

Comparative Study of Metabolites in the Imago of Silkworm, *Philosamia Ricini*

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Abstract: Acetylcholine is present in higher concentration in insects when compared with most other invertebrates & vertebrates & functions as a chemical mediator of nervous activity. In the present study, biochemical analysis of acetylcholine alongwith protein, trehalose and glucose on male and female adult moths of *Philosamia ricini* have been made during their 5 day life span. The results demonstrated that head of female adults contains quite higher concentration of acetylcholine than the male head and shows sexual dimorphism. On the converse, the pattern of protein content of heads reveals a slight increase in males over that in females. This sexual dimorphic study on *P.ricini* adult moths suggests that the cholinergic system of females is more active than that of males.

Keywords: Acetylcholine, Cholinergic system, Eriworm, Imago, Lepidopteron, Nerve conduction.

1. INTRODUCTION

The imago stage of *Philosamia ricini* represents adult stage and completes its life cycle. It is the reproductive stage where adults mate and female laid eggs ¹. Moths have limited flight capability and as functional mouth parts are missing, they are unable to feed. In comparison to vertebrates, the nervous system of invertebrates is not so well developed. The cholinergic system, concerned with the enzymes controlling the metabolism of the nerve transmitter substance, provides a fundamental insight into the initiation and elaboration of neural function during the sequential stages of insect development. Evidence for the presence and the function of the cholinergic system in insects has been reviewed by O'Brien, Colhoun, Chadwick, Smallman and Treherne ². Previous investigators have shown that various neurosecretory hormones like acetylcholine, acetylcholine esterases, choline acetylase etc. play a vital role in the nerve conduction of various insects like *Apis*, *Periplaneta* etc. Strong evidence has been forwarded for one of the functions of acetylcholine as a chemical mediator of nervous activity. It is thought of as a neurotransmitter agent. Although the presence of acetylcholine has been detected in many insects ³, the highest activity has been demonstrated in the most active insects such as *Apis* and *Musca* ⁴.

The present study was taken into account to see the sex specific differences in case of nerve conduction in the lepidopteron, *Philosamia ricini*. In vertebrates, acetylcholine, which is neurotransmitter agent ⁵, plays a vital role in preganglionic transmission of the automatic nervous system, at a nerve ending of the parasympathetic system & in the brain ⁶.

2. MATERIALS AND METHOD

Virgin male and female moths were sorted immediately after emergence. The wings and the legs of the male and female imago were clipped off carefully. Then, head and trunk region was collected separately on different days i.e. 1st day, 2nd day, 3rd day & 4th day.

Next, a 20 % (w/v) homogenate of the above collected portions was prepared in 50 mM tris buffer (pH 7.0) ⁷. The homogenate was next centrifuged at 10,000 rpm at 4^o C for 30 minutes. Supernatant was collected for the quantitative estimation of metabolites.

Acetylcholine was estimated according to Metcalf ⁸, total protein was determined by the method of Lowry et al ⁹ while trehalose was determined by that of Wyatt & Kalf ¹⁰. Glucose was assayed according to Nelson & Somogy ¹¹.

All assays were made in duplicate sets on two individual homogenates and the mean values have been calculated.

3. RESULTS AND DISCUSSION

The above study on the imago of the *Philosamia ricini* on different days was undertaken to see if any sex specific differences occurs in the case of nerve conduction. The studies on insects shown significant differences in head and trunk of both the imagoes of *Philosamia ricini*.

While comparing the data of different metabolites in male head of *P.ricini*, the significant variation of protein contents reveals a slight increase in males over that in females. High concentration of acetylcholine has been observed in the head tissue of adult *Philosamia ricini* larvae.

As shown in (Table 2; Figure 2), the very low content of acetylcholine in heads (3.0 mg/ml) revealed by a newly emerged male adult rises on day 2 to reach a concentration of 13.25 mg/ml. The content further increases significantly (27.25 mg/ml) on day 3 but decreases conspicuously on the last day of its life span. The significant increase in acetylcholine concentration could be attributed to the general increase in structural and functional proteins associated with growth. Adult female heads of *P. ricini*, show an increase in the acetylcholine content on day 1 over that in males .It again increases significantly on day 2 (38.70%) and with a sharp decrease on day 3 (4.83%), it further increases on day 4. This reveals that the heads of female adults manifest higher concentration of acetylcholine than in the male heads. This suggests that the cholinergic system of females is more active than that of males.

