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Evaluation of carbohydrates in selected silkworm races/breeds of *Bombyx mori* L. Using two mulberry varieties

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Abstract
The present study multivoltine Pure Mysore, bivoltine CSR2 and multi-bi hybrid PMXCSR2 of the mulberry silkworm, *Bombyx mori* L were selected to estimate carbohydrate content in the fat body of 5th instar larvae (day one to day six). M_5 and V1 mulberry varieties cultivated under irrigated condition was used to feed the larvae twice and four times a day. The quantification of carbohydrates was done by using spectrometer and was expressed in mg/g of sample. The results clearly showed an increasing trend with the age of the larvae and also in batches fed four times than twice a day for both the samples. V1 contributed to higher accumulation of carbohydrates than those fed with M_5. Fat body exhibited higher activity while, among the breeds carbohydrate content was higher in CSR2, followed by PMXCSR2 and PM for both the samples used for the present investigation.

Keywords: silkworm, *Bombyx mori* L, carbohydrates, fat body

1. Introduction
The mulberry silkworm, *Bombyx mori* L. is a domesticated and monophagous insect which feeds only on the leaves of mulberry. The growth and development of silkworm and cocoon productivity largely depend on the ambient environment and nutritive quality of the mulberry leaf (Yokoyama, 1963). The weight of the matured larvae during 5th instar; quality and quantity of silk produced is directly related to nutritive contents of the mulberry leaves.

A major portion (about 70%) of the silk protein produced by the silkworm is directly derived from the protein of the mulberry leaves (Fukuda, 1963) [3]. Mulberry leaf should contain not less than 3 - 35 % of the total nitrogen and 5-8 % soluble carbohydrates. The nutritional and chemical composition of the mulberry leaf depends mainly on the variety of mulberry, agronomical practices and environmental factors (Datta, 1992). Though the breeds of the mulberry silkworm differ from genotype to genotype, yet it is not conclusively found in all cases how the difference is exactly reflected in cocoon parameters (Patil, 2001) [7]. The performance, growth rate, time required for development, final body weight and probability of survival (Murugan and George, 1992) [6], are influenced by the quantity and quality of leaf consumed during the larval stage.

At present, the mulberry silkworm has several races/breeds falling under three voltine groups having distinct nutritional, developmental and cocoon characteristics. The races/breeds of the mulberry silkworm are known not only for their significant differences in the quantity and quality of the silk produced by them but also the response of the silkworm to the physical environment and food quality. Miyashita (1986) [5] observed that the productivity of the silkworm is controlled by mulberry leaf (38.20%), climate (37.00%), silkworm rearing techniques (9.30%), silkworm race (4.20%), silkworm egg (3.10%) and other factors (8.20%). The two factors that affect the successful crop production are therefore environment and quality of mulberry leaf.

In the present study, biochemical studies of carbohydrates in the fat body of the silkworm viz., PM race, CSR_2 breed and PM × CSR_2 hybrid fed with two mulberry varieties like M_5 and V_1. Nutritional constituents in the fat body of the silkworm races at similar stages of growth were related to the quality of the leaf of two selected mulberry varieties and racial differences of the races/breeds. The three races/breeds, selected were evaluated for their significant differences in productivity and survival traits, were fed on leaves of variable nutritive and moisture content, aimed to generate information on manifestation of economic traits of the silkworm, *Bombyx mori* by feedings leaves from different mulberry cultivars under hot Indian climatic conditions in popular silkworm races which represent base level of performance viz., poor, medium and good. The results are discussed in the light of physiological compensatory mechanisms observed in the three races in response to nutritional variations and adverse climatic conditions.

2. Materials and Methods
In the present study the disease free layings of multivoltine race PM, bivoltine CSR_2 and multi-bi hybrid PM×CSR_2 were procured from N.S.S.P., NSSO, Mysore and were reared following standard rearing methodology (Krishnaswami, 1978) [4]. In the present rearing V_1 and M_5 mulberry varieties were used to feed the silkworms twice and four times in a day. The studies were conducted during March, 2013 at the DoS in Sericulture Science, University of Mysore and the treatments comprised 3 replications. The temperature ranged between 26 - 30 º C and the relative humidity was in the range of 55 – 70 % during the conduct of rearing.

