International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 7; Issue 1(H); January 2018; Page No. 9258-9262
DOI: http://dx.doi.org/10.24327/ijcar.2018.9262.1524

Research Article

INFLUENCE OF FORTIFIED MULBERRY LEAF WITH METHIONINE ON THE ECONOMIC TRAITS AND AMINOTRANSFERASE ACTIVITY IN *BOMBYX MORI* (Lepidoptera; Bombycidae)

M. N. Anil Kumar* and Sunil Kumar. B

Department of Studies in Sericulture Science, University of Mysore, Mysuru, Karnataka, India, 570006

A R T I C L E I N F O

Article History:
Received 19th October, 2017
Received in revised form 10th November, 2017
Accepted 26th December, 2017
Published online 28th January, 2018

Key words:
Alanine and Aspartate aminotransferases, *Bombyx mori*, Economic traits, Methionine, Multivoltine breeds.

A B S T R A C T

The dietary proteins and amino acids are utmost important for the silkworm larvae due their active synthesis of silk protein in turn reflect on cocoon production. An attempt has been made in the current investigation to record the effect of mulberry leaves supplemented with methionine at varied concentrations *viz.,* 0.5, 1.0 and 1.5% on the economic traits as well as aminotransferase enzymes in multivoltine silkworm breeds MU303 and MU11. The results of the study revealed that, both the breeds expressed notable improvement for economic traits which include larval weight, cocoon weight, shell weight, filament length, filament weight, renditta and fecundity at 0.5 % methionine supplementation when compared to other concentrations. Further, aspartate aminotransferase activity level was relatively high when compared to alanine aminotransferase in the fat body tissue of both the breeds at 0.5 % concentration. The enzyme activity levels was maximum in prior to spinning stage followed by fifth instar 3rd day and fifth instar 1st day.

INTRODUCTION

The silkworm, *Bombyx mori* L, is sericigenous lepidopteran insect which produces silk in the form of cocoon at the end of the larval period. The mulberry leaf forms sole food and contributing nearly 70 per cent of silk proteins produced by the silkworm. The productivity in sericulture is dependent on palatability and suitability of leaf quality in different seasons. 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 (Quader, 1991; Bongale and Chaluvachari, 1993 and Anantharaman *et al.,* 1995). As the quality on mulberry leaf has an intimate relation to the healthy growth of the larvae and quality of their cocoons, the 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 of the silkworm. There is a correlation between the amount of mulberry leaves eaten by the silkworm and silk protein in the silk gland (Fakuda, 1960 and Fakuda *et al.,* 1963). The relationship between the amount of food intake and cocoon productivity was also studied by Takano and Arai (1978).

Corresponding author:* **M. N. Anil Kumar
Department of Studies in Sericulture Science, University of Mysore

These reports have confirmed some negative effects of nutritional restrictions on the larval growth and cocoon characters. The quality cocoons production is mainly depends upon nutritional composition of mulberry leaf. However, utilization of these nutrients varies among the silkworm breeds. The proper utilization of these nutrients effectively by the silkworms depends on the balance between anabolic and catabolic process with the aid of various biomolecules including enzymes.

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 were in the range of 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 composition 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 (Ito and Tanka, 1962; Ito and Mukaiyama, 1964, 1970; Kamioka *et al.,* 1971 and Horie *et al.,* 1971). 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 reported by Seol (1982).

The silkworm requires essential, non-essential and acidic amino acids for its growth and development. These essential amino acids are biosynthesized in silkworm only when precursors are provided in the diet. If the diet lacks any one of these amino acids, the growth and development of silkworm is get affected. The non-essential amino acids are inter convertible and are formed rapidly from aspartate or glutamate by transamination process. The deletion of any one of non-essential amino acid from the diet has virtually no effects on growth and development of silkworm. In recent years, adequate work has been undertaken on dietary supplementation of amino acids to improve rearing and reeling parameters. However, not much information is available on mulberry leaves supplemented with methionine on economic traits and aminotransferase enzymes of the silkworm. Hence, the present investigation is undertaken.