Trehalose, which is major carbohydrate and plays the source of energy, increases in day 3 of males head over that in females (Table 3; Figure 3) while it decreases in day 1, day 2 and day 4 when compared to heads of female imago. However, in trunk region female contains higher level on all four days when compared with male (Table 6; Figure 6).

Analytics carried out on trunk of male moth of Eri worm showed significant increase in total protein on day 2, which decreases on day 3, and again shows a remarkable increase on day 4 (Table 5 ; Figure 5), while in female moth protein content decreases on day 2 (25.35%) when compared with day 1 in trunk region of *P.ricini* imago. Again a remarkable sharp increase was observed in day 3 but decreases on last day.

Acetylcholine plays an essential role in the transmission of nerve impulses and it is found almost negligible in the abdominal tissue of male and female adults of *Philosamia ricini*.

The data also describes percentage increase and decrease over day 1 of both the imagoes in all the parameters carried out.

Table 1: Variation in protein content of head portion of *P. ricini* adults.

Age of Imago (Days)	Total Protein (mg/ml)			
	Male Imago	% increase or decrease over day 1	Female Imago	% increase or decrease over day 1
1	30.75 ± 0.74	-	33.0 ± 0.5	-
2	64.0 ± 1.0	108.13 % ↑	23.0 ± 0.5	30.3 % ↓
3	40.75 ± 0.74	32.52 % ↑	47.75 ± 0.75	44.69 % ↑
4	54.75 ± 0.25	78.04 % ↑	54.0 ± 1.5	63.63 % ↑

Values expressed as Average ± S.E.M. (N = 2)

Table 2 : Variation in acetylcholine content of head portion of *P. ricini* adults.

Age of Imago (Days)	Acetylcholine (mg/ml)			
	Male Imago	% increase or decrease over day 1	Female Imago	% increase or decrease over day 1
1	3.0 ± 0.5	-	15.5 ± 0.5	-
2	13.25 ± 0.25	341.66 % ↑	21.5 ± 0.5	38.70 % ↑
3	27.25 ± 0.25	808.33 % ↑	14.75 ± 0.27	4.83 % ↓
4	11.375 ± 0.12	279.06 % ↑	17.75 ± 0.26	14.51 % ↑

Values expressed as Average ± S.E.M. (N = 2)

Table 3 : Variation in trehalose content of head portion of *P. ricini* adults.

Age of Imago (Days)	Trehalose (mg/ml)			
	Male Imago	% increase or decrease over day 1	Female Imago	% increase or decrease over day 1
1	0.65 ± 0.05	-	0.7 ± 0.14	-
2	0.85 ± 0.05	30.76 % ↑	0.875 ± 0.13	25.0 % ↑
3	1.25 ± 0.05	92.30 % ↑	0.95 ± 0.05	35.71 % ↑
4	1.05 ± 0.05	61.53 % ↑	1.23 ± 0.03	75.71 % ↑

Values expressed as Average ± S.E.M. (N = 2)

Table 4 : Variation in glucose content of head portion of *P. ricini* adults.

Age of Imago (Days)	Glucose (mg/ml)			
	Male Imago	% increase or decrease over day 1	Female Imago	% increase or decrease over day 1
1	0.85 ± 0.15	-	1.4 ± 0.1	-
2	1.45 ± 0.15	70.58 % ↑	2.0 ± 0.2	42.85 % ↑
3	1.90 ± 0.10	123.52 % ↑	2.65 ± 0.15	89.28 % ↑
4	1.70 ± 0.1	100.0 % ↑	2.45 ± 0.25	75.0 % ↑

Values expressed as Average ± S.E.M. (N = 2)

Table 5 : Variation in total protein of trunk portion of *P. ricini* adults.

