



## Estimation of Lipid and Water Contents in *Bombyx mori* Linn. (NB<sub>4</sub>D<sub>2</sub>) During Metamorphosis Process

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### ABSTRACT

Lipid and water quantity changes in insects during metamorphosis. In silkworm (*Bombyx mori* Linn.) lipid and water percentage are showed negatively correlated during metamorphosis. It was observed that in silkworm throughout the larval to adult stage lipid percentage increases and water percentage decreases and male pupa and moth contain more lipid percentage than the female pupa and moth.

**Key words:** Silkworm, *Bombyx*, metamorphosis.

### INTRODUCTION

Many workers have been observed that lipid and water content changes in insects during metamorphosis *i.e.* in blowfly *Phormia* sp. [1], *P. sericata* [2] that in pupal stage contains higher fat percentage than that of pre-pupal stage that decreased during metamorphosis in *Phormia regina* [3]. In diptera, *Sarcophagidae*, it has been observed that the fat and water content varies from the 3rd instar to pupation stage. In adult stage it has been observed that water and dry weight was not varies much while the fat content decreases sharply on the 12th day [4]. Sex dimorphism has been observed in the fat content in *Glossinapalpolis*, R. and in *Hyalophora cecropia* L. In these species the female contains more fat than the male [2].

But this findings has not been true for Lepidopteron, where *Bombyx mori* Linn. females have been showed lower amount of fat than male [5]. The present study was an investigation of lipid and water content of bivoltine (NB<sub>4</sub>D<sub>2</sub>) breed at different growth stages from 5<sup>th</sup> instar to the moth stage.

### MATERIALS AND METHODS

In present study the silkworm breed NB<sub>4</sub>D<sub>2</sub> was taken and analyzed three different stage of metamorphosis. In first stage study was taken to the larvae. The first stage represented larvae of the 5th instar from the 1<sup>st</sup> day to the 7<sup>th</sup> day, the 2<sup>nd</sup> stage represented pupa with sexual dimorphism of early, middle and late stage and 3rd stage represented moths with dimorphism before and after mating for 3 hours. Worms were starved for 24 hours in order to clear out the gut.

Water and lipid contents were estimated by Abasa's [4] and Folch's [6] methods with slight modifications. For estimation of moisture content, 10 living silkworms were taken in a weighed aluminum box and the silkworms were doing unconscious by chloroform vapors and fresh weight was taken. The box was kept in the oven at 110°C for 12 hours and then kept overnight in a desiccator. Next day it was weighed and dry weight was calculated. This process was repeated till the constant weight was not obtained. This difference between the fresh weight and dry weight gave the water content from that water percentage was calculated.

For lipid estimation the above dry worms were crushed, shaken well with 20 ml of chloroform-methanol mixture (2:1,V/V) in a stoppered conical flask and kept overnight in a dark place. Next day this mixture solution was filtered using fat free filter paper and the residue was washed with 15 ml of chloroform-methanol mixture (1:1, V/V) and lastly with 10 ml of petroleum ether (boiling point, 60°C-80°C). Total filtrate was collected in the same aluminum box previously weighed and kept in an oven at 60°C for 3 hours. When this filtrate was completely evaporated, it was taken from the oven and kept overnight in a desiccator. Next day it was weighed and dry weight was taken. This difference between the dry weight and initial weight of box gave the lipid content from which the lipid percentage was estimated.