MATERIALS AND METHODS

The multivoltine silkworm breeds namely MU_{303} and MU_{11} were selected for the present investigation and larvae were reared by employing standard rearing techniques has advocated by Dandin and Giridhar (2010).

Supplementation of methionine

The methionine at varied concentrations *viz.,* 0.5, 1.0 and 1.5% were sprayed on ventral surface of mulberry leaf and surface dried under shade and before feeding to the silkworms. The silkworm larvae divided into five batches *viz.,* batch I (T1), batch II (T2) and batch III (T3) were reared with methionine supplementation at 0.5, 1.0 and 1.5 %, respectively along with control batch IV (T4) reared on mulberry leaves supplemented with distilled water and batch V (T5) reared on natural diet (absolute control). The treated leaves were fed to silkworms once in a day (morning) during fourth and fifth instars. In each treatment three replications were maintained. A maximum of ten larvae were used from each batch to record the economic traits namely larval weight, cocoon weight, shell weight, shell ratio, filament length, filament weight, denier, renditta and fecundity. The parameters like shell percentage, filament length, denier and renditta were calculated by using following formulae.

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

Filament Length (L) = R×1.125

R = Number of revolutions recorded by an epprouvette.

1.125= Circumference of epprouvette in meter

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

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

Estimation of alanine and aspartate aminotransferase enzymes

The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity were estimated in fifth instar 1st day, 3rd day and prior to spinning stage (5th day) of silkworm breeds MU_{303} and MU_{11} in respective treatments and control batches. 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 supernatant was collected and used as a enzyme source. Both the enzymes was estimated by the method of Reitman and Frankel (1957). Aspartate aminotransferase was estimated by using 1ml of tissue extract was incubated with 0.5ml of glutamic oxalo acetate (substrate) at 37^0 C for 1 hour. To this reaction mixture, 0.5ml of 2,4-D and 5ml of 0.4 N NaoH was added. The colour intensity was measured at 510 nm by using spectrophotometer. For alanie aminotransferase, 0.5 ml glutamate pyurvate was used as a substrate and standard curve was used for calculation. The enzyme activity was expressed in terms of μ moles /g protein/h. The data obtained were analyzed by standard deviation (±) method and mean values were expressed.

RESULTS AND DISCUSSION

The nitrogen, which is the main component of amino acids and protein, is one of the essential elements for the growth and development of insects, which is generally obtained by feeding although some insects are able to maintain their need in some other processes. Silkworm uses 65% of absorbed nitrogen through 5th instar for silk production. Therefore nitrogen sources present in the diet can have high effects on larval growth and cocoon production (Horie and Watanabe, 1983; Uni *et al.,* 2000).

The 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. The 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 (Lu and Jiang 1988).Studies on mulberry leaves supplementation with nitrogenous compound and amino acids and the evaluation of their effects on silkworm rearing have been conducted and different conclusions have been obtained by several workers.

Matured larval weight

Silkworm breeds reared on fortified mulberry leaves with methionine at different concentrations exhibited notable influence on larval weight. The breeds MU_{11} and MU_{303} recorded higher larval weight of 2.071 and 2.056 g (Table.1) at 0.5 % of methionine supplementation as against absolute control. The increase in larval weight might be due to additional supplementation of methionine along with mulberry leaves. The present findings are in conformity with the finding of Chapman (1998) who has reported that, in order to have best larval growth, the insects needs optimum level of amino acids, being used for structural purposes such as enzymes and transport receptors. In the present study, lower concentration of methionine supplementation at 0.5% was found promising than their higher concentration. Etibari *et al.* (2007) observed that supplementation of glutamic acids and glycine at varied concentrations enhance larval weight in the bivoltine hybrid (107' 110). Similar results were also observed in some other amino acids by Kabila *et al.* (1994), Ravi *et al.* (1994) and Rahman Khan and Saha (1995).

Cocoon weight

The larvae reared on methionine at 0.5% expressed higher cocoon weight of 1.320 and 1.313g (Table.1) in MU_{11} and $MU_{303,}$ respectively. The increase in cocoon weight in both the breeds might be due to increase in absorption of methionine by midgut epithelial cells and transformation to cellular structure by the body cells. These results are in agreements with those of Daniel Nicodemo and Juliano Olivera (2014) who 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 by Rouhollah Radjabi (2010), Proline and amino acid mixture supplementation with mulberry leaves by Indira Bhojnae *et al.* (2014).

