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Estimation of amino acids, urea and uric acid in tasar silkworm, *Antheraea mylitta* Drury

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Abstract: *The tasar silkworm, Antheraea mylitta Drury, Andhra local ecorace is an exclusive race of Andhra Pradesh. It is on the verge of extinction due to difficulty of acclimatisation at breeding and rearing stages. As an attempt to protect this race, a method of total indoor rearing has been done. In this context, the estimation of free amino acids, excretory products- urea and uric acid were compared during the fourth and fifth instars of tasar silkworm, reared under outdoor and indoor conditions. The study has revealed that amino acids decreased in the fat body in outdoor and indoor reared larvae in contrast to that in the haemolymph where it has gradually increased from first to third crops. This is an important finding as it reveals that indoor worms seem to adopt proteolytic activity in the haemolymph. Secondly, in the fifth instar the excretory products are more compared to fourth instar in the indoor reared worms. During fifth instar, formation of nitrogenous products lessens as silk synthesis enhances. The present study reveals that decrease in uric acid in fifth instar implies increase in growth rate and silk synthesis in both outdoor and indoor worms. The findings of the present investigation is helpful in the conservation and protection of the A. mylitta, Andhra local ecorace.*

Key words: *Antheraea mylitta, Indoor rearing, Estimation, Amino acids, Urea and uric acid*
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Introduction

Antheraea mylitta Drury, Andhra local ecorace is an exclusive silkworm of Andhra Pradesh. The reports from Centre for Developmental Studies (1992) of tasar area in Andhra Pradesh indicated a decline in its natural proliferation due to its difficulty in acclimatisation at breeding and rearing stages, and that if this trend continues, this race may become extinct if proper measures towards protection and conservation are not taken. Taking into consideration its superior commercial qualities such as compact and hard cocoons, high reelability, high shell ratio and low denier, indoor rearing method has been evolved in the laboratory. Earlier a few attempts like Chawki rearing (Thangavelu, 1992), prevention of massive deforestation (Sahay and Kapila, 1991) and afforestation drive (Kapila, 1989) have been suggested as an aid to improve environment and social forestry in backward areas. Recently, studies were also made in this direction in *Antheraea assama*, by interchanging the food plants during larval feeding (Singh *et al.*, 2004).

The majority of crop loss in tasar silkworm rearing is due to viral disease. Since rearing is conducted completely outdoor in the forest, there is no control over the climatic conditions (temperature and humidity) and thereby the rearings are subjected to great fluctuation in climatic conditions that often lead to viral attacks. There are also a number of predators which thrive on tasar silk worms, which lead to its crop loss.

In insects, the growth and development is associated with protein metabolism (Singh and Baquaya, 1971). In silkworms, the protein synthesis acitivity of the body wall and the midgut decreased when the larvae began to moult and increased from the midstage of the moulting period (Nagota, 1976). Marked increase in protein, pyruvate, total free amino acids, total lipids, phospholipids and triglycerols was observed in response to cold exposure (Pant, 1984). A recent study has also revealed the involvement of amino acids in interaction with tyranine in *Bombyx mori* (Ohta *et al.*, 2004).

In the silkworm larva, the nitrogenous waste products of metabolism are mainly excreted as urine, together with faecal pellets. The excretory pattern depends upon a number of environmental factors such as temperature and humidity (Alexandria and Stanchion, 1981; Dhinaker, 1990). The excretory pattern of silkworm larvae on exposure to F2 alpha increased the nitrogenous end products (Bharathi, 1993). Similarly larvae feeding with trace elements like cobalt increased the pattern of excretion (Sailaja *et al.*, 1997).

Excretion forms an important factor for the balance of nitrogen in the body. The excretion of nitrogenous waste products has been studied in a number of insects (Wigglesworth, 1950; Prosser and Brown, 1965; Craig, 1960). Uric acid contains comparatively less hydrogen than any other nitrogenous compound excreted by animals and it is therefore well adapted for conservation (Wigglesworth, 1965). Urea is present in small quantities in insects. The excretion in insects, its energetics and functional principles have also been worked out (Florey, 1982). As there is a limited information regarding tasar silkworm excretion, an attempt has been made here to compare the excretory pattern in the pellets of outdoor and indoor reared Andhra local tasar silkworms.

