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Studies on the Proteins and Proteolytic activity of indoor reared Tasar silkworm, *Antheraea mylitta*. D (Daba TV)

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Abstract

Tasar silkworm, Antheraea mylitta Drury distributed as various ecoraces, is a forest-grown, trivoltine, polyphagous insect feeding primarily on Terminalia arjuna and is commercially exploited in varied tropical zones of India. Its traditional rearing in forest plantations has many disadvantages like erratic climatic conditions, lack of supervision and easy access to pests and predators, leading to extremely low cocoon yield. As there is a need to evolve a new method of rearing to stabilize tasar production, consistent efforts are being made towards" indoor rearing", from first instar to spinning. In the present investigation, a comparative analysis of the growth and biochemical aspects of indoor reared Tasar silkworm, Antheraea mylitta.D (Daba TV) is reported. It is observed that the protein content and Proteolytic activity in the fourth and fifth instars of indoor worms, reared under lower temperature and higher humidity than that of outdoor conditions, is higher than that of the outdoor worms in all the three crops.

Keywords: Tasar silkworm, *Antheraea mylitta*, indoor rearing, protein content, proteolytic activity.

Introduction

Non-mulberry sericulture holds great economic promise for the world forestry as a supplementary activity[1], helping arrest deforestation and permitting gainful utilization of natural wealth. Tasar silkworm is completely a wild living strain, however, survival and propagation of the silkworm depends upon the race of the worms and environment, which may sometimes involve unfavorable conditions like rain, hail or storm and exposes to constant danger of being attacked by pests and predators (figure-1). Moreover, the tribals follow traditional method of rearing *i.e.,* larvae after being transferred onto the food plants, on a routine basis will select own leaves and are left uncared for, till the harvest of cocoons. This method of rearing cannot ensure provision of the proper quality of leaves during different instars.

It is mainly to overcome these hurdles, the concept of *"Indoor rearing"* was conceived many years ago[2-4] which aims at protecting the larvae during younger stages of the development. These attempts include the rearing of tasar larvae upto the third instar on the cut branches of the host plants hanging on wire from tarpaulin roof, dipping of cut ends inside water, use of polythene cover and spraying of water over the leaves. In the subsequent years some of these methods were integrated and modified effectively and proved to be successful until the first moult with reduced crop loss. Further efforts to improve the rearing conditions, reduce larval loss and increase in production rate were reported[5, 6]. Choudhari *et al.* [7] studied on the rearing of tasar silkworm *Antheraea mylitta* D (Daba TV) have adopted indoor rearing of the young age worms (Chawki worms), concluded on the basis of cocoon assessment that short-time rearing of the tender worms under indoor conditions resulted in lessening of mortality rate and betterment in the cocoon characters. Due to the problems faced by them as cited above, an urgent need to protect tasar culture is urged.

Domestication of silkworm *i.e.,* the *indoor rearing technique* at the laboratory level has already been successful and is been adopted by many scientists to carry out their research works on nutritional ecology[8-10]. Certain works have also demonstrated that silkmoths can be bred successfully under confinement and the tasar caterpillars can be successfully reared indoor, for years, on standardized methodology[11,12].

However, the concept of *"Total indoor rearing"* from first instar larva to cocoon spinning was reported for the first time in the Sericulture Lab of Kakatiya University[13] by conducting an extensive comparative account of larval behaviour, physical and biochemical parameters, post-cocoon characters including the scanning electronic microscopic studies of shell and filament of outdoor and indoor reared tasar silkworm, *Antheraea mylitta* D (Andhra local ecorace). The study proved to be encouraging from the point of ERR (Effective Rate of Rearing) and denier of the filament, as but majority of the pre and post cocoon characters were not on par with the outdoor rearing. The present investigation which is based on this concept goes further with improved rearing equipment, systematic and continuous rearing of three crops and of Tasar silkworm, *Antheraea mylitta* (Daba TV) for three successive years (2008-2011).

Material and Methods

As an improvement over traditional method of rearing of tasar silkworms, measures were taken on regular pruning of *Terminalia arjuna* plantation and also use of nets to prevent pest

and predator menace. Pruning is done after the third crop is over, so that with the arrival of first crop, fresh branches, this time more in number will be ready for the larvae. This ensures uniform foliage and healthy leaves, resulting in improvement of crop yield. The *T. arjuna* plants are pruned to two to three feet in height and a gap of two feet between the plant and the net is observed so that the predators, which sit on the net cannot attack the worms. Another advantage of this is being able to observe the worms easily, without bending the branches. The new method of indoor rearing evolved here does not involve any sophisticated machines and can be practiced without much investment. In this attempt, a simpler way of feeding has been adopted (figure-2).