Mulberry varieties used for the present study: V_1 and M_5 variety

To understand the importance of mulberry which is fed, ingested/digested and the amount of protein and carbohydrates accumulated and diverted to various tissues of the body of silkworm, two feedings at 9 am and 5 pm, and four feedings at 6 am, 11 am, 4 pm and 9 pm were given to the silkworm races/breeds.

Materials

Silkworm races

The multivoltine race PM, bivoltine CSR_2 and multi-bi hybrid PM×CSR_2 of silkworm *Bombyx mori* was selected for the present investigation.

Methodology

Estimation of carbohydrates

Collection of samples

The samples were collected from 5th instar larvae of silkworm *Bombyx mori* L. (1-6 days) by dissecting the larvae fat body was collected in eppendorf tubes then preserved in deep freezer at -20^{0}C. Appropriate dilutions of the fat body were made and samples were centrifuged at 3000 rpm for 15 minutes. The supernatant was diluted appropriately and used for the estimation of carbohydrates.

Procedure of experiment

Anthrone method was followed with slight modification for the carbohydrates estimation in the present investigation. Procedure was followed by taking 100mg of fat body was taken and dissolve in 20 ml of 5 % TCA and homogenate with a help of mortar and pestle. The homogenate was centrifuged at 3000 rpm for 10-15 minutes. 1 ml of supernatant was drawn and transferred to another test tube. 4 ml of Anthrone reagent was added and boiled exactly for 8 minutes and cooled rapidly in running water. Optical density (OD) was taken at 630nm against blank solution.

3. Experimental Results

The data pertaining to the influence of feeding frequencies of V_1 and M_5 varieties of mulberry on the selected traits of PM, CSR_2 and PM × CSR_2 is presented in Table 1 and 2. The data of all the three batches investigated using V1 variety is presented in table 1 and M5 variety in table 2.

The results of carbohydrate content in fat body of the three strains fed with V_1 and M_5 variety is presented in table 1 and 2.

Carbohydrate content in Fat body

Among the three batches selected for the present study the carbohydrate content was highest in CSR_2. It was observed that carbohydrate content in CSR_2, was lowest on 1st day (6.39 mg/g) and highest on 6th day (7.91mg/g) of tissue when the feedings was given twice (increased by 19.28%). Similarly 6.41mg/g of tissue on 1st day and on 6th day 8.08mg/g of tissue with 4 feedings V_1 variety was noticed (increased by 20.61%). With M_5 variety fed twice, the carbohydrate content was 3.42 mg/g of tissue on 1st day and on 6th day it was 6.87mg/g of tissue (increased by 50.21%). With 4 feedings, the activity on 1st day was 4.91mg/g of tissue and on 6th day it was 7.29 mg/g of tissue (increased by 32.56%).

In PM, the carbohydrate content on 1st day was 3.84 mg/g and on 6th day it increased to 4.88 mg/g when fed twice with V_1 variety (increased by 21.35%) and 3.92 mg/g of tissue on 1st day and 5.13 mg/g of tissue on 6th day when fed 4 times (increased by 23.64%). The feedings was given twice using M_5 variety resulted in 2.83 mg/g of tissue on 1st day and 4.28 mg/g of tissue on 6th day (increased by 33.76%). With 4 feedings the carbohydrate content on 1st day was 3.39mg/g of tissue and on 6th day it rose to 4.24 mg/g of tissue. (increased by 20.17%).

In PM × CSR_2, the carbohydrate content was 5.98mg/g of tissue on 1st day and 7.63 mg/g of tissue on 6th day when fed twice with V_1 variety (increased by 21.54%). Similarly with 4 feedings using same variety 6.07 mg/g of tissue was observed on 1st day and 7.74 mg/g of tissue was noticed on 6th day (increased by 21.67%). PM × CSR_2 fed with M_5 variety exhibited the carbohydrate content of 2.95 mg/g of tissue on 1st day and 6.40 mg/g of tissue on 6th day when fed twice (increased by 53.90%). The carbohydrate level was 3.29 mg/g of tissue on 1st day and 6.54 mg/g of tissue on 6th day when fed 4 times (increased by 49.73%).