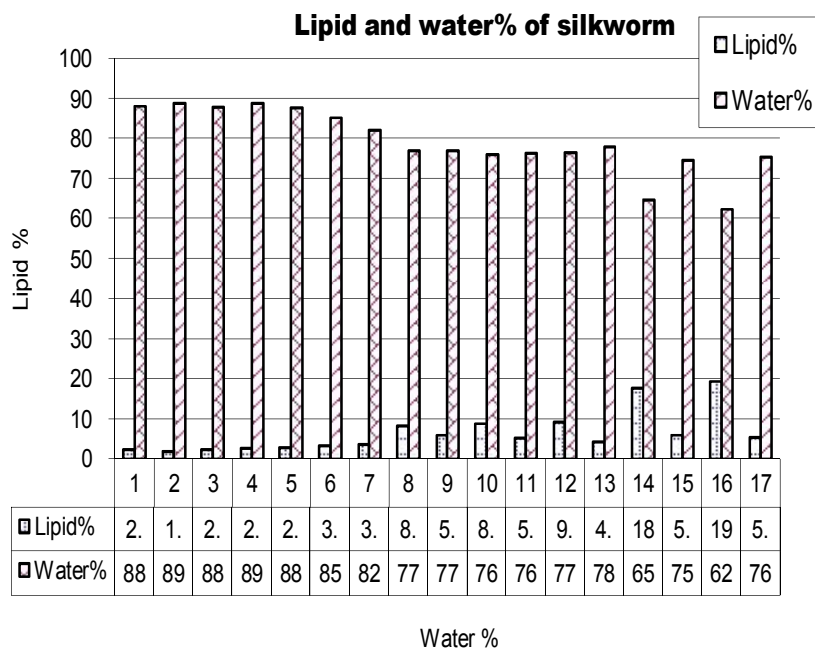
**Table 1: Lipid and water % of Silkworm**

S.N.	Stages		Lipid%	Water%
1	5 <sup>th</sup> instar /1 <sup>st</sup> day		2.28	88.04
2	5 <sup>th</sup> instar /2 <sup>nd</sup> day		1.78	88.88
3	5 <sup>th</sup> instar /3 <sup>rd</sup> day		2.28	87.92
4	5 <sup>th</sup> instar /4 <sup>th</sup> day		2.56	88.80
5	5 <sup>th</sup> instar /5 <sup>th</sup> day		2.75	87.67
6	5 <sup>th</sup> instar /6 <sup>th</sup> day		3.12	85.15
7	5 <sup>th</sup> instar /7 <sup>th</sup> day		3.55	82.06
8	Pupa early stage	Male	8.27	76.89
9		Female	5.87	76.95
10	Pupa middle stage	Male	8.71	75.94
11		Female	5.16	76.46
12	Pupa last stage	Male	9.18	76.56
13		Female	4.23	77.90
14	Adult stage	Male	17.58	64.60
15	(before mating)	Female	5.92	74.58
16	Adult stage	Male	19.25	62.28
17	(After mating)	Female	5.26	75.51
<b>S.E.</b>			<b>0.412</b>	<b>1.325</b>
<b>C.D. at 5%</b>			<b>1.302</b>	<b>4.012</b>

**RESULTS AND DISCUSSION**

The estimation of lipid and water percentages of silkworm given in Table- 1. The estimations were done in duplicate and percentages of water and lipid contents and the patterns of changes of lipid and water contents during metamorphosis are showed in Graph-1.

**Graph-1**



It may be seen that there is not much significant difference in lipid and water content in the larvae of the 5th instar from the 1st day to the 5th day. But on the 6th & 7th of the larvae there has been a significant increase in lipid content with decrease of water. This lipid is needed for the matured larvae

as energy for spinning operation and transformation into pupal stage.

The larva-pupal transformation there has been a significant change in lipid content. In male pupa the lipid content has been observed more than the female. In adult male pupa the lipid content has increased with the stage of growth but it has been just opposite in the case of adult female pupa is perhaps due to egg development in female providing the loss of a greater portion of lipid as energy source and depletion of lipid was reported during embryogenesis in the oviparous cockroach (*Leucophaea maderae*) female. In case of water content there is not much difference in dimorphism. The same trend has been observed in lipid content in the pupa-adult transformation. But in the case of water content before and after mating it has decreased in the male moth and increased in the female moth to some extent. The means values of lipid percentage were showed against water percentage in graph-1 from this we conclude that the percentage of lipid has increased and the percentage of water has decreased significantly in sexual dimorphism.

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