Shell weight

The worms supplemented with methionine at 0.5% exerted higher shell weight in MU_{11} (0.217g) and MU_{303} (0.215g), respectively (Table.1) over absolute control. The increase in shell weight might be due to additional supplementation of methionine which enhances the biosynthesis of silk protein. These results are in conformity with the findings of Kabila *et al.* (1994) who opined that supplementation of mulberry leaves with aspartic acid at 2% enhance shell weight. Similar trend was also noticed for some other amino acids by Sridhar and Radha (1986, 1987), Ravi *et al.* (1994) and Rahman Khan and Saha (1995).

Shell ratio

Silkworms nourished with mulberry leaves extra foliated with methionine exerted marked influence on shell ratio.

Table1 Influence of fortified mulberry leaves with methionine at varied concentrations on larval and cocoon characters

Treatment	Breed	Larval Weight (g)	Cocoon weight (g)	Shell Weight (g)	Shell Ratio (%)	Filament Length (m)	Filament weight (w)	Denier	Renditta	Fecundity (number)
0.5 %	MU303	2.056±0.032	1.313±0.001	0.215±0.008	16.40±0.616	508±6.245	0.103±0.003	1.819±0.023	10.649±0.310	450±2.517
	MU11	2.071±0.032	1.320±0.008	0.217±0.007	16.46±0.429	523±6.245	0.105±0.002	1.807±0.036	10.579±0.308	463±6.557
1.0 %	MU303	1.992±0.026	1.297±0.002	0.203±0.003	15.67±0.187	487±5.292	0.094±0.004	1.74±0.077	10.846±0.644	448±1.53
	MU11	2.004±0.026	1.291±0.003	0.208±0.003	16.11±0.202	498±5.033	0.098±0.003	1.77±0.046	10.83±0.657	461±7.77
1.5 %	MU303	1.971±0.045	1.213±0.010	0.185±0.008	15.28±0.675	464±5.508	0.088±0.002	1.70±0.049	11.23±0.112	448±2.082
	MU11	1.984±0.031	1.214±0.004	0.188±0.008	15.46±0.668	473±6.083	0.087±0.002	1.65±0.050	11.13±0.121	459±2.517
Distilled Water	MU303	1.92±0.009	1.201±0.005	0.176±0.004	14.63±0.232	456±13.748	0.083±0.002	1.63±0.048	11.62±0.049	450±1.528
	MU11	1.914±0.009	1.205±0.006	0.176±0.004	14.58±0.245	464±9.539	0.084±0.003	1.63±0.054	11.56±0.072	452±1.000
Absolute Control	MU303	1.854±0.043	1.120±0.020	0.162±0.003	14.44±0.067	434±9.866	0.080±0.000	1.65±0.039	11.95±0.142	445±2.646
	MU11	1.864±0.030	1.120±0.020	0.160±0.007	14.31±0.503	436±9.539	0.073±0.006	1.50±0.116	11.83±0.055	444±3.606

Table 2 Influence of fortified mulberry leaves with methionine at varied concentrations on aspartate aminotransferase activity in the fat body of multivoltine breeds

Treatment	Breed	V Instar 1st Day	V Instar 3rd Day	V Instar 6th Day
0.50%	MU303	423 ± 6.80	459 ± 7.10	508 ± 1.96
	MU11	434 ± 3.93	478 ± 1.54	518 ± 1.64
1%	MU303	359 ± 10.25	390 ± 6.85	423 ± 5.48
	MU11	380 ± 2.84	410 ± 3.90	446 ± 8.10
1.50%	MU303	314 ± 4.54	361 ± 26.98	388 ± 2.45
	MU11	332 ± 11.64	384 ± 6.42	402 ± 7.22
Distilled Water	MU303	294 ± 5.36	325 ± 636	362 ± 6.68
	MU11	310 ± 5.86	356 ± 4.92	384 ± 2.64
Absolute Control	MU303	376 ± 5.68	312 ± 2.36	340 ± 3.54
	MU11	295 ± 4.56	334 ± 5.25	369 ± 3.65

