



CHANGES IN FAT BODY LIPID LEVEL DURING PUPAL AND ADULT STAGES OF DISTINCT BIVOLTINE AND MULTIVOLTINE BREEDS OF THE SILKWORM, *BOMBYX MORI* L.

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ABSTRACT

The present investigation was carried out to understand the changes in the lipid profile during pupal and adult stages of four bivoltine and four multivoltine races/breeds belonging to different geographical/aboriginal region were drawn from the germplasm banks of Department of Sericulture, Mysuru and APSSRDI, Hindupur. The standard method of silkworm rearing was conducted under standard laboratory condition, the fat body samples of pupae and silk moth of all the races were extracted and estimated by standard procedure and recorded day to day quantitative changes in the lipid content and data subjected to the statistical analysis. Based on the statistical results, it is clear that, the variation in the lipid content was noticed in both the pupal and adult stages. Moreover, in case of pupal stage an increase of lipid level from pupation period (3rd day to 5th day) but on 6th day onwards the trend was decreased in the male and female pupae. Whereas in the adult, there was drastic decline in the fat body lipid level in females than male individual observed and lipid content were also in virgin and mated females and unmated and mated males are also herein discussed.

KEY WORDS: *Bombyx mori*, pupa, adult, lipid content.

INTRODUCTION

India is being a second leader in silk production next to the china is occupied a large producer and converter (produced silk converts into fabric) and the quality of the silk obtained only when the silkworm consumes good quality of mulberry leaf protein converts into silk protein by the way of its good growth and development and finally in-turn action takes place by wonder of biochemical changes, which varies from breeds to breeds, sex and larvae to pupae followed by adults stage of the silkworm. The total changes in the biochemical properties of fat body reflects on tissue specific in lipid content of the silkworm, there is a generalised fact that, the fat body tissue stored an energy in the form of glycogen and triglycerides in the adipocytes. The fat body adipocytes can reserve a high quality of lipid stores as cytoplasmic lipid droplets. Apart, lipid metabolism is essential for growth, reproduction and provides energy consistently and extended to the development of the non feeding stage. Moreover, proteins are essential biomolecules utilized in several types of cellular functioning of the life. On the other hand carbohydrates are again prime source of cellular energy and helps in physiological monitoring activities of the life. The lipid, generally called as fat, contains a heterogeneous group and other type of edible fat, phospholipids, steroids, vitamins, pigments, traces of metals and few kinds of toxic material and lipids in the diet survey as a energy power to the living cells of the organisms. A report is available with

reference to the silkworm pupae which contain different kinds of lipids like, triacylglycerol (TG), phosphatidylethanolamine and phosphatidylcholine (Naseema Begam *et al.*, 2014). Moreover, the biochemical parameters like, proteins, carbohydrates, nucleic acid and lipids are fluctuating during developmental stages of all living organisms. The quantitative variation in this biochemical constituents in fat body of insects depends on the quality with good nutritional value of the food and their consumption during developmental process (Nagata and Yashitake, 1989). Further, several researcher investigations were carried out with relevant to biochemical changes of the silkworm, but few were very similar as per literature survey available are (Naguchi *et al.* (1974), Bose *et al.* (1989) and Hiroshi *et al.*, 1996) clearly distinguished. Moreover, the importance of lipid and its physiological, biochemical, structural functions were the main interest prompted investigator to carry out the research on quantitative and qualitative aspects of lipid contents during post embryonic developments (D'e Costa and Birt, 1966, Gilbert, 1967, Kim & Kyung, 1975, Dunphy *et al.*, 1977 and Pant *et al.*, 1978). Besides, the variation in biochemical composition of lipid content in pupal and adults are fluctuating from day by day. Hence, the present experiment was conducted to understand the changes in the lipid content in pupal and adult stages of the silkworm, *Bombyx mori*.

