International Journal of Advanced Science and Research
ISSN: 2455-4227, Impact Factor: RJIF 5.12
www.allsciencejournal.com
Volume 1; Issue 11; November 2016; Page No. 09-14

Influence of nutritive additive on digestive physiology of silkworm *Bombyx mori* L.

*** Ananda Kumar MD, Ann Sandhya Michael**

Department of Life Science, Bangalore University, Jnanabharathi, Bangalore, Karnataka, India

Abstract
Nutritive supplement "serifeed" in optimum concentration (0.04g) has beneficiary effect on increasing the feeding and assimilation rate in silkworm. The bivoltine hybrid CSR_2 X CSR_4 and multi-bivoltine hybrid PM x CSR_2 fed on 0.04g treated leaves showed an increased macromolecule concentration in midgut and haemolymph tissues. The digestive enzyme activity and conversion efficiency of mulberry nutrients enriched with feed supplement is found to be significantly higher in bivoltine hybrid than the crossbreed. Specifically rainy season has influenced in converting dietary nutrients into silk and yolk protein.

Keywords: assimilation rate, digestive enzymes, fecundity, feeding rate, feed supplement, silk productivity

1. Introduction
Nutrition is an important growth regulating factor in any living organism. Substitutive sources of nutrients cooperating with the commonly recognized food stuff of the species are needed to fulfill the nutritional requirements in many insects [1]. Silkworm *Bombyx mori* being monophagous insects exclusively depends on the quality and quantity of mulberry leaves. Even the non-feeding stages of lepidopteron insects such as adults are affected by nutritional capacity during their larval stages [2]. Growth is manifested by accumulation of organic matter from the nutritive substances that are absorbed after digestion of food. The integrated nutritional management in sericulture needs to address nutritional requirements of silkworm *B. mori* [3]. The simplest and effective method is to enrich the normal food by adding various acknowledged supplementary nutrients. There has been considerable progress made in the field of silkworm nutrition, especially after the advent of defined diets [4-6]. It is also known that nutritional ecology play an important role in various life process of insects such as feeding, growth and development, reproduction etc., [7]. The seasonal responses of pure breeds and hybrids, pertaining to economically important trait of silkworm have been reported earlier [8]. The adaptability of different silkworm breeds to nutritive additive varies depending on their genetic makeup [9].
In the present work an effort was made to understand the impact of nutrition of silkworm when fed on feed supplemented mulberry leaves. We mainly focused on:

a) Influence of serifeed on feeding and assimilation rate in silkworm.
b) Role of seifeed on the conversion efficiency of mulberry leaf into silk and yolk protein, finally
c) The role of season and silkworm variety in the optimum utilization of nutrient supplement serifeed.

2. Materials and methods
2.1 Silkworm rearing and application of feed supplement
Silkworm races namely cross breed PM x CSR_2 and hybrid CSR_2 x CSR_4 were brought from private grainage near Bangalore. From the third instar to spinning, the silkworms were maintained in cellular rearing at Jnanabharathi campus [10]. Each silkworm larvae received mulberry leaves treated with either 0.02, 0.04 and 0.10g of nutritive additive 'serifeed' during night feeding in all days during fifth instar until spinning. The control worms were fed with 0.02 g of inert material. The experiment was done in summer, rainy and winter season.

2.2 Estimation of feeding rate and assimilation rate
4th day of the final instar was considered for the study of feeding and assimilation rate as it correlates with significant increase in the digestive enzymes. Although the feeding rate and assimilation rate decreases with further increase in the instar days, the decrease was not significant. Both the parameters were calculated by gravimetric method [11].

2.3 Assay of digestive enzymes
A 10% homogenate (w/v) of the midgut tissue was prepared in ice-cold buffer. The tissue homogenate was centrifuged at 3000 rpm for 10 minutes in a refrigerated centrifuge (Remi). The supernatant was used for the assay of amylase, invertase and protease. Amylase activity was estimated using the 3, 5, dinitro salicylic acid reagent [12]. Invertase activity [13] and protease activity [14] was assayed.