Table 3 Influence of fortified mulberry leaves with methionine at varied concentrationson alanine aminotransferase activity in the fat body of multivoltine breeds

Treatment	Breed	V Instar 1st Day	V Instar 3rd Day	V Instar 6th Day
0.50%	MU303	110 ± 1.489	158 ± 1.291	175 ± 3.941
	MU11	125 ± 3.308	167 ± 3.196	187 ± 2.589
1%	MU303	102 ± 1.747	120 ± 5.701	151 ± 7.135
	MU11	108 ± 5.798	129 ± 1.298	163 ± 2.339
1.50%	MU303	95 ± 1.219	109 ± 1.572	144 ± 2.168
	MU11	104 ± 3.843	118 ± 3.306	154 ± 7.308
Distilled Water	MU303	88 ± 1.764	102 ± 2.133	134 ± 0.643
	MU11	94 ± 1.997	106 ± 1.770	146 ± 4.463
Absolute Control	MU303	79 ± 5.263	91 ± 2.536	120 ± 3.486
	MU11	85 ± 2.369	99 ± 4.125	130 ± 1.965

A concentration of methionine at 0.5% resulted in higher shell ratio of 16.46 and 16.40% (Table.1) in MU_{11} and MU_{303}, respectively when compared to absolute control. Increase in the shell ratio might be due to increased silk production by additional supplementation of methionine. These results are also supported by the observations of Krishnappa (1987) who 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% (Kabila *et al.,* 1994).

Filament length

Filament length has positive correlation with shell weight. The silkworms fed on mulberry leaf fortified with methionine at varied concentrations registered marked influence on filament length. The multivoltine breeds MU_{11} and MU_{303} supplemented with methionine at 0.5% exerted longer filament length of 523 and 508 m (Table.1) over control batch. The increase in the filament length might be due to higher rate of silk protein synthesis by additional supplementation of methionine. These results are in conformity in the finding of Ravi *et al.* (1994) who observed that supplementation of mulberry leaf with glycine and soya powder enhances filament length over unsupplemented batch. Similar results are also reported in bivoltine breed NB_4D_2 supplemented with soya protein (Sundhar Raj *et al*., 2000a).

Filament weight

Silkworm breeds reared on fortified mulberry leaves with methionine at varied concentrations exhibited notable impact on filament weight. In the study, MU_{11} and MU $_{303}$ expressed gain in the filament weight of 0.105 and 0.103g (Table.1), respectively with methionine supplementation when compared to larvae reared on natural diet. The increase in filament weight in both the breeds might be due to higher rate of biosynthesis silk protein due to additional supplementation of methionine. These results are in agreement with the findings of Sridhar and Radha (1987) who have reported that supplementation of mulberry leaf with glycine enhances filament weight over control batch.

Denier

The larvae reared on methionine supplementation at 1.5% expressed lower denier of 1.65 in MU_{11} and 1.70 in MU_{303} (Table.1). The results are conformity in the findings of Sundhar Raj *et al.* (2001) who opined that fine denier (1.82) was recorded in Pure Mysore fed on mulberry leaves supplemented with soybean flour as against control (2.14).

Further, significantly fine denier (2.70) was registered in cocoons spun by the worms supplemented with soya bean flour over control batch (2.96) in PM×NB_4D_2 was also reported by Sundhar Raj *et al.* (2000b).

Renditta

The lowest renditta 10.57 and 10.64 (Table.1) were recorded in MU_{11} and MU_{303}, respectively. The improvement for this trait in both multivoltine breeds at 0.5% concentration of methionine 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 of Sridhar and Radha (1987) who have noticed that silkworm reared on mulberry leaf supplemented with 10ppm concentration of glycine exerted significant decrease in renditta. Further, silkworm hybrid PM × CSR_2 reared on mulberry leaves supplemented with proline and amino acid mixture at 1% improves the renditta than the 2% has reported by Indira Bhojne *et al.* (2014).