MATERIALS & METHODS

Four popular bivoltine breeds namely, CSR₂, NB₄D₂, APS₄5 and APS₁₂ and four multivoltine races namely, PM, C. nichii, APM₁ and APM₃ were drawn and collected from the germplasm banks of Department of Sericulture, Mysuru, and APSSRDI, Hindupur, Andhra Pradesh and the layings were prepared and reared by adopting the methods described by Tazima, (1978) and Krishna swami, (1978), and the good cocoons were selected for further assessment. The male and female sexes were separated during 5th instar just after resuming from 4th moult before feeding to get virgin female and unmated male on 3rd day after spinning during pupal stage. The fat body samples were extracted from male and female pupae and adult stages of 3rd day to 12th day and 1st day to 10th day respectively. The extracted samples were kept at -20°C for preservation and utilization for the analysis of lipid profiles. The total lipid content in the fat body of selected races/breeds was estimated by the methods of Bragdon, (1951) and Pande, *et al.*, (1963). Further, 15 mg of chilled samples were homogenized in 1 ml mixture of chloroform and methanol (2:1 V:V) in prechilled glass homogenizer. Then, the homogenate was filtered and was subjected to rapid evaporation. Further, for the residue, 3 ml of 2% potassium dichromate in 98% sulphuric acid (w/v) was added and boiled in water bath exactly for 15 minutes. The tubes were cooled in ice water bath and 4.5 ml of distilled water was added and the mixture was cooled again. Finally, the colour intensity was read at 590 nm with a suitable blank and lipid levels are expressed in mg of lipid per gram of wet tissue. The data was recorded and subjected to the statistical analysis with OPSTAT statistical software package of CSS Haryana, Agricultural University, Hisar.

RESULTS AND DISCUSSION

The insect development and progression is depends on the cellular and sub cellular events attributed to the conversion, transformation and mobilization energy tend to utilize at the target cells. The silkworm is not exception in which the four stages of development attained a series of changes in the modifications and the transformations of one form to another as a result the adult formation is derived for the large proposition of the accumulation of fat body content. The fat body is congregation of a mass of the cells in which the energy is derived from the lipid comprising triglycerides, steroids, saturated and unsaturated fatty acids. Each and every stages of development is programme for which a specific processes of the synthesis of different energy molecules in order to exhibit the embryogenesis, ecdysis, spinning, egg laying, *etc.* The difference in the expression of energy molecules depends on the available nutritive elements. In insects, the same events attributed for the impinging processes of metabolism, anabolism for the concurrent physiological events of the organism. The statistically analyzed data with pertaining to lipid content of the male pupae of four bivoltine and four multivoltine races/breeds was shown in Table-1 and same is shown in figure-1 and data exhibits highest level of the of 1.443 ±0.008mg/g during 5th day in CSR₂ breed, where as a

least lipid content of 1.161±0.019mg/g was recorded during same day in C.nichi. The observation of data were recorded from 3th day to 12th day and few races namely C.nichi, APM₁ and APM₃ exhibit still 9th day 11th day and 11th day respectively. The minimum lipid of 0.854 ±0.003mg/g was recorded in case of PM till the end of pupal developmental (12th day) stage. The statistical recorded data revealed all the selected races/breed shown significance CD @5% and critical variance.

Further, data pertaining to lipid content of the female pupae of bivoltine and multivoltine races/breeds of the silkworm, *Bombyx mori* (Table-2 & figure-2) was clearly indicated the variation in lipid content from 3rd day to 12th day of pupal stage. Consequently from 3th day to 5th day revealed an increase in lipid content later on 6th day onwards decrease of lipid content in pupal period. The observed data manifested a highest of 1.478 ±0.013mg/g was during 5th day in CSR₂ breed, whereas, least of 1.162 ±0.007mg/g of the lipid might be found to be noticed and recorded in C.nichi race on the same day, but the least of 0.341 ±0.002mg/g was PM during 12th day of pupal stage and statistical analysis and their significance CD at 5% and critical variance. The data with regards to Table-3 and figure-3 shown, lipid content of the unmated male adult of four bivoltine and four multivoltine races/breeds observed that, an highest lipid content (0.987 ±0.007 mg/g) was recorded in CSR₂ adult, whereas, lowest (0.724 ±0.017mg/g) was noticed in C.nichi races during 1st day of adult and remaining all the races observed intermediary lipid content during same days. On 8th and 9th day of PM (0.208 ±0.014) and C.nichi (0.150 ±0.020) races recorded lowest lipid content in adult and statistically significance observed in all the races with C.D at 5% and CV. However, in the silkworm the different voltinistic group of breeds showed the diversification, variations in day to day changes of the lipid level during pupal and adult development. The total lipid content from the day one of the spinning till the completion of emergence of silkworm a distinct changes and significant variations in the lipid content of the fat body tissue. The quantity lipid is increased in the early pupal period but the level of synthesis a slower in pace reported by Pant *et al.* (1974). This may owing to utilization as a respiratory substrate as a result an agreement of the continuous utilization of lipid in relation to ageing of both the sexes (Nowosielski and Patton, 1965). The larval growth period the quantum of lipid content decreased by 75% up to initial stage of 3rd instars therefore, the subsequent larval instars is P<0.01%, Gilbert and Schneiderman, (1961) mentioned that, the increased concentration of lipid before moulting appear to be related with increased rate of oxygen consumption during the moulting period. The Table - 4 and in figure-4 showed lipid content of the mated male of bivoltine and multivoltine adult revealed that, minimum lipid was observed in C.nichi race (0.673 ±0.012) and maximum (0.926±0.010) was in CSR₂ breed during 1st day of adult and as such lowest lipid depicted in C.nichi and PM during last day of adult and statistically showed an significant variation among all the races.

