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Studies on the amylase and protease activities in Eri silkworm *Samia cynthia ricini* based on seasonal variations

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ABSTRACT

The Eri silkworm *Samia cynthia ricini* is a non-mulberry, domesticated, multi-voltine insect, producing spun silk, which is mostly found in Assam and Meghalaya, which are known as traditional states of Eri Silkworm in India. Its primary food plants are Castor (*Ricinus communis*) and Papaya (*Carico papaya*) and the secondary food plants include like Jatropa, Tapioca etc. Of late, Ericulture is spreading to other states like Bihar, West-Bengal, Orissa, Tamil Nadu, Kerala, Karnataka, Telangana and Andhra Pradesh, owing to the availability and suitability of high-yielding castor varieties. The present study is focused on the qualitative analysis of proteins and carbohydrates, and the estimation of enzymes amylase and protease in the haemolymph of fifth instar worms during different seasons. The study revealed an increased metabolic activity during winter, providing an insight into the physiological strategy of survival adopted by this silkworm species during winter (18-28 °C).

KEYWORDS: Eri silkworm, *Samia cynthia ricini*, trehalose, protein, amylase, protease, seasonal variations

INTRODUCTION

The Eri silkworm *Samia cynthia ricini* is a multivoltine domestic moth, reared completely in an indoor environment, and it is a non-mulberry worm producing spun silk, mostly found in the North-eastern states of India, especially Assam and Meghalaya. The primary host plants are Castor (*Ricinus communis*) and Papaya (*Carico papaya*); Jatropa and Tapioca being the secondary host plants. Ericulture has a great potential to grow into a big industry if meticulous planning and strategies are adopted. The present study, undertaken in order to exploit the growing awareness and demand for Eri silk, is based on evaluating the metabolic activity of Eri silkworm during different seasons.

The Eri silkworm, like other Lepidopterans, undergoes complete metamorphosis passing through four stages namely egg, lava, pupa and adult. It completes 5-6 life cycles in an year. The environmental factors play a major role in Eri silk production. As Eri worms are quite delicate and sensitive to environmental conditions, the prospect of increasing silk production depends more on the food or host plant nutrition. In silkworms, it has been shown that, the protein synthetic activity of the body wall and the midgut decreased when the larvae began to moult and increased again from the midstage to moulting period [1]. Like all other animals, growth and development in insects is associated with protein metabolism [2].

Most of the literature available on the Eri silkworm *Samia cynthia ricini* is based on the comparative studies on the effect of castor food plants on rearing performance, biochemical analysis and seasonal variations. A study on the worms fed with castor leaves revealed that they possess predominantly higher content of the metabolites followed by jatropa and papaya [3]. In the present study, the major biochemicals such as carbohydrates, proteins, amylase and proteolytic enzymes in the silk gland of fifth instar are evaluated and analysed in relation to seasonal variations.

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MATERIALS AND METHODS

The rearing of Eri silkworm *Samia cynthia ricini* was carried out in a convenient, well-equipped rearing room with adequate ventilation. The castor leaves were freshly collected during the summer (Feb-March), rainy (July-Aug) and winter (Nov-Dec) seasons from *Ricinus communis* plantation raised at Kakatiya University, Warangal (2001-2011). The temperature and relative humidity were recorded regularly.

Castor, a crop of the tropics, is one of the major oil seed crops of India and is generally grown on sandy or clayey red loams and also on good light alluvial loams. The castor crop can withstand drought, heavy rains and floods and also slightly acidic soil conditions but not alkaline and water logged soils. The agricultural operations like weeding, irrigation and cultivation were done from time to time. The rearing site was also cleared off weeds to avoid pests.

The rearing was done by tray rearing method in which the Eri silkworms were reared in wooden trays. A paraffin paper of tray size was used to cover the rearing beds in the early stages (Figure 1). During the first three instars, care was taken to feed the worms with tender leaves, while late instar worms were fed with mature leaves (topmost leaves of stem). The optimum temperature and relative humidity under which Eri silkworm thrive the best are 25-28 °C and 80-90%, respectively and these were maintained in the laboratory.