Fecundity

It is one of the fitness parameter. A concentration of methionine at 0.5% resulted in higher fecundity of 463 and 450 eggs (Table.1) in MU_{11} and MU $_{303}$, respectively over absolute control batch. The increase in fecundity in both breeds might be due to additional supplementation of methionine with mulberry leaves. The current results are in conformity with those of Rouhollah Radjabi (2010) who has reported that the bivoltine hybrid silkworm (103X104) supplemented with aspergine and alanine at varied concentrations enhances fecundity and hatching percentage due to increase in secretion of corpora allata leads to vitellogenesis. Similarly, supplementation of methionine and tryptophan were also increased fecundity in multivoltine breed BSRI-83/3 (Rezine laz, 2010). Similar trend was also noticed for some other amino acids by Krishnappa (1987).

Influence of supplemented mulberry leaves with methionine on alanine and aspartate aminotransferase activity

The silkworm breeds MU_{11} fed on mulberry leaves supplemented with methionine recorded highest activity level of aspartate aminotransfarase (AST) in the fat body at 0.5% concentration (518 μ moles oxalo acetate g/protein/ hour), (478 μ moles oxalo acetate g/protein/ hour) and (434 μ moles oxalo acetate g/protein/ hour) (Table.2) in 5^{th} instar 6^{th} day, 5^{th} instar 3^{rd} day and 5^{th} instar 1^{st} day, respectively. Similar trend was also noticed in MU $_{303}$ at 0.5% concentration. Similarly, the silkworm breed MU_{11} fed on mulberry leaves supplemented with methionine recorded highest activity level of alanine aminotransfarase (ALT) in the fat body at 0.5% concentration (187 μ moles pyurvate g/protein/ hour), (167 μ moles pyurvate g/protein/ hour) and (125 μ moles pyurvate g/protein/ hour) (Table.3) in 5^{th} instar 6^{th} day, 5^{th} instar 3^{rd} day and 5^{th} instar 1^{st} day, respectively. Similar trend was also observed in MU $_{303}$ at 0.5% concentration.

The transaminases are the important components of amino acid metabolism which mainly involved in transferring an amino group from one amino acid to another keto acid, thus forming another amino acid. The aspartate and alanine aminotransferase which serve as strategic link between carbohydrate and protein metabolism (Martin *et al*., 1981). The free amino acid in haemolymph might be increased due to additional supplement of methionine in turn reflects the increase in the activities of both AST and ALT enzymes in the fat body of silkworm breeds. This clearly indicating that, the active transportation of amino acids which provides keto acid to serve as precursor for silk protein synthesis. These results are in agreement with the earlier observations of Sarvanan Manjula *et al.* (2010) who have reported that increase in activity of AST and ALT in haemolymph when the silkworms fed on mulberry leaves supplemented with *Dolchos lablab* flour 7.5% concentration. Similar result was observed by Manjula Sarvanan *et al.* (2011) in silkworms fed on mulberry leaves fortified with *Vigna unguiculata* at 7.5% concentration.

In both breeds, the AST and ALT were highest in 5^{th} instar 6^{th} day, followed by 5^{th} instar 3^{rd} day and 5^{th} instar 1^{st} day over absolute control batch. It shows that higher quantity of methionine required by the silkworm with the advancement of age. These results are on par with the earlier observations of Anil Kumar (2009) who has opined that increase in protease activity with advancement of age in different silkworm breeds. Further, the activity level of AST and ALT in all the larval stages were relatively high in MU_{11} than the MU_{303} at 0.5%. methionine supplementation. These results showed that even the supplementation aminoacid concentrations remain constant, but utilization pattern differs genetically among the two breeds.