Age of Imago (Days)	Total protein (mg/ml)			
	Male Imago	% increase or decrease over day 1	Female Imago	% increase or decrease over day 1
1	19.25 ± 0.75	-	26.125 ± 0.37	-
2	47.25 ± 0.25	145.45 % ↑	19.5 ± 0.5	25.35 % ↓
3	36.0	87.012 % ↑	39.0 ± 0.5	49.28 % ↑
4	42.75 ± 0.25	122.07 % ↑	33.625 ± 0.12	28.7 % ↑

Values expressed as Average ± S.E.M. (N = 2)

Table 6 : Variation in trehalose of trunk portion of *P. ricini* adults.

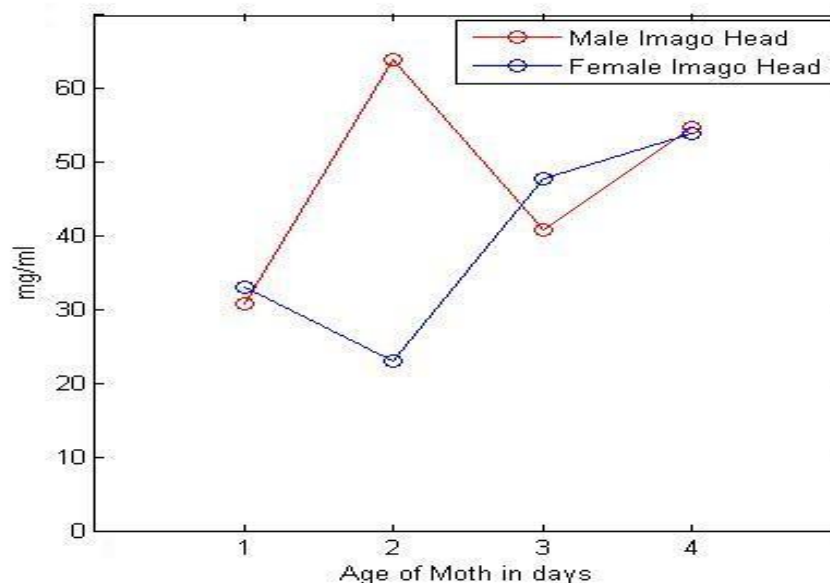
Age of Imago (Days)	Trehalose (mg/ml)			
	Male Imago	% increase or decrease over day 1	Female Imago	% increase or decrease over day 1
1	0.4 ± 0.141	-	1.1 ± 0.2	-
2	0.75 ± 0.05	87.5 % ↑	1.025 ± 0.025	6.818 % ↓
3	0.8 ± 0.14	100.0 % ↑	1.3 ± 0.14	18.18 % ↑
4	0.55 ± 0.05	37.5 % ↑	1.025 ± 0.074	6.818 % ↓

Values expressed as Average ± S.E.M. (N = 2)

Table 7 : Variation in glucose of trunk portion of *P. ricini* adults.

Age of Imago (Days)	Glucose (mg/ml)			
	Male Imago	% increase or decrease over day 1	Female Imago	% increase or decrease over day 1
1	1.35 ± 0.05	-	1.95 ± 0.25	-
2	1.9 ± 0.3	40.74 % ↑	2.2 ± 0.3	12.82 % ↑
3	2.6 ± 0.2	92.59 % ↑	2.85 ± 0.15	46.15 % ↑
4	2.2 ± 0.3	62.96 % ↑	2.6 ± 0.2	33.33 % ↑

Values expressed as Average ± S.E.M. (N = 2)

Figure [1] : Variation of Protein content of head part of adult imago of *P. ricini*

