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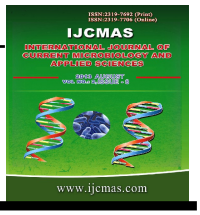

Original Research Article

Estimation of crude and purified protein from de-oiled pupae of ERI silkworm, *Philosamia ricini*

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Keywords

Eri silkworm; *Philosamia ricini*; histogenesis crude protein; Mulberry histolysis.

A B S T R A C T

In the present investigation, crude and purified protein percentage was estimated from de-oiled pupae powder at the different hours(0 hours,72 hours,144 hours,216 hours) of pupae development of Eri silkworm,*Philosamia ricini* was analysed.It was found that crude protein and protein concentrate was gradually increased from 0 hours to 216 hours both in male and female pupae. However, female pupae exhibited 45.1g of protein concentrate at 216 hours of development when compared to male pupae(39.3g).The presence of high protein concentrate in the pupae may be due to the fact that at the beginning of development the protein was utilized for histolysis and the increase of protein at 216 hours may be due to histogenesis of adult organs hence, the gradual increase of protein was observed from 0 hr-216 hrs.

Introduction

India is the only country in the world, all the four commercial varieties of natural silks namely Mulberry, Tsar, Eri and Muga. Eri culture is practiced in Assam, WestBengal, Manipur, Bihar, Orissa, Nagaland, Meghalaya, Karnataka, Andhra Pradesh and Tamil nadu states. *Philosamia ricini* which belongs to the order Lepidoptera and family Saturniidae is a domesticated, multivoltine and polyphagous insect feeding on castor, Tapoica, Kesseru, Papaya leaves. There are reports available on the quantitative and qualitative changes in the haemolymph protein in insect development (Lauffer, 1960; Chippendale and Beck 1966,Sinha *et al*, 1985). Similary there are some reports on amino acid estimation (Jolly *et al* 1972, Pant and Unni, 1978; Mohanty and Mittra 1988) and concentraction and types of carbohydrates in haemolymph of insects during the life cycle (Wyatt and Kalf 1957,Kim *et al*, 1973). There is no documentary evidence at present to the total protein concentration during the development of male and female pupae of *P.ricini*. Therefore,a crude and purified protein concentration was analysed during different hours of

pupal development in *P.ricini* has been made in present paper. This total protein analysis may provide some clue to understand the possible role of protein during the pupae development of Eri silkworm *P.ricini*.

Materials and Methods

The male and female pupae of domesticated Eri silkworm *Philosamia ricini* constituted the experimental material. The young age to late age Eri silkworm larvae reared under the standard condition of temperature and humidity fed with castor leaves. The life cycle of *P.ricini* is similar to *Bombyx mor*i in respect of development from egg to adult. The pupae at every 72 hrs of development(0 hrs,72 hrs,144 hrs and 216 hrs) were selected and the male and female pupae were separated based on genital marking.

100g wet weight of male and female pupae were dried separately at 65°C using hot air oven for 3 days and the dried male and female were powdered separately using mortar and pestle. The powdered pupae were subjected to oil extraction using chloroform adopting Soxhlet method. Now the de-oiled pupae powder is used for protein extraction.

Extraction of protein from de-oiled male and female pupae powder of Silkworm, *Philosamia ricini*

The silkworm pupae protein was extracted from male and female the oiled pupae powder of *Philosamia ricini* by changing ionic concentration (pH) and cell disruption techniques following the method of Nagaraj and Basavanna (1969).

20g of de-oiled male and female pupae powder was soaked in 0.5% aqueous sodium hydroxide solution (1 : 10 w/v) separately taken in a 250 ml glass beaker and was agitated for about 20-30 minutes and kept overnight in a refrigerator maintained at 5^{o}C. Next day the protein was coagulated at pH 4 by adding concentrated hydrochloric acid drop by drop by the side of the beaker. The precipitate was allowed to settle and the supernatant was decanted. The precipitate was washed with water to bring its pH to neutral and filtered in a Buchner funnel. The protein precipitate obtained was repeatedly pressed in blotting paper to remove the moisture content and the extracted protein per cent was estimated by using Kjeldhal method as explained below.