References

1. Quader,M.A.(1991) Varietal differences and correlation studies in the nutritional composition of the mulberry. *Sercologia,* 31(3):449-453.
2. Bongale, U.D. and Chaluvachari (1993) Evaluation of four mulberry varieties by leaf biological analysis and bioassay with *Bombyx mori. J. Indian Bot. Soc.,* 72:59-62.
3. Anantha Raman, K.V., Magadum, S.B., Shivakumar, G.R., Giridhar, K. and Datta, R.K. (1995) Correlation studies on different economic and nutritional parameters in *Bombyx mori* L. hybrids. *Indian J. Seri.,* 34 (2):118-121.
4. Fukuda, T. (1960) A semi synthetic diet for eri silkworm raising. *Agric.Biol.Chem.,* 27 (9): 601- 609.
5. Fakuda, T., Kamegama, T. and Matsuda, M. A. (1963) Correlation between the mulberry leaves consumed by the silkworm larvae in different ages of the larval growth and production of the cocoon fiber spun by the silkworm larvae and the eggs laid by the silkworm. *Bull.Seric. Exp. Stn.,* 18 : 165- 171.
6. Takano, K. and Arai, N. (1978) Studies on the food value on the basis of feeding and cocoon productivity in the silkworm, *Bombyx mori* L. Treatment of food intake and cocoon productivity. *J. Seri. Sci. J*., 47: 415-419.
7. Ito, T. and Tanaka, M. (1962) Nutrition of the silkworm, *Bombyx mori.*L. VI. Effects of concentrations of sugar and protein added in artificial diet. *Bull.Seric.Exp.Stn.* Japan., 18:1-34.
8. Ito, T. and Mukiyama. F. (1964) Relationship between protein content of diets and Xanthine Oxidase activity in the silkworm *Bombyx mori*. L. *J. Insect Physiol.,* 10:789-796.
9. Ito, T. and Mukiyama. F. (1970) Relationship between protein content of diets and cocoon quality in the silkworm. *Acta. Seric.* Japan., 77:77-86.