2.4 Estimation of carbohydrates and proteins
The haemolymph was collected, by cutting the caudal horn of larvae on 4th day of V instar into a haemolymph tube containing a pinch of thiourea to prevent oxidation and diluted 10 times in ice-cold distilled water. A 10% homogenate (w/v) of the midgut tissue was prepared as above. The supernatants of homogenate or haemolymph samples were used for the estimation of total proteins and carbohydrates. The quantitative estimation of proteins in haemolymph and midgut tissues was carried out, [15] the reducing sugar by using dinitro-salicylic acid (DNS) [16] and total sugar by Anthrone method [17].

2.5 Estimation of silk productivity and fecundity
Silk productivity [18] and fecundity was calculated [19].

Statistical analysis

The experimental results were subjected to ANOVA and significant of the values were indicated at 0.05 level [20].

3. Results and Discussion

The feed supplement contains different fortifying agents such as sucrose (4%), defatted soyabean powder (84%), glycine (6%), chloromphenicol (0.5 %), Vitamin B complex (0.5%), magnesium (2%), iron (2%) and traces of iodine and cobalt. Apart from these, morin (0.5%) being a phagostimulant, helps the *B. mori* for continuous feeding. Figure-1 shows the feeding rate of silkworm fed with mulberry leaves that were treated with different concentration of serifeed. The feeding rate in CSR_2xCSR_4 hybrid was significantly higher when compared to PMxCSR_2 hybrid. Silkworms devour more mulberry leaves when treated with 0.04g serifeed when compared to 0.1g. Feeding rate was significantly higher in rainy season (fig-1) in comparison with other seasons studied and serifeed significantly increased the feeding rate in hybrid when compared to crossbreed. Phagostimulants such as morin influences the feeding rate in the silkworm as reported elsewhere [21] and the feeding rate performance of *B. mori* was also dependent on nutritional value of leaves [22]. Importance of leaf moisture in the palatability of mulberry leaves by silkworm had been reported earlier [23]. In the present study significant increase in feeding rate in rainy season may be due to increased moisture content. The feeding rate in mulberry silkworm varied among the breeds [24].

Significantly higher assimilation rate is observed on the 4th day V instar of silkworm in both the races studied. 0.04 g of serifeed significantly increased the assimilation rate in both the races and was higher during the rainy season (fig- 2). The optimum utilization of serifeed treated leaves by silkworms was at 0.04 g rather than the 0.1 g. Rainy season also influenced assimilation rate significantly in hybrid. Increased assimilation rate had been reported earlier in the silkworm fed on mulberry leaves supplemented with single cell protein, Spirulina [25, 26]. In the present study, serifeed was fortified with vitamin complex and increased assimilation rate in *B. mori* was observed and these results were similar to Chinese variety [27]. The dietary water content also influences the assimilation efficiency of silkworm [28].

Digestive enzymes such as protease, amylase and invertase are studied in the V instar larvae of *B. mori*. Interestingly the digestive enzymes activity was also found to be high on 4th day V instar (results not shown). Significant increase in the proteolytic enzyme activity was found in the larval midgut when fed with mulberry leaves treated with 0.04 g serifeed in both the breeds irrespective of the season (fig- 3). Although protease activity was not significantly different among the seasons studied, the proteolytic enzyme activity was found to be higher in bivoltine hybrid worms over multi- bivoltine hybrid in rainy season. Amylase activity of V instar was found to be higher on 4th day and 0.04 g of serifeed influenced the activity significantly in both the races studied (fig- 4). Amylase activity was not been influenced by the season in crossbreed however it was surprising to note that the hybrid have highest activity in winter. 0.04g of serifeed treated leaves significantly increased the invertase activity of the silkworm midgut in both the races studied (fig- 5). There was no significant difference in the enzymic activity in the seasons studied within the breeds; however, rainy season influenced enzymic activity in hybrid over crossbreed. Digestive enzymes in insects are synthesized in the midgut epithelium and secreted into the gut lumen [29]. The enzyme activity was found to be higher in the later stage of larval development and the dietary intake has a role to play [30]. The present study depicts that silkworms fed on mulberry leaves treated with 0.04 g serifeed showed significant increase in the proteolytic enzyme in both the breeds studied irrespective of the season. Highest activity of protease in silkworm fed on treated mulberry leaves could be due to a greater utilization of exogenous proteins [11]. The midgut protease activity varied significantly among bivoltine, multivoltine and the crossbreed variety [31, 32] whereas our present results indicate higher protease activity in bivoltine hybrids over multi-bivolne hybid.