Collection of digestive fluid to determine the enzyme activity of amylase and protease *in vitro* conditions

Digestive fluid was collected from the 4th day of fifth larva, during which the larvae were starved initially for 4 h and digestive juice was collected from midgut after dissection of larvae. Digestive fluid was collected in pre-cooled tubes. Five individual larvae were used for sample collection. As the sex was not discernible during the larval stage, it is probable that the samples were derived from both sexes. The digestive fluid was centrifuged at 10,000 rpm for 10 min to remove undigested leaf particles and stored at -20 °C until use.

Quantitative analysis

In the present study, the quantification of protein content was done by the Lowry method [4] and total protein content was expressed in terms of mg/ml or mg of 50 mg tissue. Estimation of trehalose content was done by the Mokrash method [5] and trehalose content was expressed in terms of mg/ml or mg of 50 mg tissue. The digestive amylase activity was measured by the dinitrosalicylic acid procedure using soluble starch as substrate [6, 7]. The method of [8, 9, 4], was used to estimate the activity of digestive protease.

RESULTS

Protein content

The season-wise total protein content and its standard deviation in the haemolymph, fat body and silk gland of the fifth instar of the Eri silkworm *Samia cynthia ricini* during winter, rainy and summer seasons of 2009 were 11.96 ± 0.94 (S. D), 5.79 ± 0.81 (S. D) and 4.86 ± 0.50 mg/ml, 3.29 ± 0.26 (S. D), 3.64 ± 0.38 (S. D) and 3.48 ± 0.233 (S. D) mg/ml and 14.36 ± 0.73 (S. D), 7.10 ± 1.53 and 5.44 ± 0.41 (S. D) mg/ml, respectively.

The season-wise total protein content and its standard deviation in the haemolymph, fat body and silk gland of the fifth instar of the Eri silkworm *Samia cynthia ricini* during winter, rainy and summer seasons of 2010 were 9.88 ± 2.29 (S. D), 6.84 ± 0.67 (S. D) and 5.83 ± 0.45 (S. D) mg/ml, 2.46 ± 0.89 (S. D), 3.62 ± 0.38 (S. D) and 4.17 ± 0.93 (S. D), mg/ml and 8.92 ± 5.76 (S. D), 7.47 ± 1.05 (S. D) and 6.17 ± 0.37 (S. D) mg/ml, respectively.

The season-wise total protein content and its standard deviation in the haemolymph, fat body and silk gland of the fifth instar of the Eri silkworm *Samia cynthia ricini* during winter, rainy and summer seasons of 2011 were 8.8 ± 1.87 (S. D), 8.16 ± 1.01 (S. D) and 6.04 ± 0.43 (S. D) mg/ml, 2.56 ± 0.40 (S. D), 4.23 ± 0.46 (S. D) and 4.29 ± 0.80 (S. D) mg/ml and 3.08 ± 0.55 (S. D), 5.63 ± 0.38 (S. D) and 6.10 ± 0.42 (S. D) mg/ml, respectively.

From the above results it is observed that the protein content in the haemolymph and silk gland was higher during winter season than during rainy and summer seasons, while occasional increase in the protein content was found in the silk gland during summer. Whereas, among all the tissues, protein content was mostly found to be higher in

Figure 1. Castor plantation and tray rearing method of Eri silkworm *Samia cynthia ricini*.

the silk gland than in the haemolymph and fat body (Table 1).

Trehalose content

The season-wise total trehalose content and its standard deviation in the haemolymph, fat body and silk gland of the fifth instar of the Eri silkworm *Samia cynthia ricini* during winter, rainy and summer seasons of 2009 were 43.37 ± 4.89 (S. D), 33.51 ± 5.39 (S. D) and 26 ± 2.36 (S. D) mg/ml, 39.37 ± 2.29 (S. D), 18.12 ± 5.04 (S. D) and 9.45 ± 3.26 (S. D) mg/ml and 26.42 ± 1.38 (S. D), 17.89 ± 2.79 (S. D) and 9.19 ± 1.75 (S. D) mg/ml, respectively.