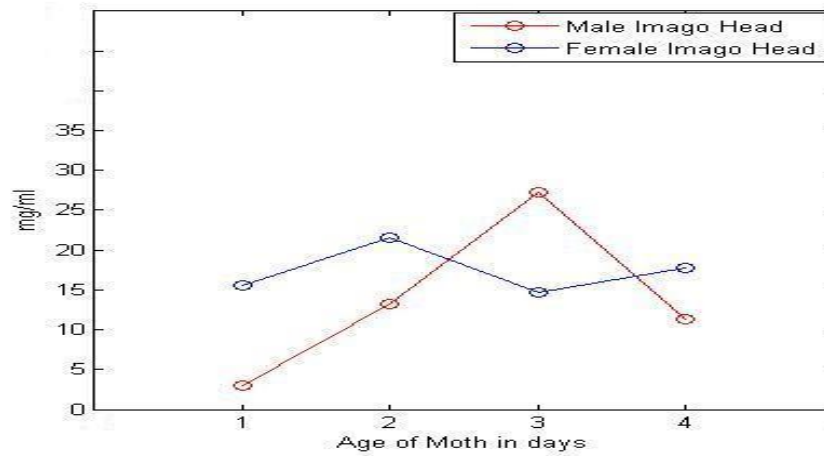


Figure [2] : Variati on in Acetylcholine in head part of adult imago of *P. ricini*.

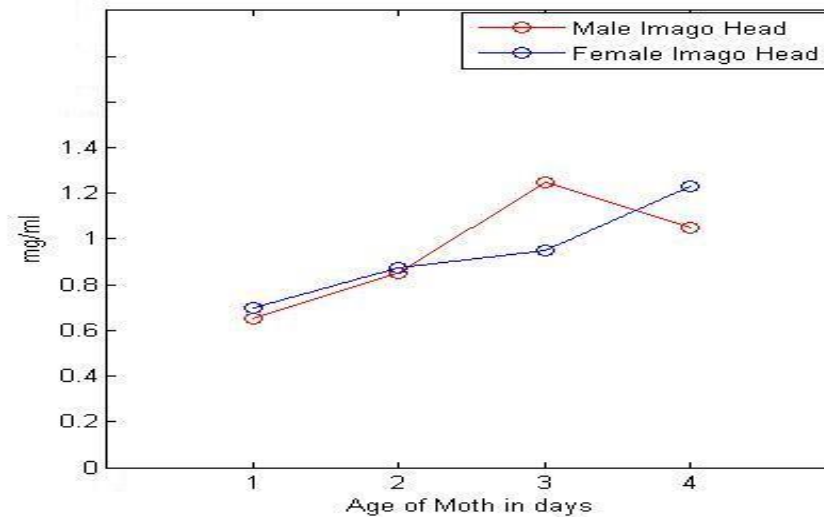


Figure [3] : Variati on in Trehalose content of head part of adult imago of *P. ricini*

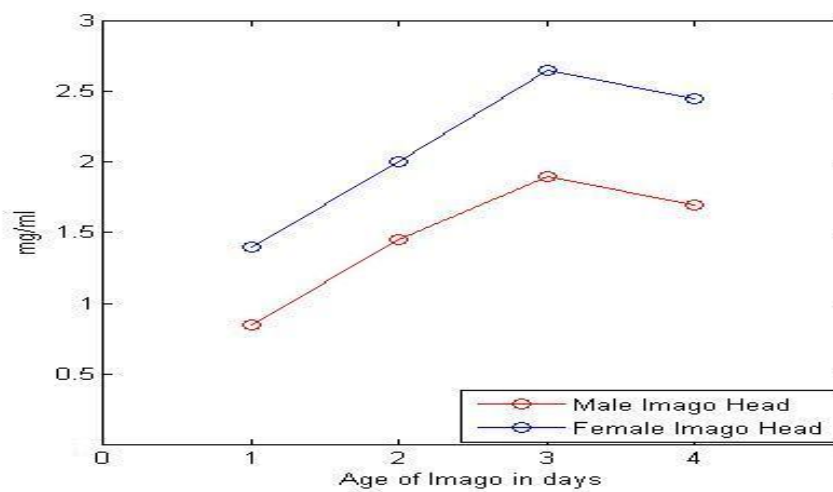


Figure (4) : Variati on in Glucose content of head part of adult imago of *P. ricini*.