Estimation of protein per cent in the de-oiled male and female pupae powder of Eri silkworm, *Philosamia ricini* following Kjeldhal method [IS : 7874 (Part I) 1975]

0.5 grams of de-oiled male and female pupae powder of Eri silkworm,*Philosamia ricini* was transferred separately to the Kjeldahl flask, to this 5 g potassium sulphate, 0.25 g of copper sulphate and 25ml of concentrated sulphuric acid was added. The flask was placed in an inclined position and heated below the boiling point of acid until frothing ceases. Increase the heat until the acid boils vigorously for about 2 hours till oxidation was completed. The contents of the flask were cooled and transferred separately to the round bottom flask with 200ml water. Further, 45% of Sodium hydroxide (NaOH) solution was added to make the solution alkaline by the side of the flask (so that it does not mix at once with the acid solution, but forms a layer below the acid layer). The apparatus was arranged in

such a way that the tip of the dip-tube extends below the surface of the standard Sulphuric acid solution in receiver. The contents of the flask was mixed by shaking the distilled until ammonia passed over into the standard Sulphuric acid (0.25N) solution when end point is reached (red to yellow). The blank experiment was carried out to remove the experimental error. The protein percent was calculated by multiplying the per cent of nitrogen with animal protein factor (N x 6.25) the total nitrogen was calculated using the following equation:

Total Nitrogen = Vol. of NaOH consumed × Normality of Sodium Hydroxide × 100 × 0.014 /Weight of the sample

The above method was also followed to estimate the nitrogen per cent and crude protein per cent of the de-oiled pupae powder.

Result and Discussion

The analysis of total crude protein per cent in deoiled pupae powder of male and female Eri silkworm *Philosamia ricini* at different hours of development revealed that there is a gradual increase from 0 hrs-216 hrs of development i.e. 71.37%-79.375% in male and similar changes was seen in female with 71.5%-88% (Table-1) However, the amount of protein extracted from 100g of deoiled pupae powder at different hours of development showed variation in male with 21.6g, 24.15g, 43.85g, 39.3g and female with 17.7g, 41.7g, 37.45g, 45.1g at 0hrs,72 hrs,144 hrs and 216 hrs.(Table-1).

The analysis of purified protein from deoiled pupae powder revealed gradual increase in both male and female during different hours of development from 0 hrs-216 hrs. Male showed variation with 72.375%-80.56% and similar increase in protein per cent was seen in female with 72.5%-93.875% (Table-1).

At present Indian sericulture industry has mainly concentrated on the silk production for the commercial exploitation. However, there is no evidence to show that waste material generated during silkworm rearing and silk reeling is used for large scale commercial utilization in India. Therefore in the present study an attempt has been made to estimate the protein percent and extraction of protein in deoiled pupae powder of male and female Eri silkworm.

Protein have always been interesting biochemical tool for insect biochemists because of their potential role in growth,development,morphogenesis and many intermediary of metabolic pathway of insects Kar *et al.,* (1994). The insect haemolymph protein was observed by Lauffer (1943) on silkworm *Bombyx mori.* Afterwards a series of detailed physico-chemical studies of silkworm was made by Wyatt (1961) and Tojo *et al.,* (1980).

The analysis of the result with regard to estimation of protein per cent in the deoiled pupae of male and female Eri silkworm was shown the lowest percent in male and highest in female.This is in agreement with the finding of Doira and Kawaguchi (1972).

The analysis of the result of the extraction of protein concentrate of deoiled powder of male and female pupae at different hours revealed at about 50% of protein can be extracted. It is also observed that there is variation in protein concentrate of male and female pupae of Eri silkworm. The presences of higher protein concentrate in male female are due to the histolysis and histogenesis of adult organs.