TABLE 1: Changes in fat body lipid content of male pupae (mg/g wet weight) of selected silkworm races / breeds

Races/Breeds	CSR ₂	NB.D ₂	APs45	APs12	PM	Cnichi	APM1	APM3
Days								
3	1.370±0.001	1.342±0.001	1.365±0.002	1.332±0.002	1.175±0.002	1.086±0.003	1.283±0.003	1.277±0.006
4	1.396±0.004	1.383±0.002	1.392±0.004	1.380±0.002	1.239±0.002	1.121±0.009	1.306±0.009	1.286±0.008
5	1.443±0.008	1.418±0.006	1.425±0.009	1.411±0.005	1.276±0.015	1.161±0.019	1.354±0.009	1.322±0.006
6	1.264±0.001	1.230±0.010	1.247±0.003	1.211±0.004	1.087±0.003	0.956±0.003	1.141±0.003	1.125±0.002
7	1.215±0.001	1.191±0.002	1.200±0.002	1.184±0.002	1.032±0.002	0.922±0.003	1.111±0.004	1.105±0.004
8	1.152±0.001	1.132±0.001	1.143±0.003	1.121±0.003	1.005±0.003	0.890±0.003	1.068±0.003	1.046±0.003
9	1.117±0.001	1.100±0.001	1.104±0.002	1.092±0.003	0.987±0.004	0.835±0.004	1.001±0.003	0.988±0.003
10	1.072±0.002	1.049±0.002	1.062±0.003	1.045±0.003	0.926±0.003	-	0.974±0.003	0.963±0.004
11	1.013±0.002	1.011±0.002	1.015±0.003	1.006±0.003	0.900±0.004	-	0.936±0.005	0.922±0.005
12	1.000±0.001	0.981±0.002	0.986±0.003	0.975±0.003	0.854±0.003	-	-	-
F-test	*	*	*	*	*	*	*	*
C.D@ 5%	0.009	0.012	0.012	0.010	0.016	0.021	0.015	0.014
SE(m)±	0.003	0.004	0.004	0.003	0.005	0.007	0.005	0.005
SE(D)±	0.004	0.005	0.006	0.005	0.008	0.010	0.007	0.006
C.V. (%)	0.444	0.567	0.579	0.482	0.891	1.775	0.861	0.792

TABLE 2: Changes in fat body lipid content of female pupae (mg/g wet weight) of selected silkworm races / breeds