Larval midgut and haemolymph proteins and carbohydrates of the two breeds in different seasons were presented in table 1 and 2. Δ change in protein content is was significantly high in midgut as well as in haemolymph in the 4th day of V instar when fed with 0.04 g serifeed applied leaves. The protein content was also influenced by rainy season in both the breeds of the silkworm. Total sugar and reducing sugar had also shown similar trends in midgut and haemolymph. Among PM x CSR_2 and CSR_2 X CSR_4, the hybrid showed better macromolecule concentrations irrespective of the season in both the tissues studied. Digestion of carbohydrates and its influence in increase the enzyme activity [33] was reported earlier. In present study amylase activity was found to be higher in silkworms fed on leaves treated with 0.4g serifeed in both PM x CSR_2 (multi- bivoltine) and CSR_2 x CSR_4 (bivoltine hybrid) and more so in bivoltine hybrid. The increased enzymic activity in hybrid silkworms are reported elsewhere [9] and the production of digestive enzymes linked with feeding in all insects [34]. Significant increase of invertase activity was found in silkworm fed on 0.04g of serifeed treated leaves in both the races studied.

Macromolecules like protein, total sugar and reducing sugar concentration was significantly high in midgut and haemolymph tissues in both the breeds studied when fed on mulberry leaves treated with 0.04g serifeed especially during rainy season. Accumulation of carbohydrate in the present study could be due to the presence of sucrose in serifeed and the increase in the total protein content could be due to the vitamin complex present in feed supplement. The increased protein content in silkworm on treatment with thiamine was already reported [35]. The concentration of carbohydrates and proteins in bivoltine was higher than the crossbreed [36] where as in the present result bivoltine bhybrid have increased macromolecule concentrations over multi- bivoltine hybrid. Influence of season on macromolecular concentrations is the unique finding of the present study.

Season has no relevance on silk productivity irrespective of the race and was optimum at 0.04 g serifeed applied leaves. However, the silk content of the hybrid was significantly higher than crossbreed (fig -6). Increased concentration of serifeed (0.04g) influenced the egg laying capacity of the silk moth significantly in both races studied (fig-7) and it was worth noting that the egg production was influenced by rainy season. Serifeed being fortified with beneficial nutrients, enhanced the conversion of macronutrients into proteins like silk and yolk. The silk productivity was found to be higher in

CSR_2 x CSR_4 (bivoltine hybrid) than the PM x CSR_2 (multi-bivoltine hybrid). Vitamins present in serifeed induced the increased silk productivity [37]. In the present study, increased concentration of serifeed (0.04g) influenced the egg laying capacity of the silk moth significantly in both races mainly during rainy season. The eggs laying capacity and silk productivity in hybrid silkworms were increased when fed on mulberry leaves enriched with feed supplement *kohiko silcare* rich in protein [6]. Increased fecundity in hybrid silkworms fed on treated mulberry leaves in the present study may be due to the proteins present in serifeed. The fecundity was found to be highest during the rainy season having cooler temperature [38] and also in the present study rainy season was found to influence the fecundity.

4. Tables and Figures

Table 1: Δ change in the amount of macromolecules: total protein, total sugar and reducing sugar in haemolymph and midgut of PM x CSR_2 (cross breed) silkworms fed on feed supplement serifeed treated mulberry leaves (0.02g, 0.04g and 0.1g) during different seasons.

Tissue used	Macromolecules	Season	0.1g	0.2g	0.5g
Haemolymph	Total protein (mg/ml)	Summer	2.63	4.34	3.23
		Rainy	2.94	5.76	5.04
		Winter	2.78	4.75	3.84
	Total sugar (mg/ml)	Summer	0.24	0.55	0.43
		Rainy	0.64	1.48	1.18
		Winter	0.55	1.21	0.94
	Reducing sugar (mg/ml)	Summer	1.02	1.18	1.09
		Rainy	1.12	1.37	1.22
		Winter	1.08	1.25	1.15
Midgut	Total protein (mg/g)	Summer	3.1	4.86	4.06
		Rainy	4.14	6.14	5.32
		Winter	3.64	5.18	5.05
	Total sugar (mg/g)	Summer	0.28	0.69	0.45
		Rainy	0.73	1.56	1.26
		Winter	0.62	1.33	1.16
	Reducing sugar (mg/g)	Summer	1.08	1.35	1.19
		Rainy	1.23	1.52	1.45
		Winter	1.12	1.46	1.28

Table 2: Δ change in the amount of macromolecules: total protein, total sugar and reducing sugar in haemolymph and midgut of CSR_2 x CSR_4 (hybrid) silkworms fed on feed supplement serifeed treated mulberry leaves (0.02g, 0.04g and 0.1g) during different seasons.