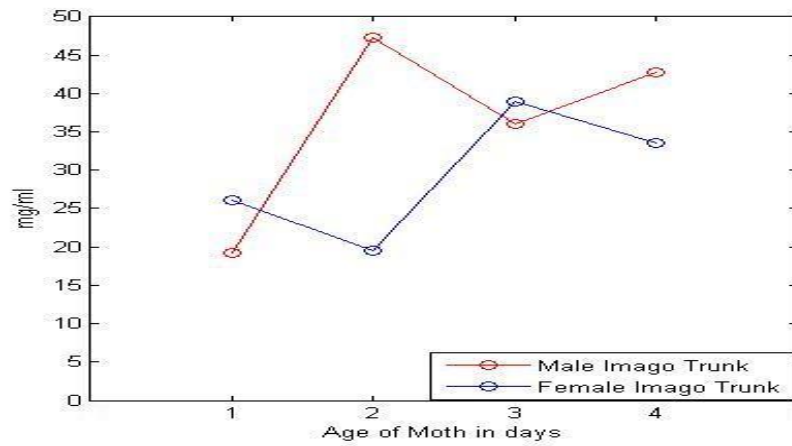


Figure [5] : Variation in Protein concentration in the trunk part of adult imago of *P. ricini*

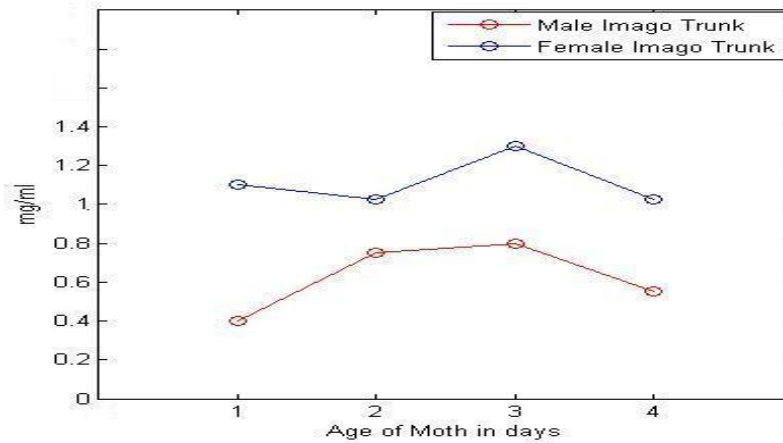


Figure [6] : Variation in Trehalose content of trunk region of adult imago of *P. ricini*.

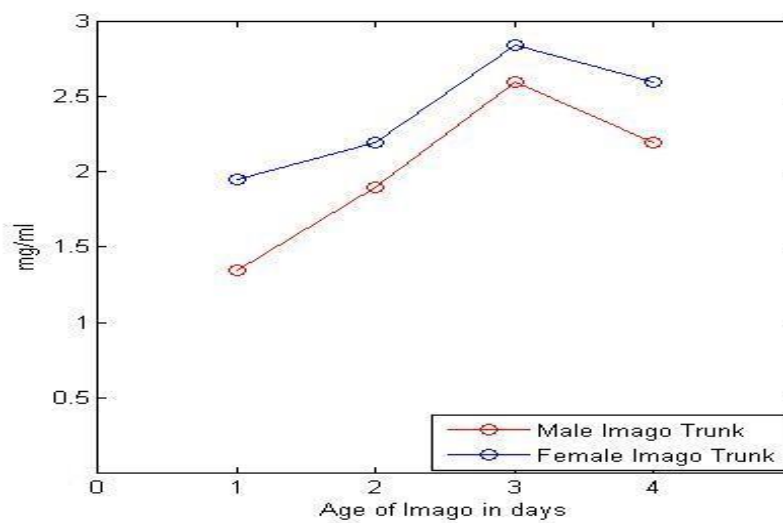


Figure (7): Variation in Glucose content of trunk region of adult imago of *P. ricini*.

4. CONCLUSION

The results revealed that head of female adults contain very higher concentration of acetylcholine than the male head. Variation pattern of protein content of heads reveals a slight increase in males over that in females.

It is concluded that the presence of the cholinergic system and the proteins of head tissue is sex dependant in addition to involving central nervous system. The presence of acetylcholine and protein in the abdominal tissue of *Philosamia ricini* adults was also found to be sex dependent but not involving central nervous system. The cholinergic system of females seems to be relatively more active than that of males. So, it is concluded that the presence of acetylcholine & protein in imago of *Philosamia ricini* is sex dependent.

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