In the present investigation, total indoor rearing of silkworms has been undertaken from brushing stage to the cocooning stage in the controlled conditions for the three crops. Simultaneously, outdoor rearing was also done in the field of *Terminalia arjuna* plantation and various physical, biochemical and post cocoon characters were observed.

Materials and Methods

A batch of tasar silkworms, Andhra local ecorace, were reared simultaneously in outdoor field feeding on *Terminalia arjuna*

Fig. 1: Fifth instar worms on the rearing set-up. Faecal pellets seen on the parrafin paper

plantation. Total indoor rearing includes a rearing tray and freshly cut *T. arjuna* branches placed in the glass jars in the rearing room. The rearing is conducted meticulously at optimum temperature (25-30°C and humidity of 60-70%). Removal of faecal matter, diseased worms and bed cleaning was done at regular intervals. The rearing was conducted for three crops from June to December in the Sericulture Unit, Kakatiya University, Warangal (Fig. 1).

For the estimation of amino acids (Moore and Stein, 1954) the fifth instar larvae of outdoor and indoor rearing are selected for the collection of haemolymph and fat body. The haemolymph was collected into the test tubes and kept in deep freezer. The fat body was isolated in cold condition and weighed in chilled state using "Dhona"electrical balance and was used for subsequent biochemical studies. Free amino acids were measured at µg 100mg^{-1} of wet weight of tissue.

The excretory pellets of fourth and fifth instar larvae of outdoor and indoor larvae were collected separately and homogenized in precooled mortar and pestle thoroughly. The measurements are done according to the standard procedures for estimation of urea (Natelson, 1971) and uric acid (Brown, 1945; Oser, 1965) estimated as µg 100mg^{-1} of wet weight of the pellets. Centrifugation was done by using Remi centrifuge T.8 model (20,000 rpm). The estimations were based on colorimetric principle of Beer- Lambert's law in which the absorbance of coloured complexes are proportional to the concentration of reaction products. The data was statistically analysed and the results have been discussed by companing outdoor and indoor rearings.

Results and Discussion

The amino acid content in the fifth instar of outdoor reared larvae in the three crops are depicted in the Table 1. From the results it is seen that amino acid content has gradually decreased in the fat body in outdoor and indoor reared larvae in contrast to that in the haemolymph where it has gradually increased from first to third crops.

The qualitative differences in the carbohydrates, trehalose, proteins and amino acids found in the present investigation in Andhra local ecorace, *A. mylitta* between outdoor and indoor reared fifth instar silkworm is considered to be mainly because of the environmental

Table -1: Amino acids in outdoor and indoor V instar tasar silkworm, *Antherea mylitta* (Andhra local ecorace) in µg 100 mg^{-1} of fat body, mg 100 ml^{-1} of hemolymph

Crop	Outdoor rearing	Indoor rearing	Percent difference
Hamolymph			
I	401.0 ± 1.0	945.0 ± 5.0	57.56
II	581.0 ± 10.5	960.0 ± 68.4	65.23
III	680.0 ± 28.2	1033.0 ± 134.3*	34.17
Fat body			
I	3.58 ± 0.032	2.38 ± 0.0321	33.51
II	1.16 ± 0.04	1.30 ± 0.145*	12.06
III	0.053 ± 0.011	0.165 ± 0.21*	67.87

No. of samples = 3, * = Higher value of urea and uric acid contents in indoor rearing

Table - 2: Urea content in the excretory pellets of tasar silkworm, *Antherea mylitta* Drury (Andhra local) in µg 100 mg^{-1} of excretory pellets during the third crop

Instar \ Rearing method	Outdoor	Indoor	Percent difference
Fourth (IV)	9.2 ± 0.081	13.46 ± 0.12*	46.30
Fifth (V)	3.07 ± 0.06	10.46 ± 0.09*	240.71*

No. samples = 3, * = Higher value of urea and uric acid contents in indoor rearing

Table - 3: Uric acid in the excretory pellets of tasar silkworm *Antherea mylitta* Drury (Andhra local) in µg 100mg^{-1} of excretory pellets

Instar \ Rearing method	Outdoor	Indoor	Percent difference
Fourth (IV)	5.19 ± 0.073	12.2 ± 0.118*	135.0*
Fifth (V)	2.59 ± 0.051	6.38 ± 0.078*	146.3*

No. of samples = 3, * = Higher value of urea and uric acid contents in indoor rearing

factors and qualitative changes in the leaf and photoperiodism (Shamitha and Purushotham Rao, 2005). Sinha *et al.* (1987) has concluded that variations in the concentration of amino acids during rearing seasons in the larval and pupal haemolymph of *A. mylitta* was due to the variations in climatic changes during two rearing seasons.