Table 1: Carbohydrate content in multivoltine, bivoltine and multi × bi hybrid of the silkworm *Bombyx mori* fed with V_1variety

V_1variety	**Tissue taken – Fat body from 5th instar larvae (Day 1 - 6) (carbohydrates content - mg/g of tissue taken)**																	
Treatments	**Pure Mysore**						**CSR_2**						**Pure Mysore × CSR_2**					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
2 Feedings	3.835± 0.060	4.013± 0.067	4.100± 0.205	4.591 ± 0.047	4.783 ± 0.076	4.878± 0.057	6.387 ± 0.118	6.583± 0.194	6.757 ± 0.150	6.996 ± 0.202	7.00 ± 0.321	7.913± 0.079	5.983 ± 0.250	6.474± 0.072	6.539 ± 0.141	6.822 ± 0.141	7.226 ± 0.120	7.626± 0.123
4 Feedings	3.917± 0.015	4.187 ± 0.113	4.578± 0.173	4.670 ± 0.035	5.022 ± 0.060	5.130± 0.064	6.413 ± 0.124	6.730± 0.173	6.817 ± 0.269	7.087 ± 0.28	8.012 ± 0.358	8.078± 0.079	6.065 ± 0.225	6.504± 0.033	6.639 ± 0.128	6.904 ± 0.224	7.374 ± 0.366	7.743± 0.096

Table 2: Carbohydrate content in multivoltine, bivoltine and multi × bi hybrid of the silkworm *Bombyx mori* fed with M_5 variety

M_5 variety	**Tissue taken - Fat body from 5th instar larvae(Day 1 - 6) (carbohydrates content - mg/g of tissue taken)**																	
Treatments	**Pure Mysore**						**CSR_2**						**Pure Mysore × CSR_2**					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
2 Feedings	2.835 ± 0.049	2.878 ± 0.105	3.400 ± 0.136	3.439± 0.033	3.843 ± 0.067	4.28 ± 0.131	3.422 ± 0.064	4.830± 0.219	5.174± 0.284	5.648± 0.163	5.774± 0.136	6.874± 0.069	2.952 ± 0.268	4.743 ± 0.144	5.087± 0.143	5.561 ± 0.072	5.939 ± 0.131	6.404 ± 0.060
4 Feedings	3.387 ± 0.157	3.674± 0.158	3.822 ± 0.141	3.983 ± 0.053	4.161 ± 0.035	4.243± 0.053	4.914 ± 0.257	5.396± 0.200	6.339 ± 0.065	6.700 ± 0.212	6.791 ± 0.151	7.287± 0.098	3.287 ± 0.307	5.187 ± 0.040	5.374± 0.039	5.674 ± 0.237	6.152 ± 0.232	6.539 ± 0.319

Table 3: Day-wise changes in carbohydrate content in fat body of 5th instar larvae showing percentage level fed with V_1 variety

V_1 variety	**Day-wise changes in carbohydrate content in fat body of 5th instar larvae (Day 1 - 6) expressed in percentage level**																	
Treatments	**Pure Mysore**						**CSR_2**						**Pure Mysore × CSR_2**					
	Day2 × Day1	Day 3 × Day 1	Day 4 × Day1	Day 5 × Day1	Day 6 × Day 1	Mean change	Day2 × Day1	Day 3 × Day 1	Day 4 × Day1	Day 5 × Day1	Day 6 × Day 1	Mean change	Day2 × Day1	Day 3 × Day 1	Day 4 × Day1	Day 5 × Day1	Day 6 × Day 1	Mean change
2 Feedings	4.43	6.46	16.46	19.82	21.35	13.70	2.97	5.47	8.70	5.95	19.28	8.47	7.58	8.50	12.29	17.20	21.54	13.42
4 Feedings	6.44	14.43	16.12	22.00	23.64	16.53	4.71	5.92	9.51	8.67	20.61	9.88	6.74	8.64	12.15	17.75	21.67	16.36

Table 4: Day-wise changes in carbohydrate content in fat body of 5th instar larvae showing percentage level fed with M_5 variety

M_5 variety	**Day-wise changes in carbohydrate content in fatbody of 5th instar larvae (Day 1 - 6) expressed in percentage level**																	
Treatments	**Pure Mysore**						**CSR_2**						**Pure Mysore × CSR_2**					
	Day 2 × Day1	Day 3 × Day 1	Day 4 × Day1	Day 5 × Day1	Day 6 × Day 1	Mean change	Day 2 × Day1	Day 3 × Day 1	Day 4 × Day1	Day 5 × Day1	Day 6 × Day 1	Mean change	Day 2 × Day1	Day 3 × Day 1	Day 4 × Day1	Day 5 × Day1	Day 6 × Day 1	Mean change
2 Feedings	1.49	16.91	17.56	26.22	33.76	17.18	29.15	33.86	39.41	40.73	50.21	38.67	37.76	41.96	46.91	50.29	53.90	46.16
4 Feedings	73.81	11.38	14.96	18.60	20.17	14.58	8.93	22.47	26.65	27.63	32.56	23.64	36.63	38.83	42.20	46.57	49.73	42.79