Table.1 Estimation of protein percent, extraction of protein(g) and estimation of protein percent from extracted protein in male and female de-oiled pupae powder in Eri silkworm, *Philosamia ricini*

S.No	Nitrogen% and Protein	0 Hrs		72 Hrs		144 Hrs		216 Hrs	
		Male	Female	Male	Female	Male	Female	Male	Female
1	Nitrogen % in crude de-oiled powder(N)	11.42	11.44	11.88	11.89	11.94	13.3	12.7	14.08
2	Estimation of protein(N×6.25) per cent in de-oiled pupae powder	71.37 (11.42×6.25)	71.5 (11.44×6.25)	74.25 (11.88×6.25)	74.31 (11.89×6.25)	74.625 (11.94×6.25)	83.125 (13.3×6.25)	79.375 (12.7×6.25)	88 (14.08×6.25)
3	Amount of protein obtained de-oiled pupae powder per 100g	21.6	17.7	24.15	41.7	43.85	37.45	39.3	45.1
4	Nitrogen % in purified protein from de-oiled pupae powder(N)	11.58	11.60	11.90	11.93	12.23	13.82	12.89	15.02
5	Estimation of extracted protein(N×6.25) per cent from de-oiled pupae powder	72.375 (11.58×6.25)	72.5 (11.60×6.25)	74.375 (11.90×6.25)	74.562 (11.93×6.25)	76.43 (12.23×6.25)	86.375 (13.82×6.25)	80.56 (12.89×6.25)	93.87 (15.02×6.25)

Figure.1 Estimation of crude protein percent in deoiled pupae powder.

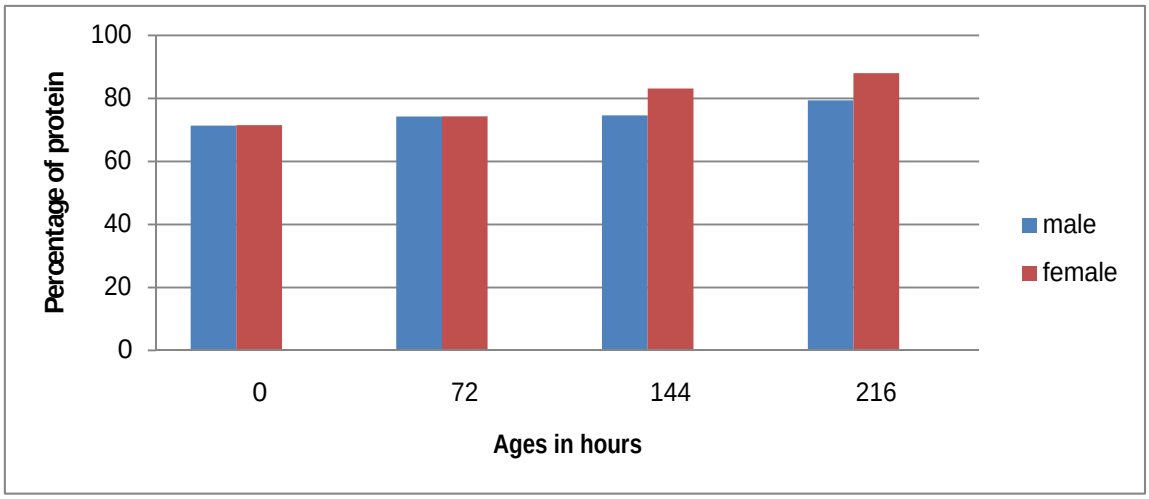


Figure. 2 Extraction of protein from deoiled pupae powder (100 gm).