Races	CSR ₂	NB.D ₂	APs45	APs12	PM	Cnichi	APM1	APM3
Days								
3	1.397±0.003	1.378±0.004	1.391±0.003	1.386±0.003	1.214±0.006	1.058±0.004	1.234±0.003	1.223±0.003
4	1.423±0.012	1.405±0.013	1.408±0.013	1.409±0.008	1.288±0.011	1.117±0.003	1.299±0.008	1.285±0.008
5	1.458±0.013	1.458±0.015	1.465±0.0015	1.464±0.014	1.312±0.007	1.162±0.007	1.348±0.013	1.327±0.009
6	1.305±0.003	1.211±0.003	1.225±0.002	1.205±0.003	1.153±0.004	0.987±0.003	1.172±0.004	1.160±0.004
7	1.260±0.003	1.178±0.003	1.210±0.004	1.171±0.003	1.131±0.003	0.673±0.003	1.147±0.003	1.14±0.004
8	1.212±0.004	1.147±0.002	1.187±0.003	1.142±0.004	1.110±0.005	0.471±0.003	1.122±0.004	1.118±0.004
9	1.185±0.003	1.125±0.003	1.157±0.003	1.121±0.004	1.096±0.003	0.391±0.004	1.103±0.003	1.104±0.008
10	1.149±0.004	1.102±0.003	1.116±0.003	1.094±0.006	1.071±0.003	-	1.085±0.004	1.078±0.003
11	1.114±0.002	1.095±0.003	1.101±0.004	1.081±0.004	1.042±0.004	-	1.05±0.003	1.048±0.004
12	1.074±0.003	1.072±0.003	1.086±0.003	1.060±0.002	0.341±0.002	-	-	-
F-test	*	*	*	*	*	*	*	*
C.D@ 5%	0.019	0.021	0.020	0.018	0.320	0.010	0.017	0.016
SE(m)±	0.006	0.007	0.007	0.006	0.108	0.003	0.006	0.005
SE(D)±	0.009	0.010	0.010	0.009	0.153	0.005	0.008	0.007
C.V. (%)	0.860	1.003	0.948	0.879	17.367	1.015	0.917	0.872

TABLE 3: Changes in fat body lipid content of unmated male moth (mg/g wet weight) of selected silkworm races/breeds

Races/breeds	CSR ₂	NBD ₂	APS45	APS12	PM	Catchi	APMI	APM3
Days								
1	0.987±0.007	0.973±0.012	0.981±0.011	0.968±0.011	0.783±0.011	0.724±0.017	0.882±0.010	0.872±0.012
2	0.822±0.012	0.810±0.016	0.818±0.012	0.730±0.045	0.688±0.011	0.625±0.015	0.709±0.013	0.704±0.019
3	0.726±0.010	0.641±0.012	0.688±0.019	0.637±0.015	0.504±0.011	0.455±0.019	0.528±0.017	0.524±0.015
4	0.619±0.007	0.593±0.012	0.605±0.010	0.518±0.014	0.429±0.019	0.346±0.019	0.443±0.018	0.427±0.018
5	0.554±0.013	0.520±0.012	0.532±0.013	0.439±0.020	0.348±0.017	0.290±0.010	0.362±0.018	0.362±0.018
6	0.486±0.008	0.464±0.012	0.469±0.014	0.421±0.011	0.280±0.012	0.227±0.016	0.284±0.020	0.280±0.013
7	0.389±0.013	0.375±0.014	0.380±0.011	0.365±0.015	0.227±0.017	0.207±0.012	0.246±0.021	0.231±0.018
8	0.306±0.044	0.265±0.014	0.265±0.013	0.254±0.017	0.227±0.014	0.191±0.011	0.226±0.019	0.210±0.013
9	0.226±0.013	0.218±0.014	0.222±0.011	0.207±0.014	-	0.150±0.020	0.184±0.013	0.170±0.013
10	0.206±0.010	0.197±0.010	0.196±0.009	0.187±0.008	-	-	-	-
F-test	*	*	*	*	*	*	*	*
C.D@ 5%	0.051	0.038	0.038	0.058	0.038	0.045	0.048	0.044
SE(m)±	0.017	0.013	0.013	0.020	0.013	0.015	0.016	0.015
SE(d)±	0.024	0.018	0.018	0.028	0.018	0.021	0.023	0.021
C.V. (%)	5.593	4.379	4.277	7.205	6.398	8.092	7.172	6.856

TABLE 4: Changes in fat body lipid content of mated male moth (mg/g wet weight) of selected silkworm races / breeds