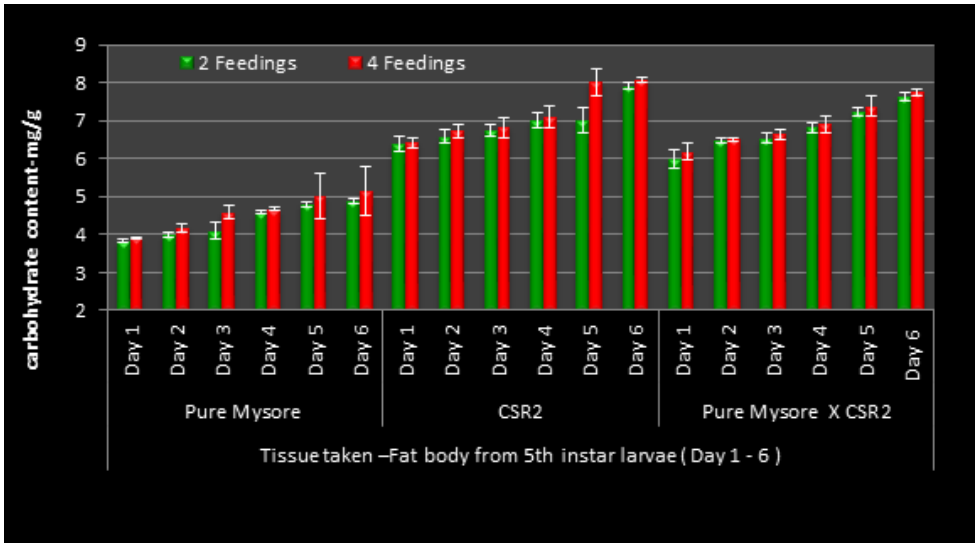

Fig 1: Carbohydrates content in the fat body of multivoltine, bivoltine and multi × bi hybrid of the silkworm *Bombyx mori* fed with V_1 variety

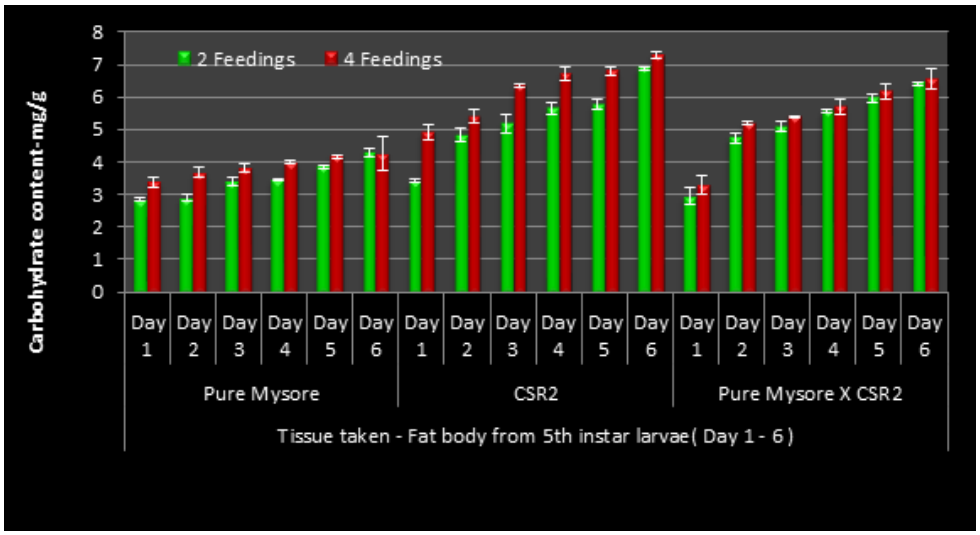

Fig 2: Carbohydrates content in the fat body multivoltine, bivoltine and Multi ×bi hybrid of the silkworm *Bombyx mori* with M_5 variety

4. Discussion

The silkworm *Bombyx mori*, being a monophagous insect, draws all its nutrition from mulberry leaves. Therefore, the growth and development of the silkworm depends on the quantity and quality of leaves provided to them. In addition the silkworm genotype also contributes to the growth and development of the insect interacting with the environment.