Tissue used	Macromolecules	Season	0.1g	0.2g	0.5g
Haemolymph	Total protein (mg/ml)	Summer	2.86	4.84	4.12
		Rainy	3.26	6.07	5.34
		Winter	2.94	4.83	4.48
	Total sugar (mg/ml)	Summer	0.41	0.68	0.54
		Rainy	0.83	1.72	1.35
		Winter	0.68	1.42	1.23
	Reducing sugar (mg/ml)	Summer	1.16	1.36	1.24
		Rainy	1.31	1.51	1.38
		Winter	1.24	1.37	1.25
Midgut	Total protein (mg/g)	Summer	3.11	5.24	4.36
		Rainy	3.52	6.43	5.62
		Winter	3.27	5.12	4.72
	Total sugar (mg/g)	Summer	0.57	0.75	0.64
		Rainy	1.04	2.13	1.68
		Winter	0.85	1.74	1.47
	Reducing sugar (mg/g)	Summer	1.38	1.65	1.53
		Rainy	1.63	1.95	1.74
		Winter	1.46	1.73	1.43

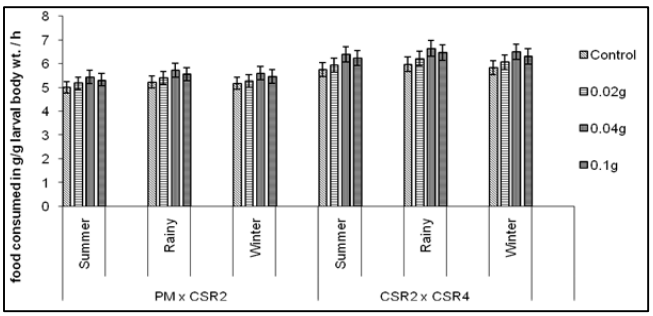

Fig 1: Feeding rate of 4th day, V instar PMxCSR2 (Crossbreed) and CSR_2 x CSR_4 (hybrid) silkworm larvae fed on control and feed supplement treated mulberry leaves (0.02 g, 0.04g and 0.1g) during different seasons. The insert shows that the feeding rate is peak on 4th day of V instar in both crossbreed and hybrid. * represents Significance at 0.05

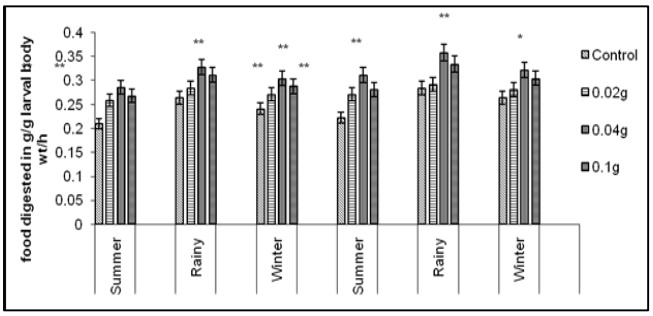

Fig 2: Assimilation rate of 4th day, V instar PM x CSR_2 (crossbreed) and CSR_2 x CSR_4 (hybrid) silkworm larvae fed on control and feed supplement treated mulberry leaves (0.02g, 0.04g and 0.1g) during different seasons. The insert shows that the assimilation rate is peak on 4th day of V instar in both crossbreed and hybrid. * represents Significance at 0.05