Figure–1
The pests attacking the tasar silkworm, *Antheraea mylitta*

Conical flasks can also be used for indoor rearing as they ensure continuous supply of water to twigs[14]. In early instars one conical flask can accommodate up to 200 – 300 young worms but in late instars only about 15-20 worms, each weighing 25-30gms can be grown. Such worms, when reach the end of the inserted twig of conical flask tend to fall down by loosing grip on the twigs. Hence the present study suggests preferring earthen pots instead of conical flask. It also contributes to maintain humidity level by constant evaporation of water in the rearing environment and prevent displacement of worms from the rearing set.

Figure–2
Indoor rearing set-up of Tasar silkworm, *Antheraea mylitta* (Daba TV)

The estimation of protein content was carried out by Lowry method[15] and the method of Anson[16], Kunitz[17], Lowry *et al.* [15] was used for the estimate the activity of digestive proteases, casein as substrate.

Results and Discussion

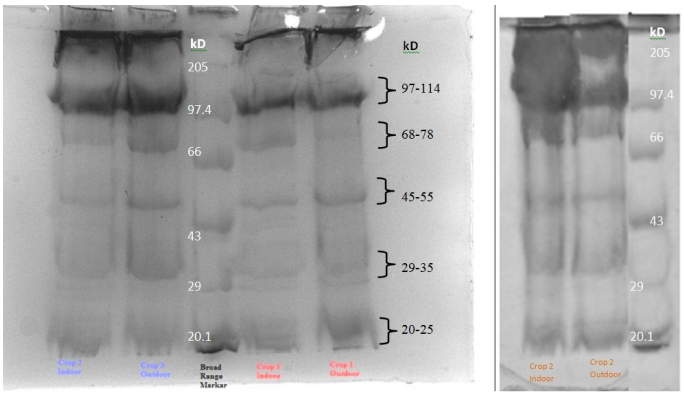
The total protein content in the haemolymph, fat body and silk gland of tasar silkworm, *Antheraea mylitta* Drury (Daba TV) during the three crops of 2008 recorded and presented in the table-1.

The first crop total protein content in the haemolymph of tasar silkworm, *Antheraea mylitta* Drury outdoor rearing of 2008 were 4.74 ± 0.42 (S. D) and 5.33 ± 0.39 (S. D) while indoor rearing were 5.6 ± 0.42 (S. D) and 8.65 ± 1.33 (S. D) mg/ml for fourth and fifth instars respectively. Total protein content in the fat body of outdoor rearing were 3.55 ± 0.46 (S. D) and 4.91 ± 0.61 (S. D) while indoor rearing were 2.2 ± 0.56 (S. D) and 3.52 ± 0.69 (S. D) mg/50mg tissue for fourth and fifth instar respectively. Total protein content in the silk gland of outdoor rearing was 6.58 ± 1.06 (S. D) while indoor rearing was 4.68 ± 0.85 (S. D) mg/50 mg tissue for fifth instar larvae (table-1).

The second crop total protein content in the haemolymph of tasar silkworm, *Antheraea mylitta* Drury outdoor rearing of 2008 were 6.71 ± 1.29 (S. D) and 8.55 ± 1.01 (S. D) while indoor rearing were 8.16 ± 0.83 (S. D) and 9.88 ± 1.52 (S. D) mg/ml for fourth and fifth instars respectively. Total protein content in the fat body of outdoor rearing were 3.9 ± 0.43 (S. D) and 5.88 ± 0.61 (S. D) while indoor rearing were 3.1 ± 0.56 (S. D) and 4.8 ± 0.57 (S. D) mg/50mg tissue for fourth and fifth instar respectively. Total protein content in the silk gland of outdoor rearing was 9.5 ± 1.0 (S. D) while indoor rearing was 8.44 ± 0.81 (S. D) mg/50 mg tissues for fifth instar larvae (table 1).