A comparative analysis of the data presented in table 1 and 2 clearly shows that the expression of the economic traits not only depends on the feeding frequency (quantity of leaves fed) but more so on the quality of mulberry leaves provided. In this context, it can be drawn that V_1 mulberry variety aided in the better expression of productive traits when compared to M_5 variety of leaves. In addition, the results indicates that CSR_2 is a better productive breed when it comes to absorption and conversion efficiency of nutrition than PM and PM × CSR_2 hybrid.

Carbohydrate is stored as glycogen which is built up in the fat body during periods of active feeding. This storage becomes depleted during sustained activity or during moult when the insect is not feeding or starved. (Chapman, 2005).

The data presented in table 1 pertaining to carbohydrates content in the fat body of 5th instar larvae fed withV_1 variety of mulberry leaves, the results clearly indicate that the carbohydrate content in the fat body increased significantly in all the three genotypes when the feeding frequency was a steady increased from 2 to 4 feeds/day also there was a steady increase of carbohydrate content from day 1 through day 6 with a maximum increase from day 3 to day 4 in case of PM (2 feedings) and from day 2 to day 3 and day 4 to day 5 in case of PM with 4 feedings.

The data present in table 2 pertaining to carbohydrate content in fat body of silkworms fed with M_5 variety leaves. The results exude similar findings as that of V_1 variety leaves except for the fact that M_5 variety leads to better absorption of carbohydrate although the initial carbohydrate content in fat body is less on day 1, than that of V_1 variety. It is interesting to note that the carbohydrate content of PM×CSR_2 increased at a steady rate in the fat body from day 4 onwards in case of 4 feedings/day, indicating earlier spinning in the hybrid when

fed with M_5 Variety leaves.

The carbohydrate in fat body was observed to be maximum in CSR_2 with four feedings. On the other hand, the carbohydrate content in fat body showed decreased trend in PM and PM × CSR_2. Irrespective of the silkworm race/breed/hybrid the carbohydrate activity in fat body being highest in 5th instar 6th day in CSR_2 followed by PM × CSR_2 and PM. There was a gradual increase from day 1- day 6 as discussed above with respect to day-wise changes expressed in percentage levels (Table 3 & 4).

5. Conclusion

More number of feedings, higher is the accumulation of carbohydrates in the tissues selected for the present investigation. The same is also true with the age of the larvae shown during the 5th instar.

The carbohydrate content was found to be higher in the race/breed/hybrid understudy when fed with V1 variety compared to M5 variety.

The carbohydrate content is highest in CSR2 breed followed by PM X CSR2 hybrid and Pure Mysore race, among the three race/breed/hybrid understudy for fat body.

There is a gradual increase in carbohydrate content with the advancement of the age of the larvae *i.e.*, from day 1 to 6 in all the three race/breeds understudy for both mulberry varieties.

Among the two mulberry varieties used, the results have shown that V1 variety contributed to higher carbohydrate accumulation in the fat body as compared to M5 variety in all the three silkworm race/breeds.

The percentage improvement of carbohydrate content is gradual in day 1 and 2. There is sudden spurt in the activity during day 3 and 4 followed by gradual decrease of activity in day 5 and 6 as the larvae show maturity and are ready for spinning.

6. References

1. Chapman RF. The insect structure and function. In: Comprehensive Insect Physiology, Biochem. and Pharmacology (Eds. GA. Kerkut and LI. Gilbert). Pergamon press, New York. 1983; 1:8-99.
2. Datta RK. Influence of mulberry varities and seasons on the economic traits of popular hybrids of silkworm, *Bombyx mori* L. fed with different mulberry varieties. Environment and Ecology. 1995; 13(2):263-268.
3. Fukuda T, Kameyama, Matsuda M. A correlation between the mulberry leaves consumed by the larvae in different ages of the larval growth and the production of cocoon fiber spun by the silkworm larva and of the eggs laid by the silkworm moth. Bull. Seri. Exp. Stn. 1963; 18(3):165-171.
4. Krishnaswami S. New Technology of Silkworm Rearing. CSR&TI Bulletin No.2, