gland. In the present investigations, significantly highest protein content in silk gland of 56.34 mg/g was observed when the larvae were fed on mulberry leaves enriched with Glycine @ 10 ppm. This was followed by 20 ppm which recorded silk gland protein content of 54.21 mg/g. The next better treatments were 50 and 100 ppm, which were statistically on par with each other and significantly differed from the control (31.34 mg/ml). The increase in silk gland protein might be due to the incorporation of glycine into the silk gland as observed by Shimura *et al.* (1976). Further, Sasaki and Noda (1973) observed that 44.45 per cent of the fibroin was glycine residue, which also strengthens the present observation. In addition to above, the present result fall in parallel with the findings of Vadivel (1995) and Saad *et al.* (2019), who registered increased silk gland protein level on supplementation of Glycine compared to the untreated control.

The same trend was observed in the haemolymph protein content, which was found to be significantly higher in 10 ppm glycine (39.85 mg/g) than all other concentrations. This was followed by 20 ppm. Here, the concentrations *viz.,* 50 and 100 ppm were found to be on par with each other. These observations were supported by the finding of Etebari *et al.* (2004), who reported that the silkworm larvae fed on mulberry leaves treated with 0.5 per cent glycine increased protein content in the haemolymph.

The silk proteins such as fibroin and sericin content in the cocoon showed greater improvement due to the application of Glycine. Glycine @ 10 ppm increased the fibroin and sericin to 356 and 83.50 mg/shell from 277 and 72.75 mg/shell, respectively in the control. This concentration was followed by 20, 50, 100 and 200 ppm. In the case of fibroin, 100 ppm was found to be statistically on par with 50 and 200 ppm, whereas, in the case of sericin, 50 ppm was found in parity with 100 ppm alone. The enhancement in silk fibroin might possibly be due to addition of glycine residues with the silk fibroin in the posterior region of silk gland. This was strengthened by the result is in parity with Asakura *et al.* (1991), who found that radioactive glycine immediately after administration entered from the midgut to haemolymph and then incorporated into the silk fibroin. The present observations can also be corroborated with the findings of Krishnapa (1987), who revealed that the supplementation of glycine at 1.5 per cent twice daily to fourth and fifth instars was helpful in improving the cocoon weight and proteins.

Saad *et al.* (2019) recorded significantly increased silk filament length due to enhancement in the cocoon protein contents on supplementation of mulberry leaves with glycine @ 1 per cent and feeding to the fifth instar silkworm larvae. This finding also falls in line with the present observations.

Alanine

Studies clearly showed that enrichment of mulberry leaves with varied concentrations of Alanine considerably increased the protein content of *B. mori* (Table 2). The maximum silk gland protein content of 54.50 mg/g was observed in the larval batch fed with 100 ppm Alanine sprayed mulberry leaves. This treatment was followed by 200 (53.15 mg/g), 50 (51.92 mg/g) and 20 (51.10 mg/g) ppm. Here, the treatment with 50 ppm was found to be statistically on par with 200 & 20 ppm. The minimum protein content of 48.36 mg/g was observed in the control. The present results are in accordance with the findings of Mustafa and Elkaraksy (1990), who proved that the administration of alanine to silkworm larvae through mulberry leaves significantly enhanced the crude protein in silk gland.

Highest quantity of haemolymph protein of 38.20 mg/ml was registered in Alanine @ 100 ppm which was found to be statistically superior over all other concentrations tested. The next better treatments were in the order of 200, 50, 20 and 10 ppm. The

increment in haemolymph protein due to the application of Alanine ranged between 5.15 and 19.90 per cent. The present observations fall in parallel with findings of Horie and Watanabe (1982); Asakura *et al.* (1991) and Radjabi *et al.* (2010), who registered increased protein level in haemolymph due to supplementation of amino acid particularly Alanine.

The application of Alanine elevated the cocoon proteins *viz.,* fibroin and sericin to a greater level over the control. Significantly higher fibroin and sericin content of 340 and 79.00 mg/shell, respectively were observed when the larvae were fed with Alanine @ 100 ppm fortified mulberry leaves, than the control. This was followed by 200 and 50 ppm, which were found to be statistically different in case of fibroin and on par in case of sericin. The least fibroin and sericin content of 235 and 68.59 mg/shell, respectively were registered in the control. The result of experiments undertaken by Mustafa and Elkaraksy (1990), and Vadivel (1995) showing an improvement in the fibroin and sericin content in the cocoon strengthen the present observations.

Serine

Supplementation of mulberry leaves with Serine invariably increased the protein content in silkworm larva and cocoon over the untreated control (Table 3). *Per os* application of Serine @ 100 ppm significantly enhanced the silk gland protein to 55.25 mg/g from 46.76 mg/g in the control. Here, the enhancement is 18.16 per cent over the control. This was followed by 50 (53.38 mg/g) and 200 (52.90 mg/g) ppm, which were found to be statistically on par with each other. The next better treatments were in the order of 20 and 10 ppm. Vadivel (1995) observed a significant increase in the silk gland protein content to 78.71 mg/g from 73.32 mg/g (Control) due to the administration of Serine @ 100 ppm to silkworm larvae during the fifth instar. Ramesh *et al.* (2018) observed that supplementation of silkworm with serine @ 0.25 per cent during late-age significantly increased the protein content over the control. This finding strengthens the present observation.