10. Kamioka, S., Mukaiyama, F., Takei, T. and Ito, T. (1971) Digestion and utilization of artificial diet by the silkworm, *Bombyx mori* L. with special references to the efficiency of the diet at varying levels of dietary soyabean meal. *J. Seric. Sci.,* 40:473-483.
11. Horie, Y., Watanabe, K. and Nakasone, S. (1971) Effect of dietary composition on growth, silk gland and components in haemolymph of the silkworm, *Acta.Seric. Japan,* 78: 251-254.
12. Seol, G.Y. (1982) Studies on the effects of various levels of protein in the artificial diet on nutritional physiology of the silkworm, *Bombyx mori* L. *Seri. J.* Korea., 23 (2):37-49.
13. Dandin, S. B. and Giridhar, K. (2010) *Handbook of Sericulture Technologies*. Central Silk Board, Bangalore, p. 427.
14. Reitman, S. and Frankel, S. (1957) A colorimetric method for the determination of serum glutamate oxaloacetic and glutamate pyurivic transaminases *Am.Clin. Pathol*., 28(1): 56-63.
15. Horie,Y and Watanabe, K. (1983) Effect of various kinds of dietary protein and supplementation with limiting amino acids and growth, haemolymph components and uric acid excretion in the silkworm, *Bombyx mori* L. *J.Insect Physiol*., 29:187-199.
16. Unni, B.G., Das, P and Gosh A.C. (2000) Silk gland and the biosynthesis of silk by silkworm ; in *Sericulture in India* Agrwal, H.O and Seth M.K (eds.), pp.251-269, Bishen Singh Mahendra Pal Singh, Dehradum, India.
17. Lu, S.L. and. Jiang, Z.D (1988) Absorption and utilization of amino acids in mulberry leaves by *Bombyx mori* L. *Act. Seri col.sin*.,14:198-204
18. Chapman, R.F. (1998) *The insect structure and function*, Cambridge University Press, Cambridge.
19. Etebari, K. Ebadi, R. and Matindoost, L. (2007) Physiological changes of silkworm, (*Bombyx mori* L.) larvae feed on mulberry leaves supplemented with nitrogenous compounds. *J.Ent.Res.Soc.,* 9(2):1-15.
20. Kabila, V., Subburathinam, K.M. and Chitty, J.S. (1994) Growth and economic characters of silkworm, *Bombyx mori* L. on feed enriched with neutralized aspartic acid. *Indian J. Seric.,* 33 (1): 80-81.
21. Ravi, K.N., Shekarappa, M.A., Puttaraju, T.B. and Puttaswamy, B. (1994) Effect of feed supplementation of silkworm growth, cocoon weight and silk quality, *Second Nat.Symp.Prosp.Prob.Seric.,* India (March, 7-9). Madras Univ. Vellore, pp.47.
22. Rahman Khan, A. and Saha, B.N. (1995) Growth and development of the mulberry silkworm, *Bombyx mori* L. on feed supplement with alanine and glutamine, *Sercologia,* 35(4):657-663.
23. Daniel Nicodemo and Juliano Oliveira (2014) Impact of different silkworm dietary supplements on its silk performance. *J. Master Sci*., 49:6302-6310.
24. Rouhollah Radjabi. (2010) Effect of mulberry leaves enrichment with amino acids supplementary nutrient on silkworm, *Bombyx mori* L. *Academic Journal of entomology,* 3(1):45-51.
25. Indira Bhojne., Rajiv L. Naik and Satapa B. Kharbade. (2014) Effect of leaf supplementation with secondary metabolites on economic traits of mulberry silkworm. *Int. J Entomol. Res.,* 2(01):29-32.
26. Sridhar, P. and Radha, N.V.(1986) Effect of supplementing glycine to the feed of silkworm, *Bombyx mori* L. National Seminar on *Prospectus and Problems of Sericulture in India*, March 27-30.pp.18.
27. Sridhar, P. and Radha, N.V. (1987) Effect of supplementing glycine to the feed of silkworm, *Bombyx mori* L. *Proc. Seric. Symp. Semi.,* Coimbatore, pp.88-98.
28. Krishnappa, J.B. (1987) Influence of amino acids supplementation on growth and development of mulberry silkworm, *Bombyx mori L*. M.Sc. Thesis, Univ. of Agricultural Science. Bangalore, India.
29. Sundar Raj, S., Chinnaswamy, K.P. and Sannapa, B. (2000a) Effect of feeding mulberry leaves fortified with protein supplements on the productivity of silkworm, *Bombyx mori* L. *Bull. Ind Acad. Seri.,* 4 (2): 34-38.
30. Sundar Raj, S., Neelu Nangia, Chinnaswany, K.P. and Sannappa, B. (2001) Enrichment of rainfed mulberry leaves with protein and its influence on rearing performance of *Bombyx mori* L.. *Res.on Crops.,* 2 (2); 179-184.
31. Sundar Raj, S., Neelu Nangia, Chinnaswany, K.P. and Sannappa, B. (2000b) Influence of protein supplements on performance of PM x NB4D2 silkworm breed. *Mysore J. Agric. Sci.,* 34: 302-307.
32. Rezina Laz (2010) Effect of methionine and tryptophan on some quantitative traits of silkworm, *Bombyx mori L.* (Lepidoptera: Bombycidae).*Univ. J. zool. Rajshahi. Univ., 28:* 15-19.
33. Martin, M., Osborn, K.E., Billing, P and Glickstein, N. (1981) *Bombyx mori* supplemented with *Vigna unguiculata* Marine Pollution Bulletin; 12:305-308.
34. Saravanan Manjula, Selvi Sabhanayakam, Veernarayanan Mathivanan and Nadanam Saravanan, (2010) Biochemical alterations in the haemolymph of silkworm [*Bombyx mori* L.(Lepidoptera: Bombycidae)] fed with mulberry leaves enriched with Indian bean (*Dolichos lablab*). *Recent Research in Science and Technology*, 2(3): 32-37.
35. Saravanan Manjula, Selvi Sabhanayakam, Veernarayanan Mathivanan and Nadanam Saravanan, (2011) Modulations in the haemolymph of silkworm [*Bombyx mori* L. (Lepidoptera: Bombycidae)] fed with mulberry leaves augmented with cowpeas (*Vigna unguiculata*). *Int. J. of Nutri. Pharmacol. Neurologic. Diseases,* 1(2): 64-68.
36. Anil Kumar, M.N. (2009), Studies on the seasonal variations in protease activity and their correlation with the economic characters in the popular silkworm breeds and their hybrids of *Bombyx mori* L. *Ph. D. Thesis*, University of Mysore, Mysore.

****