Races/breeds	CSR ₂	NBD ₂	APS45	APS12	PM	Catchi	APMI	APM3
Days								
1	0.926±0.010	0.910±0.014	0.914±0.017	0.882±0.012	0.746±0.018	0.673±0.012	0.839±0.024	0.826±0.013
2	0.804±0.010	0.779±0.013	0.773±0.012	0.755±0.014	0.619±0.012	0.605±0.020	0.682±0.021	0.677±0.017
3	0.712±0.009	0.638±0.015	0.686±0.014	0.604±0.013	0.487±0.021	0.440±0.018	0.538±0.019	0.525±0.017
4	0.591±0.012	0.573±0.019	0.581±0.013	0.488±0.011	0.408±0.014	0.363±0.018	0.429±0.013	0.409±0.019
5	0.516±0.017	0.493±0.015	0.502±0.012	0.434±0.015	0.329±0.018	0.274±0.014	0.361±0.019	0.331±0.020
6	0.365±0.014	0.340±0.016	0.350±0.016	0.343±0.019	0.260±0.018	0.224±0.015	0.283±0.020	0.278±0.016
7	0.314±0.017	0.303±0.011	0.308±0.013	0.304±0.017	0.218±0.014	0.206±0.019	0.241±0.017	0.238±0.018
8	0.226±0.015	0.220±0.014	0.220±0.012	0.220±0.013	0.172±0.016	0.167±0.017	0.226±0.017	0.213±0.021
9	-	-	-	-	-	-	-	0.177±0.014
F-test	*	*	*	**	*	*	*	*
C.D@ 5%	0.035	0.039	0.036	0.038	0.044	0.045	0.050	0.047
SE(m)±	0.012	0.013	0.012	0.013	0.015	0.015	0.017	0.016
SE(d)±	0.017	0.018	0.017	0.018	0.021	0.021	0.024	0.022
C.V. (%)	4.544	5.292	4.906	5.567	7.939	8.857	8.116	7.846

TABLE 5: Changes in fat body lipid content of virgin female moth (mg / g wet weight) of selected silkworm races/breeds

Races/breeds	CSR ₂	NB: D ₂	APS45	APSI2	PM	C.nichi	APM1	APM3
Days								
1	0.433±0.016	0.431±0.019	0.419±0.011	0.419±0.014	0.379±0.014	0.350±0.021	0.396±0.014	0.391±0.012
2	0.428±0.018	0.422±0.014	0.410±0.012	0.410±0.014	0.360±0.016	0.336±0.021	0.377±0.014	0.372±0.011
3	0.423±0.021	0.407±0.020	0.403±0.022	0.407±0.023	0.338±0.021	0.318±0.013	0.347±0.014	0.340±0.015
4	0.379±0.014	0.369±0.019	0.369±0.015	0.366±0.018	0.322±0.016	0.298±0.015	0.325±0.016	0.320±0.012
5	0.357±0.023	0.341±0.020	0.334±0.015	0.330±0.018	0.306±0.019	0.265±0.017	0.310±0.014	0.302±0.012
6	0.323±0.017	0.313±0.018	0.304±0.021	0.275±0.018	0.261±0.017	0.245±0.021	0.277±0.014	0.273±0.011
7	0.268±0.020	0.259±0.019	0.262±0.018	0.257±0.016	0.232±0.017	0.227±0.018	0.249±0.020	0.238±0.016
8	0.246±0.017	0.238±0.017	0.227±0.015	0.230±0.020	0.206±0.018	0.195±0.013	0.226±0.017	0.216±0.009
9	0.212±0.021	0.206±0.019	0.196±0.012	0.188±0.020	0.178±0.013	0.175±0.014	0.189±0.020	0.182±0.011
10	0.163±0.023	0.162±0.022	0.156±0.014	0.153±0.010	0.143±0.018	0.117±0.009	0.152±0.019	0.143±0.015
F-test	*	*	*	*	*	*	*	*
C:D@ 5%	0.057	0.056	0.047	0.052	0.051	0.049	0.049	0.037
SE(m)±	0.019	0.019	0.016	0.017	0.017	0.017	0.016	0.012
SE(d)±	0.027	0.027	0.022	0.025	0.024	0.023	0.023	0.018
C.V. (%)	10.209	10.323	8.942	9.956	10.930	11.339	9.932	7.738