The amino acid content of haemolymph and fat are found in the reverse order for three crops (Fig. 1 and 2). In the haemolymph the indoor worms have significantly high levels of amino acids showing more in the third crop, while in the fat body the amino acid content of indoor worms is less in the first crop, but increased in the second and third crops, showing a decline by third crop. An increase in the amino acid content indicates either low transaminase activity (Watanabe and Kobayashi, 1976) or high proteolytic activity of enzymes.

The amino acid content of the haemolymph in the indoor worms was found very high which means high proteolytic activity or possibly reduced breakdown of amino acids due to slow and restricted movements of the worm. It gives a clear picture of the worms under indoor conditions which perhaps leads to the quality of the cocoons produced. The worms under indoor conditions are less active and consume leaf in which the quality goes down with time after harvest.

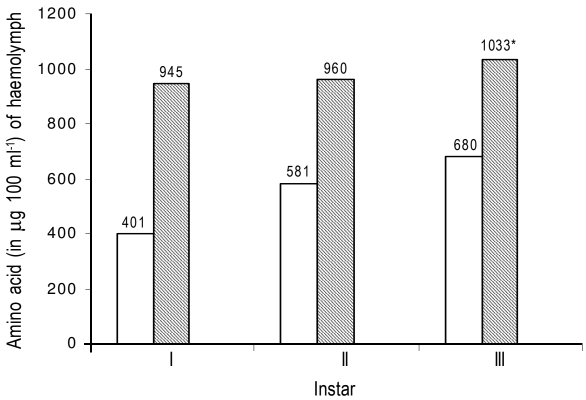

Fig. 2: Amino acids in the haemolymph of V instar tasar silkworm, *A. mylitta* (Andhra local ecorace)

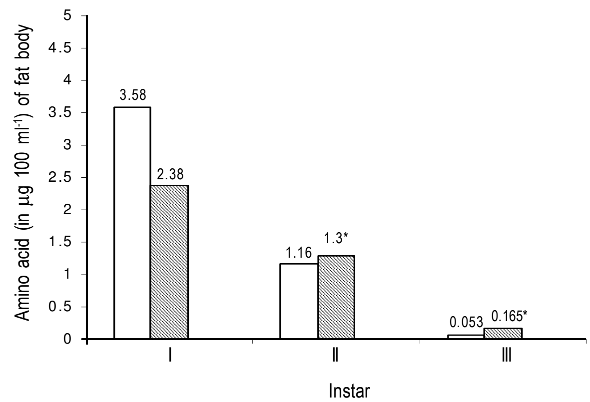

Fig. 3: Amino acids in fat body of V instar tasar silkworm, *A. mylitta* (Andhra local ecorace)

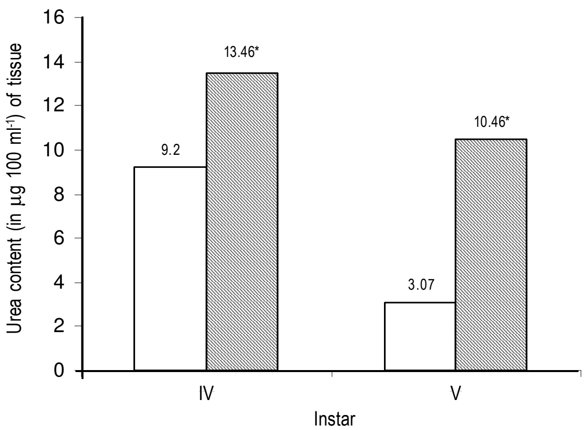

Fig. 4: Urea content in excretory pellets of *A. mylitta* (Andhra local ecorace)

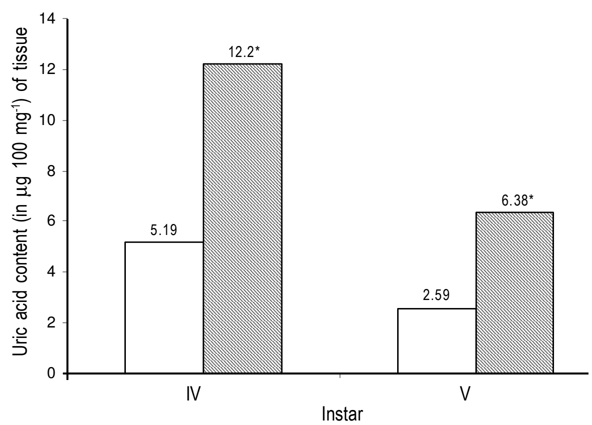

Fig. 5: Uric acid content in excretory pellets of *A. mylitta* (Andhra local ecorace)