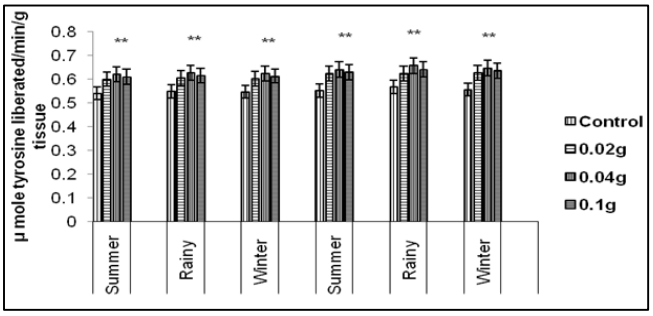

Fig 3: Protease activity on 4th day, V instar PM x CSR_2 (crossbreed) and CSR_2 x CSR_4 (hybrid) silkworm larvae fed on control and feed supplement treated mulberry leaves (0.02g, 0.04g and 0.1g) during different seasons. * represents Significance at 0.05

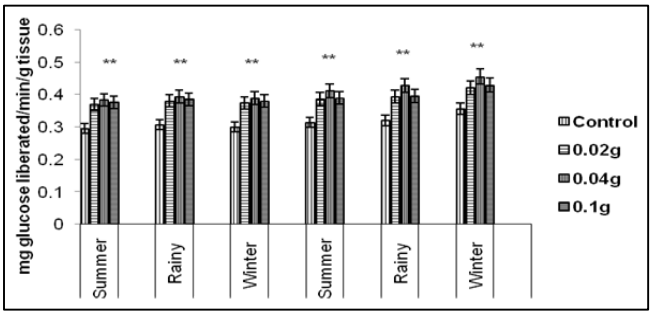

Fig 4: Amylase activity on 4th day, V instar PM x CSR_2 (crossbreed) and CSR_2 x CSR_4 (hybrid) silkworm larvae fed on control and feed supplement treated mulberry leaves (0.02g, 0.04g and 0.1g) during different seasons. * represents Significance at 0.05

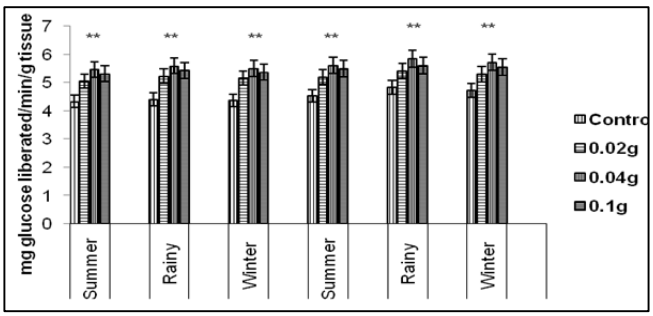

Fig 5: Invertase activity on 4th day, V instar PM x CSR_2 (crossbreed) and CSR_2 x CSR_4 (hybrid) silkworm larvae fed on control and feed supplement treated mulberry leaves (0.02g, 0.04g and 0.1g) during different seasons. * represents Significance at 0.05

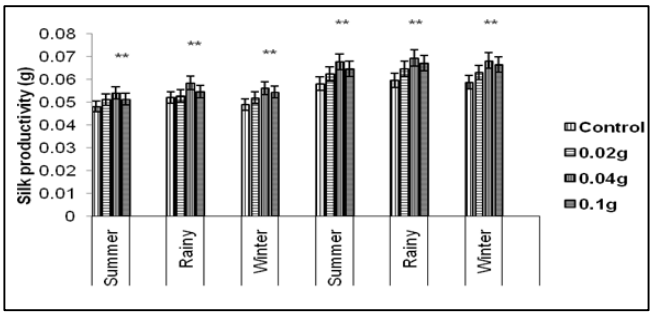

Fig 6: Silk productivity of PM x CSR_2 (cross breed) and CSR_2 x CSR_4 (hybrid) silkworm fed on control and feed supplement treated mulberry leaves (0.02g, 0.04g and 0.1g) during different seasons. * represents Significance at 0.05

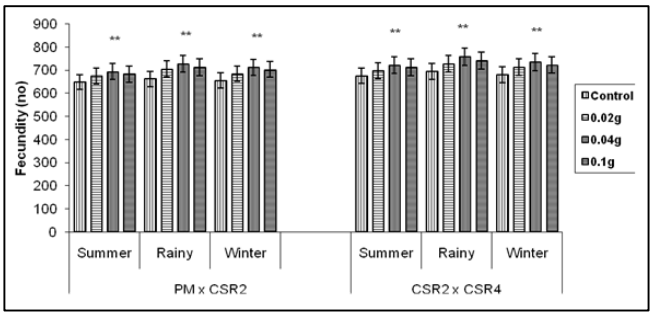

Fig 7: Fecundity of PM x CSR_2 (cross breed) and CSR_2 x CSR_4 (hybrid) silkworm fed on control and feed supplement treated mulberry leaves (0.02g, 0.04g and 0.1g) during different seasons.* represents Significance at 0.05