TABLE 6: Changes in fat body lipid content of mated female adult (mg/g wet weight) of selected silkworm races / breeds

Races/breeds	CSR ₂	NB: D ₂	APS45	APSI2	PM	C.nichi	APM1	APM3
Days								
1	0.428±0.018	0.417±0.019	0.410±0.018	0.390±0.015	0.365±0.015	0.345±0.016	0.382±0.011	0.372±0.016
2	0.413±0.022	0.411±0.022	0.402±0.024	0.376±0.014	0.342±0.017	0.325±0.015	0.362±0.010	0.355±0.016
3	0.391±0.021	0.372±0.017	0.371±0.017	0.360±0.013	0.324±0.016	0.307±0.010	0.325±0.015	0.325±0.016
4	0.365±0.015	0.337±0.019	0.333±0.014	0.329±0.018	0.291±0.015	0.282±0.011	0.308±0.014	0.297±0.016
5	0.333±0.022	0.320±0.012	0.320±0.016	0.309±0.012	0.258±0.020	0.253±0.016	0.325±0.021	0.273±0.016
6	0.315±0.020	0.302±0.016	0.290±0.014	0.278±0.013	0.226±0.016	0.216±0.009	0.249±0.015	0.235±0.017
7	0.254±0.019	0.250±0.017	0.248±0.022	0.241±0.018	0.208±0.016	0.193±0.012	0.224±0.015	0.209±0.014
8	0.227±0.020	0.220±0.015	0.211±0.016	0.207±0.016	0.182±0.011	0.180±0.011	0.197±0.014	0.188±0.008
9	0.197±0.031	0.175±0.018	0.178±0.014	0.172±0.016	0.166±0.015	0.156±0.013	0.172±0.016	0.169±0.014
10	-	-	-	-	-	-	0.126±0.009	0.149±0.043
F-test	*	*	*	*	*	*	*	*
C:D@ 5%	0.060	0.049	0.050	0.043	0.044	0.036	0.042	0.043
SE(m)±	0.020	0.017	0.017	0.014	0.015	0.012	0.014	0.015
SE(d)±	0.029	0.023	0.024	0.020	0.021	0.017	0.020	0.021
C.V. (%)	11.967	10.244	10.464	9.379	10.902	9.394	9.245	9.969

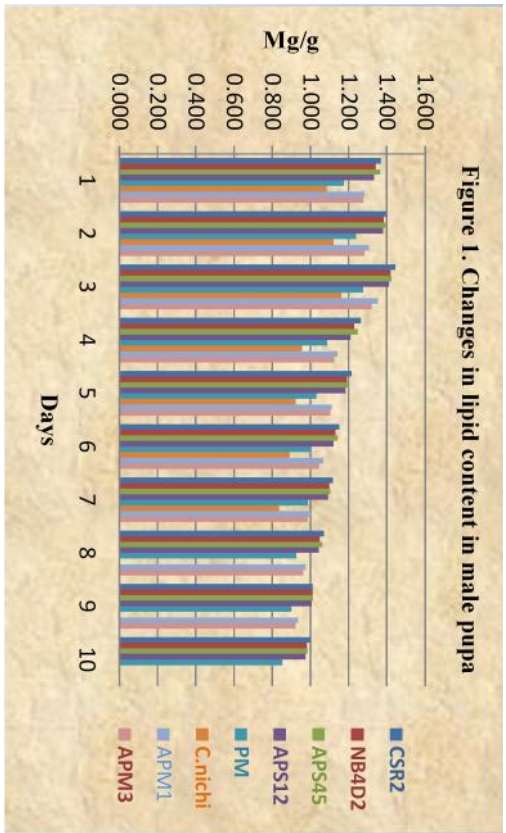


FIGURE 1 & 2. Changes in fat body lipid content (mg/g wet weight) of male pupae of selected silkworm races/breeds