The third crop total protein content in the haemolymph of tasar silkworm, *Antheraea mylitta* Drury outdoor rearing of 2008 were 8.16 ± 0.54 (S. D) and 10.81 ± 1.11 (S. D) while indoor rearing were 10.14 ± 0.67 (S. D) and 12.89 ± 1.17 (S. D) mg/ml for fourth and fifth instars respectively. Total protein content in the fat body of outdoor rearing were 5.19 ± 0.33 (S. D) and 6.47 ± 0.57 (S. D) while indoor rearing were 4.81 ± 0.83 (S. D) and 5.87 ± 1.01 (S. D) mg/50mg tissue for fourth and fifth instar respectively. Total protein content in the silk gland of outdoor rearing was 10.64 ± 0.64 (S. D) while indoor rearing was 9.79 ± 0.34 (S. D) mg/50 mg tissue for fifth instar larvae (table-1).

The haemolymph proteins of outdoor and indoor reared tasar silkworm, *Antheraea mylitta* Drury (Daba TV) have been analysed by 10% SDS Polyacrylamide Gel Electrophoresis (PAGE). Five bands were observed in the range between 20-114 kD. In the haemolymph of outdoor and indoor fifth instar larvae, there is no significant variation and dissimilarity observed in bands of haemolymph proteins. The bands appeared in the range of 20-25, 29-35, 45-55, 68-78, and 97-114 kD (figure-3).



Figure–3
SDS-PAGE showing the band patterns in the haemolymph

The estimation of proteolytic activity of digestive juice of outdoor and indoor reared tasar silkworm, *Antheraea mylitta* Drury (Daba TV) during the three crops of 2009 recorded and presented in the table-2.

The first crop Proteolytic activity of digestive juice of tasar silkworm, *Antheraea mylitta* Drury outdoor rearing of 2009 were 0.021 ± 0.003 (S. D) and 0.034 ± 0.007 (S. D) while indoor rearing were 0.029 ± 0.007 (S. D) and 0.037 ± 0.004 (S. D) mg of tyrosine released mg/ml/min at 37°C for fourth and fifth instars respectively (table-2).

The second crop Proteolytic activity of digestive juice of tasar silkworm, *Antheraea mylitta* Drury outdoor rearing of 2009 were 0.029 ± 0.001 (S. D) and 0.037 ± 0.002 (S. D) while indoor rearing were 0.035 ± 0.003 (S. D) and 0.039 ± 0.002 (S. D) mg of tyrosine released mg/ml/min at 37°C for fourth and fifth instars respectively (table-2).

The third crop Proteolytic activity of digestive juice of tasar silkworm, *Antheraea mylitta* Drury outdoor rearing of 2009 were 0.046 ± 0.008 (S. D) and 0.062 ± 0.005 (S. D) while indoor rearing were 0.064 ± 0.009 (S. D) and 0.079 ± 0.005 (S. D) mg of tyrosine released mg/ml/min at 37°C for fourth and fifth instars respectively (table-2).

The *protein content* in haemolymph of fourth and fifth instars was found to be higher in all the three crops of the indoor reared tasar silkworms. On the contrary, in the fat body it was higher in outdoor reared ones. The protein content in the silk gland of fifth instar larvae of outdoor reared worms was also found to be higher.

The present analysis of lymph proteins by SDS shows the range of molecular weights that are present in the haemolymph. A study on haemolymph amino acids and protein profile by SDS – PAGE on *Antheraea mylitta* suggested that main role in growth and development of the larva[18]. This method was characteristic sericin molecular[19] in *Bombyx mori*. Some studies have also shown the anti oxidant potential of sericin extracted by SDS – PAGE from cocoon of *A. mylitta* exposed to H_2O_2 for 24 hrs[20].

As this is a preliminary work, specific proteins (*e*.g., Storage, proteins, sericin, fibroin, *etc.,)* are required to be done to know the protein differences in the haemolymph of outdoor and indoor reared tasar silkworm *Antheraea mylitta* Drury Daba TV.

As stated earlier, haemolymph which serves as a reservoir of a number of nutrients and metabolites, undergoes physiological fluctuations in its composition at different developmental stages. Nutrition of proteins is important for silkworm larvae because of their active utilization of nitrogen substances involving the synthesis of silk protein. The increased protein content in the haemolymph and its decrease in fat body in the indoor worms might be due to the release of excess of proteins by the fat body into the haemolymph and simultaneous increase in the silk gland.