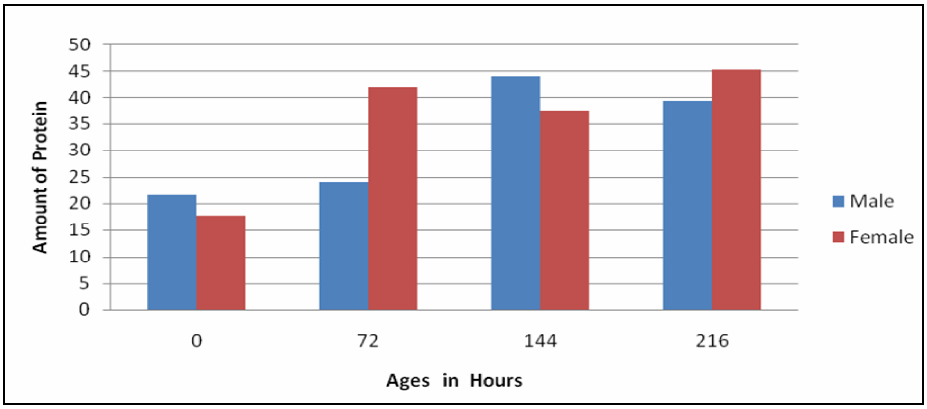
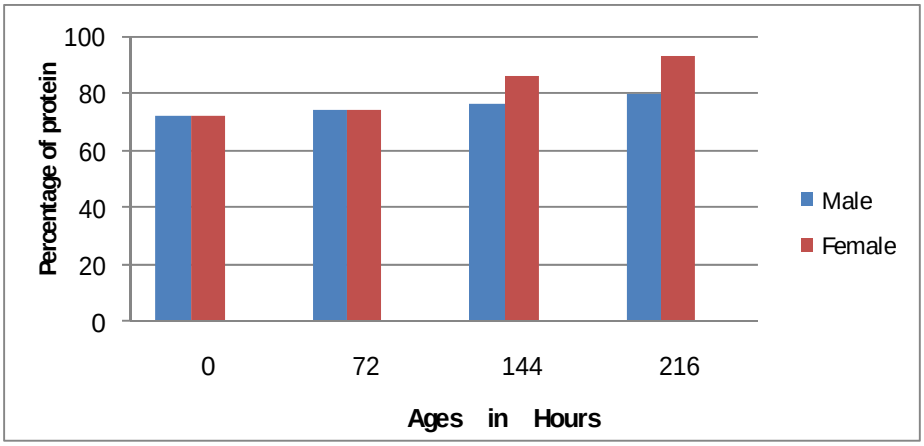


Figure.3 Estimation of extracted protein percent in deoiled pupae powder.



These events are mediated by pupae fat body which serves as a transient depot of cell constituent as suggested Yamashita and Hasegawa (1974). It has been reported that silk protein hydrolyses are one ingredients in cosmetics industries which can improve skin look and cover skin problem (Kato *et al.*,1998;Yamada *et al.*,2001; Chang Kee *et al.*, 2002; Hu *et al.*, 2005). Since pupae is rich in protein it can be sold for fertilizer, biogas (Viswanath and Nand, 1994, feed stuff (Nandeesha *et al.*,1990) and other agricultural purposes.

Furthermore, Yang (2002) also reported that silkworm pupas have been used as Chinese traditional medicines since ancient time.

Pharmacological studies show that silkworm pupas are alimental for increasing immunity, protecting the liver and preventing cancer. Proximate analysis of pupa showed that it contains 55-60% protein, 25-30% lipid, 4.96% fiber, and other substances, thus indicating that it could be a good protein source for various purposes (Yang *et al.*, 2002; Rangacharyulu *et al.*, 2003).

From the above experimental analysis of extraction of protein concentrates of deoiled male and female pupae at different stages of development revealed the presence of 35-45% proteins. This experiment was repeated using Lowry's method which gave a similar result of 35-

45% protein concentrates in both male and female pupae (Anil, N.T.,1999; Priyadarshini, P.A.,2013).

The present work can be extended for purification of different types of proteins in insect metabolism, metamorphosis and growth. It can be further extended for understanding sequencing of proteins and other molecular analysis.

References

Anil, N.T., B. 1999. Ph.D Thesis. Extraction of oil and aminoacids from silkworm pupae. Bangalore University.