The season-wise total trehalose content and its standard deviation in the haemolymph, fat body and silk gland of the fifth instar of the Eri silkworm *Samia cynthia ricini* during winter, rainy and summer seasons of 2010 were 35.12 ± 0.81 (S. D), 34.25 ± 2.31 (S. D) and 22.38 ± 1.42 (S. D) mg/ml, 35.62 ± 3.02 (S. D), 28.39 ± 4.85 (S. D) and 19.12 ± 1.56 (S. D) mg/ml and 23.62 ± 4.31 (S. D), 23.5 ± 4.20 (S. D) and 15.25 ± 2.59 (S. D) mg/ml, respectively.

The season-wise total trehalose content and its standard deviation in the haemolymph, fat body and silk gland of the fifth instar of the Eri silkworm *Samia cynthia ricini* during winter, rainy and

summer seasons of 2011 were 36.5 ± 5.35 (S. D), 34.25 ± 1.20 (S. D) and 20.47 ± 0.81 (S. D) mg/ml, 25.29 ± 3.06 (S. D), 39.37 ± 2.29 (S. D) and 12.29 ± 1.25 (S. D) mg/ml and 24.02 ± 4.77 (S. D), 13.62 ± 1.49 (S. D) and 9.66 ± 1.13 (S. D) mg/ml, respectively.

From the above results it is observed that the carbohydrate content in the haemolymph, silk gland and fat body were higher in winter season than rainy and summer seasons. Whereas, among all the tissues, carbohydrate content was mostly found to be higher in the haemolymph than in the silk gland and occasionally higher in the fat body (Table 2).

Proteolytic activity

The season-wise proteolytic activity in the digestive juice of the fifth instar of the Eri silkworm *Samia cynthia ricini* during winter, rainy and summer

Table 1. Estimation of total protein content in Eri silkworm *Samia cynthia ricini* during various seasons in the years 2009 to 2011 (mg/ml or mg of 50 mg tissue).

Year	Seasons	Protein content		
		Haemolymph	Silk gland	Fat body
2009	Winter	11.96 ± 0.94*	14.36 ± 0.73*	3.29 ± 0.26
	Rainy	5.79 ± 0.81	7.10 ± 1.53	3.64 ± 0.38*
	Summer	4.86 ± 0.50	5.44 ± 0.41	3.48 ± 0.23
2010	Winter	9.88 ± 2.29*	8.92 ± 5.76*	2.46 ± 0.89
	Rainy	6.84 ± 0.67	7.47 ± 1.05	3.62 ± 0.38*
	Summer	5.83 ± 0.45	6.17 ± 0.37	4.17 ± 0.93
2011	Winter	8.8 ± 1.87*	3.08 ± 0.55	2.56 ± 0.40
	Rainy	8.16 ± 1.01	5.63 ± 0.38	4.23 ± 0.46
	Summer	6.04 ± 0.43	6.10 ± 0.42*	4.29 ± 0.80*

The values are expressed in terms of Standard Error of the Mean.
*Significant values of total protein content in Eri silkworm *Samia cynthia ricini* during various seasons in the years 2009 to 2011.

Table 2. Estimation of trehalose content in Eri silkworm *Samia cynthia ricini* during various seasons in the years 2009 to 2011 (mg/ml or mg of 50 mg tissue).

Year	Seasons	Trehalose content		
		Haemolymph	Silk gland	Fat body
2009	Winter	43.37 ± 4.89*	26.42 ± 1.38*	39.37 ± 2.29*
	Rainy	33.51 ± 5.39	17.89 ± 2.79	18.12 ± 5.04
	Summer	26 ± 2.36	9.19 ± 1.75	9.45 ± 3.26
2010	Winter	35.12 ± 0.81*	23.62 ± 4.31*	35.62 ± 3.02*
	Rainy	34.25 ± 2.31	23.5 ± 4.20	28.39 ± 4.85
	Summer	22.38 ± 1.42	15.25 ± 2.59	19.12 ± 1.56
2011	Winter	36.5 ± 5.35*	24.02 ± 4.77*	25.29 ± 3.06
	Rainy	34.25 ± 1.20	13.62 ± 1.49	39.37 ± 2.29*
	Summer	20.47 ± 0.81	9.66 ± 1.13	12.29 ± 1.25

The values are expressed in terms of Standard Error of the Mean.
*Significant values of trehalose content in Eri silkworm *Samia cynthia ricini* during various seasons in the years 2009 to 2011.

seasons of 2009 were 0.069 ± 0.024 (S. D), 0.046 ± 0.005 (S. D) and 0.031 ± 0.005 (S. D) mg, respectively, of the tyrosine (expressed in mg/ml/min) released at 37 °C.