The exposure to low temperature stimulates the accumulation of low molecular weight cryoprotectants and synthesis of some antifreeze proteins[21,22]. Above study revealed increase in the protein content in the haemolymph tissue of cold-stressed larvae reflects on the reduced amount of food intake at low temperature. This confirms that proteins are not a source of energy in colder environments, but are involved in lowering the super cooling and freezing points and thus protect the larvae from injury caused by ice crystals[23, 24]. This reason can substantiate as to why the protein content was more in the haemolymph but not the silk gland of indoor reared worms. Haemolymph protein concentration in Bombyx mori suggested its role in growth and metamorphosis of the larvae rather than contributing in silk protein synthesis and also been established that haemolymph proteins differ from silk proteins in molecular weight, mRNAs and tRNAs and genes[25].

Like all other animals, growth and development in insects is associated with protein metabolism[26]. In silkworms, the protein synthetic activity of the body wall and the midgut decreased when the larvae began to moult and increase again from the midstage of the moulting period[27]. Marked increase was observed in the tissues of the silkworm in cooler temperatures[28]. This finding can also be substantiated with the earlier studies that the quantitative differences in the carbohydrates, trehalose, proteins and amino acids in the fifth instar indoor reared *Antheraea mylitta* D, Andhra local ecorace[29] is mainly because of the environmental factors and qualitative changes in the leaf, photoperiodism *etc*. The production of good quality and quantity of silk depends on larval nutrition and healthiness, which is influenced by the quality of leaves given for food. Thus, it can be inferred that though healthy leaves were selected for indoor rearing, in order to increase the protein content in the silk gland, the leaves can be dipped in trace amounts of minerals (potassium, calcium, magnesium *etc.*) as they are found to stimulate the enzyme activity and metabolic processes[30].

Table–1
Estimation of total protein content in the outdoor and indoor reared tasar silkworm, *Antheraea mylitta*. D

Haemolymph				
Instar	IV		V	
1	4.74 ± 0.42	5.6 ± 0.42	5.33 ± 0.39	8.65 ± 1.33
2	6.71 ± 1.29	8.16 ± 0.83	8.55 ± 1.01	9.88 ± 1.52
3	8.16 ± 0.54	10.14 ± 0.67	10.81 ± 1.11	12.89 ± 1.17
Fat body				
4	3.55 ± 0.46	2.2 ± 0.56	4.91 ± 0.61	3.52 ± 0.69
5	3.9 ± 0.43	3.1 ± 0.56	5.88 ± 0.61	4.8 ± 0.57
6	5.19 ± 0.33	4.81 ± 0.83	6.47 ± 0.57	5.87 ± 1.01
Silk gland				
7	Developing Silk glands		6.58 ± 1.06	4.68 ± 0.85
8			9.5 ± 1.0	8.44 ± 0.81
9			10.64 ± 0.64	9.79 ± 0.34

Table–2
Estimation of digestive Proteolytic activity in outdoor and indoor reared tasar silkworm, *Antheraea mylitta* Drury, DabaTV (expressed in mg/ml/min)

Expressed in mg/ml/min		**IV instar**		**V instar**	
Enzyme activity	**Rearing Crop**	**Outdoor**	**Indoor**	**Outdoor**	**Indoor**
Proteolytic activity	I	0.021 ± 0.003	0.029 ± 0.007*	0.034 ± 0.007	0.037 ± 0.004
	II	0.029 ± 0.001	0.035 ± 0.003	0.037 ± 0.002	0.039 ± 0.002
	III	0.046 ± 0.008	0.064 ± 0.009*	0.062 ± 0.005	0.079 ± 0.005*

The ***proteolytic activity*** in the fourth and fifth instars of indoor reared tasar silkworms was significantly higher than that of the outdoor reared ones in all the three crops.

The proteolytic activity in the fourth and fifth instars of indoor reared tasar silkworms was significantly higher than that of the outdoor reared ones in all the three crops. Reports available on the increased protease activity of midgut of fifth instar *Bombyx mori*, has been attributed to quality of mulberry leaves for digestion and absorption of sugar and protein content of the mulberry leaves with subsequent increase in the haemolymph and silk gland[31].

Conclusion

The present studies includes provision of selected leaf and optimum conditions of temperature and RH, minimized pest and predator menace, improved crop production and decreased mortality. However, further efforts to produce robust indoor reared silkworms are the need of the hour.

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