□ Outdoor rearing ▧ Indoor rearing

The excretory products, urea and uric acid contents were found to be at a higher level in the silkworms reared under indoor conditions (Tables 2, 3). Instar-wise, in the fourth instar, ammonia and glutamine content were found at a significantly high level in the indoor worms as compared to the fifth instar (Shamitha and Purushotham Rao, 2001) while urea and uric acid content are in the reverse order showing higher content in the fifth instar. Of the four nitrogenous products, urea in the fifth instar larvae of indoor rearing is found to be maximum. The fourth instar silkworms had more significant difference between the outdoor and indoor worms (Fig. 3 and 4). It is well known that the terrestrial insects generally excrete uric acid exhibiting uricotelism (Corrigan, 1970; Prosser and Brown, 1965).

It is also reported that excretion in insects is variable depending upon habitat (Yamada and Kato, 1991). The higher level of nitrogenous products during fourth instar indicate higher breakdown of the end products which is correlated with high growth rate.

During fifth instar, formation of nitrogenous products lessens due to depleted growth rate while silk synthesis in the glands takes place at a rapid pace. In both the instars an increase of the excretory material in the indoor worms has been observed. In the present investigation, the outdoor worms are more active and growth rate has been higher than indoor worms while the excretory products are at a higher level in the indoor worms. A negative correlation between the growth rate and excretory pattern of silkworm has also been reported (Rath *et al.*, 2004).

A few workers have attempted to demonstrate the excretory products of genus *Antheraea* (Leifert, 1935; Heller and Jezeweska, 1959). Some of them include excretory metabolism of lepidopteran larvae at different stages of larval life (Barsagade and Tembhare, 2004). These studies reveal that there is a lability within the life history of the individual where the ratio of major three end products may fluctuate enormously from day to day and the major end product is invariably the uric acid. The present studies also show relatively constant higher content of uric acid in the indoor reared worms of both fourth and fifth instars.

The tasar silk industry has contributed substantially towards the economic development of tribes in India. The unscientific exploitation of *Antheraea* species, its multiplication and rearing on forest plants for cocoon production resulted in heterogeneity. The distribution, phenotypic and behavioural expression of various eoctypes of *Antheraea* species existing in different parts of India are based on environment- food plant, forest type, soil type and altitude of the area. Some of the eco-races of *Antheraea mylitta viz.,* Raily, Modal, Bhandara, Sukinda, Daba, Andhra local are being maintained in different locations in forest area. However, it lacked scientific approach of conservation of species in natural condition. Genetic identity of these species through bio-chemical studies and their regular monitoring in every generation give precise information about the homozygosity in the population of these insect species. As many ecotypes of tasar silkworm have been lost due to inbreeding, adverse climate and sudden outbreak of diseases, an establishment of 'breeding parks' has been suggested to overcome the problem (Siddiqui *et al*., 2006).

The present studies has successfully paved the way towards a scientific approach for the conservation of the tasar silkworm, *Antheraea mylitta* (Andhra local ecorace). The methodology of total indoor rearing followed by bio-chemical studies of various substrates like trehalose, glucose, proteins, lipids, lactic acid and pyruvic acid (Shamitha and Purushotham Rao, 2000, 2005) and DNA fingerprinting of ecoraces of *A. mylitta*- Andhra local and Daba TV (Shamitha and Purushotham Rao, 2000) have contributed to the research field of tasar culture. The present studies on amino acids, urea and uric acid are based on nitrogen metabolism and amino acids, the constitutents of silk proteins -fibroin and sericin.

It is said that environment is helpful for economic development; but economic development is harmful to environment. Tasar culture is an eco-friendly avocation because, soil quality, prevailing climatic and geographical conditions of the region are conducive to the race of that region (Dutta, 2006). In the present studies, as a measure of conservation of *A. mylitta*, Andhra local ecorace, an attempt towards total indoor rearing has been made in order to overcome a few environmental hazards and disease outbreak.

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