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Lactic Acid and Pyruvic Acid Content in Diapausing Pupae of Outdoor and Total Indoor Reared Tasar Silkworm, *Antheraea mylitta*.D

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Abstract : *Antheraea mylitta* Drury is a species widely distributed from West Bengal in the East to Karnataka in the South. Andhra local ecorace is a tropical tasar variety available only in the state of Andhra Pradesh. In spite of its superior commercial characters due to certain weaknesses the development of this ecorace found not keep pace with the exotic races. As a measure of preservation of this ecorace, an attempt has been made for its total indoor rearing. In this communication a comparative account of lactic acid and pyruvic acid has been reported from the pupae of outdoor and total indoor reared tasar silkworm. The biochemical estimations have revealed certain interesting findings. More pyruvic acid in the fat body of indoor reared pupae suggests a positive feedback for indoor rearing of tasar worms as pyruvic acid occupies a central position in the metabolism, linking the metabolic pathways of proteins, carbohydrates and fats.

Key words : Indoor and outdoor rearing, Diapause pupae, Lactic acid, Pyruvic acid, Anaerobic respiration.

Introduction

Andhra local ecorace is available only in the state of Andhra Pradesh. It is well known for its superior commercial characters like compact and hard cocoons, high reelability, high shell ratio and low Denier (Thangavelu, 1992). Owing to its weaknesses like climatic hazards, prolonged larval period, heavy larval mortality, indefinite period of diapause and erratic moth emergence and poor egg-laying behaviour (Sen *et al*.,1967), it has resulted in low productivity and thorough negligence leading to its extinction. Hence an attempt on total indoor rearing of the test insect has been done, which includes hatching, larval development, cocoon spinning, moth emergence, coupling and egg-laying. Recently a comparative account of proteins and lipid content has been made between the larvae of outdoor and total indoor reared tasar worms (Shamitha and Purushotham, 2005). Earlier, efforts were also made to prevent pupal mortality due to high temperature (Ananthbandu, 1994). Chawki worm rearing was attempted (Jayaprakash *et al*., 1993). in order to prevent the early stage larval loss. A comparative studies on diapause and non-diapause pupae of tasar silkworms has been done (Madhu , 1997).

Material and Methods

A batch of tasar silkworms were grown simultaneously in the outdoor and indoor conditions on the food plant *Terminalia arjuna* (Fig 1 and 2). The diapause cocoons of outdoor and indoor rearing were collected and the pupae were taken out of the cocoons. This was done by cutting them open at the peduncle end very carefully so as not to injure the pupae (Fig 3). Haemolymph was collected by puncturing the pupae and after measurement it was kept in deep freezer and was used for biochemical tests.

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Fig. 1 : Out door rearing of Tasar Silk Worm (*Terminalia arjuna Plantation*)

Fig. 2 : Total Indoor Reading set-up showing (a: Rearing Tray; b: Conical flask; c: Parafin paper; d: Thermocoal; e: *T. arjuna* twings; f: Bleaching powder spread around the rearing set-up)

Fig. 3 : Cocoons of Tasar Silk Worm Andhra Local Ecorace

The estimation of lactic acid was done based on the principles of Barker and Summerson, modified by Huckbee (1961) and pyruvic acid estimation by Freidman Hangen method (1943). The contents of lactic acid and pyruvic acid were expressed as micrograms/100 mg wet weight.

Centrifugation was done by using Remi centrifuge T.8 model. These estimations were based on calorimetric 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 comparing outdoor and indoor rearings.

Results

The amount of pyruvic acid content in outdoor and indoor reared diapause pupae was 88.82 and 70.8 mg per 100 ml of haemolymph and 0.365 and 0.146 mg per 100 mg of fat body respectively. The outdoor reared diapause pupae have more pyruvic acid content than that of indoor reared ones which is about 20.2 and 60 percent in haemolymph and fat body respectively (Table 1).

The lactic acid content in the outdoor and indoor reared diapause was 204 mg and 189 per 100 ml of haemolymph and 0.635 and 0.241 mg per 100 mg of fat body respectively. The lactic acid content in outdoor reared diapause is greater than that of indoor reared ones by 7.35 and 62.0 percent in haemolymph and fat body respectively (Table 2).

Discussion

The pyruvic acid and lactic acid levels of haemolymph (Fig 4) signify increase of the latter both in the indoor and outdoor diapause pupae. While in the fat body lactic acid was found less than pyruvic acid in the indoor and more than pyruvic acid in the outdoor. (Fig 5) During the larval development in preparation for metamorphosis, insects usually attain maximum carbohydrate content which is present as a reserve material in the form of glycogen in the fat body besides having trehalose at higher concentration in the haemolymph (Lenarto *et al*., 1967).