Feeding the silkworm larvae on mulberry leaves sprayed with Serine at different concentrations significantly improved the protein content in larval haemolymph. The haemolymph protein was maximum in 100 ppm (37.90 mg/ml) which was found to be statistically superior over all other concentrations. This was followed by 50 and 200 ppm which showed statistical parity with each other. The minimum haemolymph protein of 31.00 mg/ml was observed in the control. The present observations were supported by Horie and Watanabe (1982) and Gokul (2015), who recorded increased haemolymph protein content on supplementation of mulberry leaves with amino acids.

Fibroin content was found to be significantly highest when the larvae were fed on mulberry leaves fortified with Serine @ 100 ppm (337 mg/shell). This was followed by 50 ppm (320 mg/shell) which was found to be on par with 200 ppm (312 mg/shell). The enhancement in fibroin content varied between 9.23 and 29.62 per cent over the control. This might be due to the incorporation of serine residues directly into fibroin in the posterior silk gland as observed by Shimura (1978). Vadivel (1995) recorded significantly higher fibroin content of 162.60 mg/shell in the larval batch fed with serine 100 ppm fortified mulberry leaves than the control (135.28 mg/shell).

Ramesh *et al.* (2018) observed that the feeding the late-age silkworm larvae on mulberry leaves treated with 0.25 per cent serine pronounced a significant improvement in the length of silk filament by secreting more quantity of fibroin. These findings more or less fall in line with the present observations.

Sericin was also significantly altered due to the administration of Serine to silkworm larvae. Highest amount of sericin (93.85 mg/shell) was observed in the treatment with Serine @ 100 ppm. This was followed by 50 and 200 ppm, both of which did not differ statistically. The lowest sericin content of 85.89 mg/shell was registered in the control. This might be due to the active participation of serine in sericin synthesis in the middle region of silk gland (Sinohara, 1979). Higher sericin content of 140 mg/shell was recorded when the larvae were fed on mulberry leaves sprayed with serine 100 ppm (Gokul, 2015). This result supports the present observation.

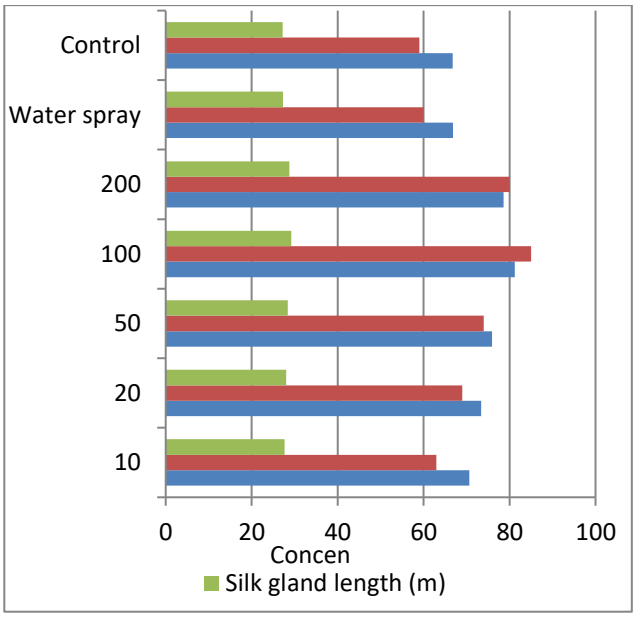

Fig. 3 Effect of Serine on CFU and silk gland traits

Table 1. Effect of Glycine on protein content

Concentration (ppm)	Silk gland protein (mg/g)	Haemolymph (mg/ml)	Fibroin (mg/shell)	Sericin (mg/shell)
10	56.34	39.85	356	83.50
20	54.21	38.00	338	81.25
50	52.00	36.24	321	78.92
100	51.25	35.16	314	78.05
200	49.04	33.22	300	76.00
Water spray	46.53	31.45	281	73.25
Control	46.27	31.34	277	72.75
SEd	**1.05**	**0.84**	**8.20**	**1.00**
CD (P=0.05)	**2.00**	**1.70**	**16.00**	**2.00**

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

Table 2. Effect of Alanine on protein content

Concentration (ppm)	Silk gland protein (mg/g)	Haemo lymph (mg/ml)	Fibroin (mg/shell)	Sericin (mg/shell)
10	49.85	33.50	263	71.50
20	51.10	34.96	284	74.10
50	51.92	35.48	297	75.00
100	54.50	38.20	340	79.00
200	53.15	36.85	320	76.25
Water spray	48.50	31.94	242	68.76
Control	48.36	31.86	235	68.59
SEd	**0.60**	**0.65**	**8.50**	**1.25**
CD (P=0.05)	**1.20**	**1.30**	**18.00**	**2.50**

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

Table 3. Effect of Serine on protein content

Concentration (ppm)	Silk gland protein (mg/g)	Haemo lymph (mg/ml)	Fibroin (mg/shell)	Sericin (mg/shell)
10	49.05	32.95	284	88.17
20	51.00	34.39	300	89.24
50	53.38	36.45	320	92.00
100	55.25	37.90	337	93.85
200	52.90	35.88	312	91.05
Water spray	46.82	31.15	265	86.00
Control	46.76	31.00	260	85.89
SEd	**0.90**	**0.70**	**7.05**	**0.88**
CD (P=0.05)	**1.80**	**1.40**	**14.00**	**1.75**

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

CONCLUSION

It is clear from the present study that supplementation of mulberry leaves with amino acids *viz.*, Glycine @ 10 ppm, Alanine @ 100 ppm and Serine @ 100 ppm, once daily in the morning from first day of fifth instar to spinning significantly increased the co-efficient of food utilization, silk gland traits such as weight and length as well as protein content in haemolymph, silk gland and cocoon of the silkworm.

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