Chippendale, G. M. and Beck, S. D. 1966. Haemolymph proteins of *Ostrinia nubilalis* during diapause and prepupae differentation J Insect Physiol. 12: 16-29

Chang-kee, H., J. Yuk-hyun, L. Sung-hee, P.Geum-ju and Decock-hyoung, C. 2002. Anticancer agents containing antigenotoxic and immunostimulative peptides produced from the hydrolysate of silkworm cocoon.Patent No. WO02076487.

Doira, H., and Kawaguchi, Y. 1972. Insect plasma proteins. J.Fac. Agric.

Hu, D., Q. Liu,H. Cui,H . Wang,D.Han and Xu, H. 2005. Effects of amino acids from selenium-rich silkworm pupas on human hepatoma cells. Life sci. 77(17): 2098-2110.

Indian standard, 1975.Methods of tests for animal feeds and feeding stuffs.Part I General methods. IS : 7874 (Part 1) 3 - 4 (Eds) Bureau of Indian Standards, New Delhi.

Kato ,N., S. Sato, A. Yamanaka, H. Yamada, N.Fuwa and Nomura, M. 1998 Silk protein, inhibits lipid peroxidation and tyrosinase activity, Biosci. Biotech. Biochem.62(1): 145-147.

Kim ryul, H., S.M. Yoe, J. K. Yu and Kum, K. M. 1973. Carbohydrates and inorganic ions in haemolymph and fat body during metamorphosis of the silkworm *Bombyx mori L.*The Korean J Entomol. 13: 55-59

Kar, P.K., P.P. Srivastava, R.K. Sinha and Sinha,B.R.R.P. 1994. Protein concentration in the pupal haemolymph of different races and F_1 stop cross of *Antheraea myllitta* D.Indian .J.Seric. 33(2): 174 -175.

Lauffer,M.A., 1943. Ultra centrifugation studies on the blood of normal and jaundice diseased silkworms . Propc. Soc. Exp. Biol. Med. 52: 330-332

Mohanty, A. K and Mittra, A. 1988. A comparative biochemical study of the haemolymph in bivoltine and trivoltine pupae of the tasar silkworm *Antheraea mylitta* Drury during development. Sericult. 28: 125-132

Nandeesh, M.C., G.K. Srikanth, P. Keshavanath, T.J. Varghese, N.Basavaraja and Das, S.K.1990. Effects of non-deflated silkworm-pupae in diets on the growth of common carp, *Cyprinus carpio.* Biological wastes. 33(1): 17-23

Nagaraj, G., and Basavanna, H.M. 1969. Proteins from silkworm pupae. Silkworm information Bull. 1(1): 29-30

Pant, R., and Unni, B. 1978. Some biochemical studies on *Philosamia ricini* during fifth instar development and spinning period. Ind J Exp Biol. 16: 705-706

Priyadarshini, P.A. 2013. Quantification of crude and extracted protein from deoiled male and female pupae of Eri silkworm, *Philosamia ricini*. (Unpublished)

Rangacharyulu, P.V., S.S. Geri, B.N. Paul, K.P.Yashoda, R . Jagannatha rao, N.S. Machendra, S.N. Mohanty and Mukhopadhyay, M.K. 2003. Utilization f the fermented silkworm pupae silage in feed for carps. Bioresource Technol. 86:29-32

Sinha, A. K., S.K. Chaudhury and Sengupta, K. 1985. Changes in protein content in the larval and pupal haemolymph of *Antheraea mylitta* D Sericult. 25: 39-43

Tojo, S., Nagata, M and KobayshI, M.1980. Storage proteins in the silkworm, *Bombyx mor*i L. Insect Biochem. 10(30): 289-303

Viswanath, P., and NAND, K. 1994. Anaerobic digestion of silk industry waste. Bioresource Technol. 49(3): 273-276.

Wyatt, G. R., and Kalf, G. F. 1957. The chemistry of insect haemolymph II Trehalose and other carbohydrates J Gen Physiol. 40: 833-847

Wyatt,G.R., 1961. The Biochemistry of insect haemolymph . Ann.Rev. Entomol. 6: 75-105