The season-wise wise proteolytic activity in the digestive juice of the fifth instar of the Eri silkworm *Samia cynthia ricini* during winter, rainy and summer seasons of 2010 were 0.097 ± 0.041 (S. D), 0.053 ± 0.008 (S. D) and 0.035 ± 0.005 (S. D) mg, respectively, of the tyrosine (expressed in mg/ml/min) released at 37 °C.

The season-wise proteolytic activity in the digestive juice of the fifth instar of the Eri silkworm *Samia cynthia ricini* during winter, rainy and summer seasons of 2011 were 0.077 ± 0.031 (S. D), 0.049 ± 0.007 (S. D) and 0.033 ± 0.005 (S. D) mg, respectively, of the tyrosine (expressed in mg/ml/min) released at 37 °C.

Amylase activity

The season-wise amylase activity in the digestive juice of the fifth instar of the Eri silkworm *Samia cynthia ricini* during winter, rainy and summer of 2009 were 0.044 ± 0.0054 (S. D), 0.031 ± 0.001 (S. D) and 0.030 ± 0.003 (S. D) mg, respectively, of the maltose (expressed in mg/ml/min) released at 37 °C.

The season-wise amylase activity in the digestive juice of the fifth instar of the Eri silkworm *Samia cynthia ricini* during winter, rainy and summer seasons of 2010 were 0.049 ± 0.002 (S. D), 0.043 ± 0.002 (S. D) and 0.026 ± 0.003 (S. D) mg, respectively, of the maltose (expressed in mg/ml/min) released at 37 °C.

The season-wise amylase activity in the digestive juice of the fifth instar of the Eri silkworm *Samia cynthia ricini* during winter, rainy and summer seasons of 2011 were 0.039 ± 0.004 (S. D), 0.034 ± 0.002 (S. D) and 0.028 ± 0.001 (S. D) mg, respectively, of the maltose (expressed in mg/ml/min) released at 37 °C.

It is observed that the proteolytic and amylase activity in the digestive juice was higher during winter season and gradually decreased during rainy and summer seasons (Table 3).

DISCUSSION

India is one of the countries that harbour wild silkmoths of commercial value in the forests of

Table 3. Estimation of digestive amylase activity and proteolytic activity in Eri silkworm *Samia cynthia ricini* during various seasons in the years 2009 to 2011 (mg/ml or mg of 50 mg tissue).

Year	Seasons	Expressed in mg/ml/min	
		Proteolytic activity	**Amylase activity**
2009	Winter	0.069 ± 0.024*	0.044 ± 0.0054*
	Rainy	0.046 ± 0.005	0.031 ± 0.001
	Summer	0.031 ± 0.005	0.030 ± 0.003
2010	Winter	0.097 ± 0.041*	0.049 ± 0.002*
	Rainy	0.053 ± 0.008	0.043 ± 0.002
	Summer	0.035 ± 0.005	0.026 ± 0.003
2011	Winter	0.077 ± 0.031*	0.039 ± 0.004*
	Rainy	0.049 ± 0.007	0.034 ± 0.002
	Summer	0.033 ± 0.005	0.028 ± 0.001

The values are expressed in terms of Standard Error of the Mean.
*Significant values of digestive amylase activity and proteolytic activity in Eri silkworm *Samia cynthia ricini* during various seasons in the years 2009 to 2011.

North-East regions. Mulberry sericulture is a tradition and culture of the tribal people. At present efforts are focussed on Eri silkworm, which could be reared and bred for commercial spun silk. Andhra Pradesh, considering its climatic and geographical conditions, is found to be suitable for establishing sericulture industry owing to the availability of both primary and secondary food plants. Assam and other Northern states of India have been successful in developing sericulture and silk rearing can also be found to a small extent in Bihar, West Bengal, Orissa, Tamil Nadu, Kerala, Karnataka and Andhra Pradesh.