5. Conclusion

The optimum utilization of feed supplement was found to be during rainy season. Although rainy season coincides with infections of silkworm, serifeed can effectively be used for silkworm rearing since it was fortified with antibiotics like chloromphenicol. Increased larval weight and better rearing performance of NB_4D_2 race on treatment with antibiotics was reported earlier. Optimum utilization of serifeed by silkworm was at 0.04g rather than 0.1g and the rainy season influences increased protein productivity. CSR_2 X CSR_4 bivoltine hybrid was found to be better in utilizing the feed supplement than the PM x CSR_2 crossbreed.

6. Acknowledgement

We thank Dr. S. R. Ananthanarayana and Mr. Ram Rao for technical discussions and Mr. Ramchanrdriah for technical assistance.

7. References

1. Sundar RS, Chinnaswamy KP, Nangia N. Soyabean to boost cocoon production, Indian Silk, 2000; 39:11.
2. Ravikumar G, Balasubramanian U, Krishnan N. Effect of casitose (P and PH) supplementation on the haemolymph protein profile and the reproductive characters of silkworm, *Bombyx mori* L, Sercologia, 2002; 42:377.
3. Bhargav SV, Sindagi SS, Santha PC, Kamble CK. Nutritional management of silkworm (*Bombyx mori* L.) through food supplements for quality improvement in sericulture – A review, Indian academy of sericulture, 2008; 12:1.
4. Gowda R. Impact of Seripro on cocoon production and productivity in silkworm *Bombyx mori* L., Advances in Indian Sericulture research, edited by Dandin, S. B., Mishra, R. K; Gupta, V. P and Reddy, Y. S, (CSR and TI, Mysore, India), 2002, 399-401.
5. Venkataramana P, Suryanarayana N, Dutta RK. The effect of *'Seri guard'* on rearing performance and loss due to disease in silkworm, *Bombyx mori* L, Sericologia, 2003; 43:191.
6. Narayanaswamy TK, Shankar MA, Sannappa B. Mulberry leaf enrichment through *Kohiko Silcare* feed supplement and its effect on rearing performance of silkworm hybrid. Sericologia, 2005; 45:309.
7. Radakrishna PG, Delvi MR. Effect of ration level on food utilization in silkworm, *Bombyx mori* L, Sericologia, 1987; 27:347.
8. Radhakrishnan PG, Sekharappa BM, Gururaj CS. Seasonal response of the new multi-bivoltine hybrids of the silkworm, *Bombyx mori* L, Indian Journal of Sericulture, 2001; 40:174.
9. Trivedy K, Nair SK. Feed conversion efficiency of improved multi x bivoltine hybrids of silkworm, *Bombyx mori* L, Indian Journal of Sericulture, 1999; 38:30.
10. Krishnaswami S, Noamani KR, Ahsan M. Studies on the quality of mulberry leaves and silkworm crop production, Part I: Quality differences due to varieties, Indian Journal of Sericulture, 1970; 9:1.
11. Lakshmikumari B, Ananathanarayana SR, Jayaprakash B. Effect of radiation on the activity of digestive enzymes in the silkworm, *Bombyx mori* L, Sericologia, 1997; 37:221.
12. Noelting G, Bernfeld P. Surles enzymes amylolyteques-III La-B-amylase dosage. D. activitite'et controle de L; abrence d' L-amylase, Helv-chimica Acta, 1948; 31:296.
13. Bernfeld P, Amylases αβ. In methods in Enzymology edited by Colowick, S. P. and Kaplan, N. O. Academic press. New York, 1955, 149-158.
14. Eguchi M, Iwamoto A. Comparison of three alkaline proteases from digestive fluid of the silkworm, *Bombyx mori*. L, Comparative Biochemistry and Physiology, 1982; 71B:663.
15. Lowry OH, Rosebrough NF, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. Journal of Biological Chemistry. 1951; 193:267.
16. Burton K. A study of the condition and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid, Biochemistry Journal. 1956; 62:315.
17. Dubois M, Giller KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances, Academy of Chemistry. 1956; 28:350.