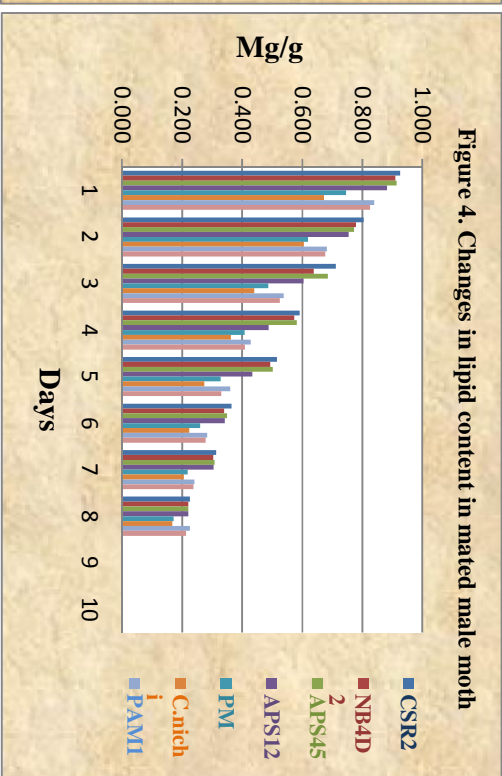
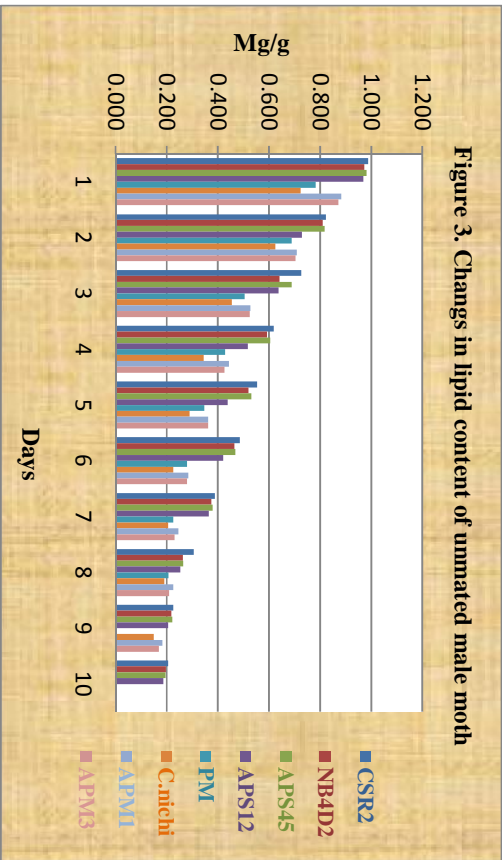
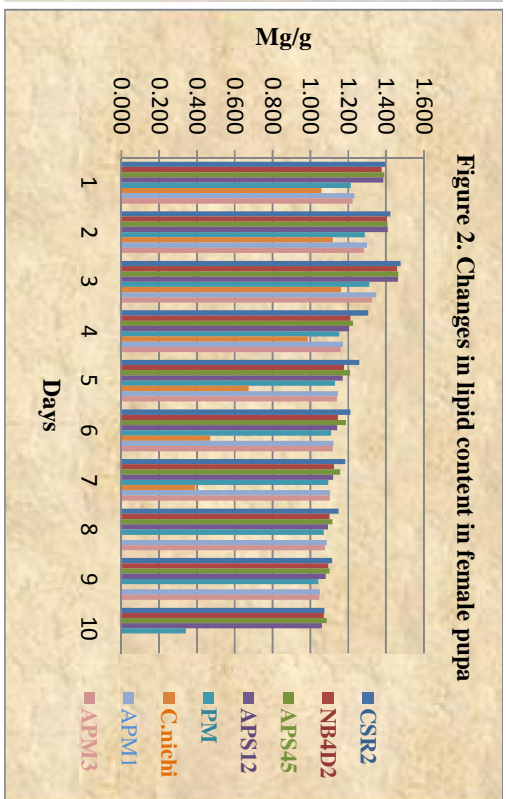


FIGURE 3 & 4: Changes in adult fat body lipid content (mg/g wet weight) of unmated and mated male of selected silkworm races/breeds