The present study has revealed that the protein content in the haemolymph was higher during the winter in all the three crops in all the three years (2009-11); the same trend was observed in silk glands except in the third year, where the protein content was higher during summer. However, it has shown variations in fat body in that it was slightly low in the three winter seasons and higher in two summer seasons. The protease activity was found to be highest during winter in all the three years, followed by rainy season and the least during summer season. From the literature it is evident that fluctuations in the protein content are mainly due to the inhibitory action of proteolytic enzymes or reduction in the synthesis of proteins [10].

The amylase and protease activity of the fifth instar larvae depends on the digestion and absorption of sugar and protein content of mulberry leaves. These will in turn increase the protein content in the haemolymph. Consequently the protein content in the silk glands will increase, which ultimately influence the silk productivity [11]. From the present studies it is evident that the highest protein content is mostly seen in the silk glands, which will be contributing to silk fibre, followed by haemolymph. The production of good quality silk and in large quantities depends on larval nutrition and healthiness, which is influenced by the quality of leaves fed to the larvae. Haemolymph protein concentration in *Bombyx mori* suggested its role in growth and metamorphosis of the larvae.

It has also been established that the silk gland proteins (sericin and fibroin) are different from the haemolymph proteins (storage proteins and 30 KDa) in molecular weight. The genes coding for fibroin and sericin expressions are found in the cells of posterior and middle silk glands, respectively [12, 13, 14]. Several studies have reported the roles of mRNA and tRNA found in the posterior silk gland in the synthesis of fibroin [15, 16].

Carbohydrates are the major components in the food of all the living organisms, which are either directly or indirectly used as the source of energy for all vital activities. The energy requirement of the larva serves as a determinant factor for the normal growth and development of the larva, which ultimately determines the quality of the silk produced. In the present study, the trehalose content in haemolymph, silk glands and fat body were found to be greater during winter season in all the three years, followed by rainy and summer seasons. Trehalose is the major and metabolically active, non reducing disaccharide in the insect blood [17] which is synthesized in the fat body [18] and utilized during spinning, flight and starvation of insects [19, 20]. It is well known that haemolymph, the only extra cellular fluid in insect, has diverse functions. It is the reservoir for most of the biochemicals that are required for nearly every physiological activity of the insect and the change in the composition of haemolymph reflects the morphogenic and biochemical changes taking place in insect tissues [21]. The quantitative differences in the trehalose and proteins of the fifth instar Eri silk worm observed in the present investigation are considered to be mainly because of environmental factors and qualitative changes in the leaf, Photoperiodism etc. [22].

The amylase activity was also found to be highest during winter in all the three years, followed by rainy season and the least during summer season. Marked increase was observed in the tissues of the silkworm in cooler temperatures [23]. Amylase is one of the key enzymes involved in digestion and carbohydrate metabolism in insects [24, 20]. According to Tanaka and Kusana [25], the amylase activity increases steeply during II and IV instars and reaches maximum in IV and fifth instars. As amylases play a very important role in starch digestion and in insect survival, a gradual increase of amylase activity in the fifth instar from summer to rainy to winter in all the three years may be attributed to better survivability of Eri silkworm in winter season. Studies on relationship between amylase activity and quantitative characters in

Bombyx mori revealed that silkworm strains which have more amylase activity showed better cocoon weight, shell weight and shell percentage [26]. A study on the correlation of yield and biochemical parameters has shown that effective rate of rearing was positively correlated with both digestive amylase and protease [27].

CONCLUSION

It is concluded that the high content of trehalose in haemolymph followed by fat body and silk gland maybe due to the energy demand of the insect, and the increase in protein content may be attributed to higher rate of protein synthesis via conversion of the nutrients of consumed leaves mainly into the silk gland followed by haemolymph and fat body. The corresponding increase in protease and amylase activity in winter season when compared to summer and rainy seasons also suggests effective digestion and absorption of carbohydrate and protein content and climatic adaptability. As the present work is focused mainly on the castor food plants, the study opens an avenue to further investigation based on the effect of nutrients in other food plants of Eri silkworm.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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