18. Nagesh S, Devaiah MC. Effect of 'Sericare'- a feed additive on silk productivity in silkworm *Bombyx mori* L. Indian Journal of Sericulture. 1996; 35:67.
19. Nair S, Vijayan K, Jula VA, Thrivedy K. Juvenilomimic compounds for enhanced productivity in silkworm *Bombyx mori* L. –a screening. Indian Journal of Sericulture. 1999; 38:119.
20. Khan IA, Khanum A. Fundamentals of bio statistics. Ukaaz publication Hyderabad, India, 1994, 473-489.
21. Chapman RF. Contact chemoreception in feeding by phytophagous insects, Annual Review of Entomology, 2003; 48:455.
22. Jeyapaul C, Padmalatha C, Singh RAJ, Murugesan AG, Dhasarathan P. Effect of Plant extracts on Nutritional efficiency in mulberry silkworm *Bombyx mori* L, Indian Journal of Sericulture. 2003; 42:128.
23. Gokulamma K, Reddy SY. Role of nutrition and environment on the consumption, growth and utilization indicies of silkworm races of *Bombyx mori* L. Indian Journal of Sericulture. 2005; 44:165.
24. Remadevi OK, Magadum SB, Shivashankar N, Benchamin KV. Evaluation of the food utilization efficiency in some polyvoltine silkworm *Bombyx mori* L, Sericologia. 1992; 32:61.
25. Naik PR, Delvi MR. Food utilization in different races of silkworm, *Bombyx mori* L. Lepidoptera: Bombycidae, Sericologia, 1987; 27:391.
26. Subburthinum KM, Krishnan M. Role of soyabean protein in silkworm nutrition, Indian Silk. 1998; 36:9.
27. Cui WZ, Yan-Wen W, Zhi-Mei M. Gustation electrophysiological response to vitamin-C of silkworm, Acta Sericologica Sinica, 2001; 27:92.
28. Paul DC, Subbarao G, Deb DC. Impact of dietary moisture on nutritional indices and growth of *Bombyx mori* L. and concomitant larval duration. Journal of Insect Physiology. 1992; 38:229.
29. Eguchi M, Arai M. Relationship between alkaline proteases from the midgut lumen and epithelium of the silkworm: solubilisation and activation of epithelial protease, Comparative Biochemistry and Physiology, 1983; 75B:589.
30. Chatterjee SN, Datta RK. Hierarchical clustering of 54 races and strains of the mulberry silkworm *Bombyx mori* L. significance of biochemical parameters, Theoritical and Applied Genetics. 1992; 85:394.
31. Sarangi SK. Studies on silk gland of *Bombyx mori* L. a comparative analysis during fifth instar development, Indian Academy of Sciences, 1995; 94:413.
32. Sarangi SK. Studies on midgut protease activity during fifth instar development of the silkworm *Bombyx mori* L, Entomon. 1986; 11:165.
33. Yaginuma T, Kobayashi M, Kawase S. Changes in activities of several enzymes responsible for carbohydrate metabolism in mid gut epithelium of the silkworm, *Bombyx mori* L. infected with *Cytoplasamic polyhedrosis* virus. Journal of Sericulture Science Japan. 1990; 59:64.
34. Dadd RH. Digestion in insects. In Comprehensive Insect Physiology, Biochemistry and Pharmacology, edited by

Kerkut GA, Gilbert LI. Pergamon *Press*, New York, 1970; 4:313-390.
35. Nirwani RB, Kaliwal BB. Effect of thiamine on commercial traits and biochemical contents of the fat body and haemolymph in the silkworm, *Bombyx mori* L, Sericologia, 1998; 36:639.
36. Mahadev KSD, Sarangi SK. Changes in protein and reducing sugar content of the haemolymph during V instar development of *Bomyx mori* L, Bulletin of Indian Academy of Sericulture, 2002; 6:46.
37. Rai MM, Ratod MK, Khurad MA. Improvement in economic characters of silkworm, *Bombyx mori* L. by folic acid administration, Entomon. 2002; 27:99.
38. Muniraju E, Sekharappa BM, Rahguraman R. Production potential of silkworm *Bombyx mori* L. under different rearing temperatures. Indian Journal of Sericulture. 2001; 40:15.