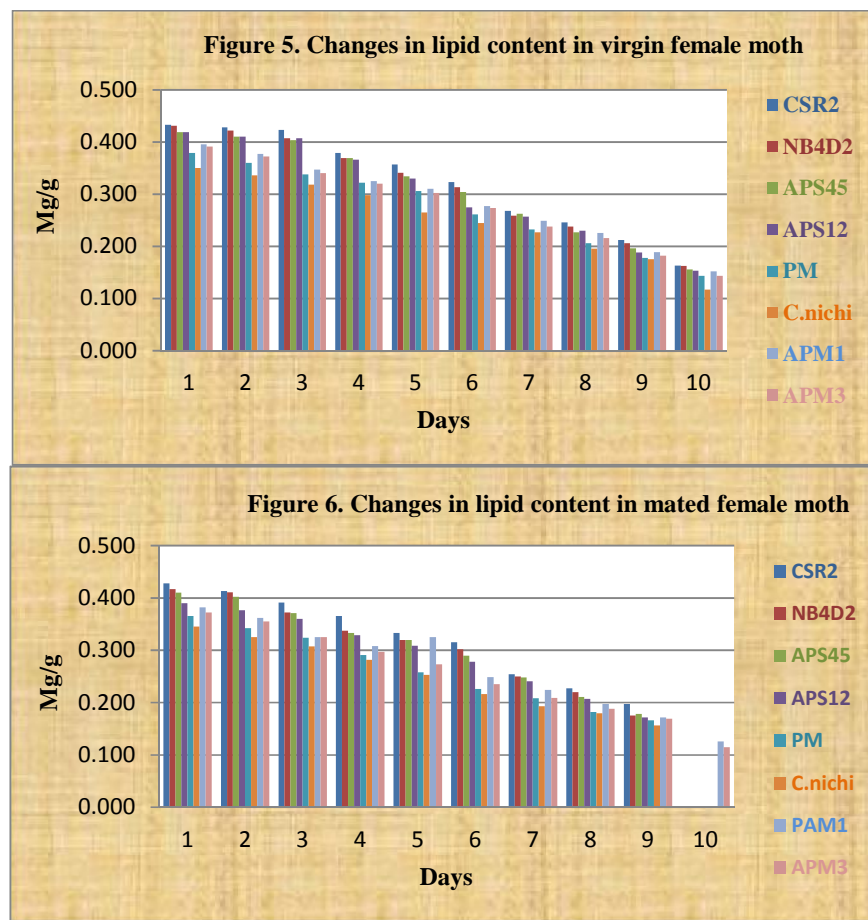


FIGURE 5&6: Changes in adult fat body lipid content (mg/g wet weight) of virgin and mated female of selected silkworm races/breeds

Moreover, the data pertaining to the lipid content of virgin female and mated female of four bivoltine and four multivoltine races (Tables-5 & 6 and s figures-5 & 6) of the silk moth revealed that CSR₂ breed adult highest of (0.433±0.016mg/g) in virgin female and 0.428±0.018mg/g in mated female during 1st day of adult, whereas, the lowest lipid was (0.117±0.009) observed in C.nichi races during 10th day in virgin female and 0.156±0.013in mated female adult and as such remaining races exhibited intermediary lipid values and virgin female and mated female of all the races showed statistically significance C.D at 5% and CV in percentage.

In insects, fat body is the main lipid storage tissue and the lipids, commonly referred as a group of naturally occurring molecules that include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides, phospholipids, and others. Small quantity of total lipid content was increased in female when compare to the male (Naseema begum *et al.*, 2014). High rate of lipid accumulation was observed in late period of the entire organism due to constant influx from the food (Islam and Roy, 1982). On the 10th day of virgin female adult development, very little lipid remains in the fat body, the adult fat body is metabolically more active than the diapausing pupal fat body (Gilbert and Chino, 1974). Moreover, General phenomenon of the lipid content observed in pupae was

increased from 3rd day to 5th day in pupation period and later on 6th day to until 12th day is recorded as decreased in all the selected races/breeds (except few races) but the races namely C.nichi, APM1 and APM3 were observed till 9th day of lipid content and 11th day respectively. But, in case of adult silkworm, there is uniformity of lipid content was exhibits from 1st day to 10th day in all the races/breeds. Interestingly, the experiment data showed that, CSR₂ breed exhibited highest lipid content and C.nichi race recorded lowest lipid content in irrespective of male, female and virgin and mated of the pupal and adult stages respectively and statistically obtained data showed significance at 5% critical differences.

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