Table 1 : Pyruvic acid in outdoor and indoor diapause pupae of tasar silkworm *Antheraea mylitta* (Andhra local) in µg/100 mg of fat tissue and µg/100 ml of haemolymph

Tissue	Outdoor rearing	Indoor rearing	Percent difference
Haemolymph	88.82 ± 0.04	70.8 ± 3.818	20.2
Fat body	0.365 ± 0.049	0.416 ± 0.028	60

Table 2 : Lactic acid in outdoor and indoor diapause pupae of tasar silkworm *Antheraea mylitta* (Andhra local) in µg/100 mg of fat tissue and µg/100 ml of haemolymph

Tissue	Outdoor rearing	Indoor rearing	Percent difference
Haemolymph	204 ± 0.03	189 ± 2.06	7.35
Fat body	0.636 ± 0.041	0.241 ± 0.025	62

The present studies also reveal significant difference in the content of glycogen and lipid of outdoor and indoor reared diapause pupae. Trehalose content of haemolymph and fat body in the diapausing pupae are found significantly depleted in the indoor pupae (Shamitha and Purushotham, 2000).

The variations in the glycogen content of diapausing pupae showing higher content in the haemolymph than lipids, higher lactic acid than pyruvic acid and higher protein content than amino acids. The higher trehalose content in the outdoor reared silkworm pupae suggest lethargic state of indoor worms. It is interesting to note higher lactic acid content of both outdoor and indoor pupae showing the hypoxic condition and the respiration in the anaerobic conditions by the pupae of tasar worms. From the literature it is found that insects undertake anaerobic respiration during which the lactic acid gets accumulated (Dinesh, 1995). In the present investigation, it is revealed that the pyruvic acid content of fat body is found to be more in the indoor

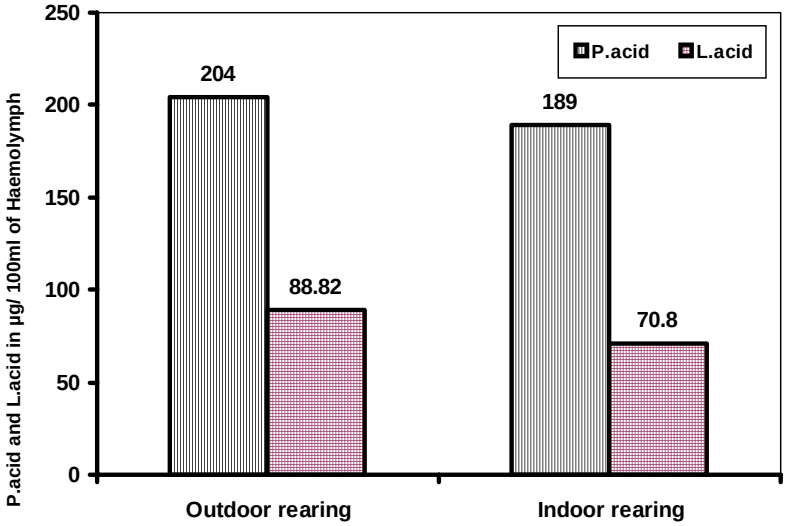

Fig. 4 : Pyruvic acid and Lacticacid content in the Haemolymph of outdoor and indoor diapause pupae of tasar silkworm *Antheraea mylitta* (Andhra local)

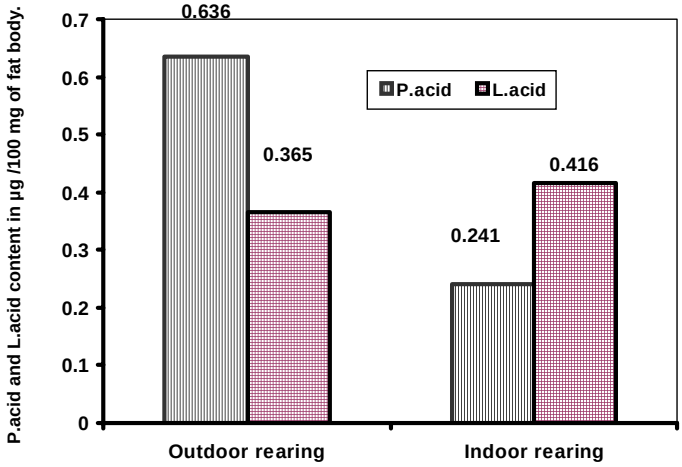

Fig. 5 : Pyruvic acid and Lactic acid content in the fat body of outdoor and indoor diapause pupae of tasar silkworm *Antheraea mylitta* (Andhra local)

diapause pupae but in the haemolymph it is in the reverse order.

Conclusion

More lactic acid in the haemolymph and fat body of outdoor reared pupae than that of indoor suggests that the rate of anaerobic respiration is more in outdoor than that of indoor rearing conditions.

More pyruvic acid in the fat body of indoor reared pupae suggests a positive feedback for indoor rearing of tasar worms as pyruvic acid occupies a central position in the metabolism, linking the metabolic pathways of proteins, carbohydrates and fats. It is also formed from proteins (by deamination) and glycogen (by glycolysis) and can give rise to glycogen (by gluconeogenesis) and fats (with acetyl coenzyme A).

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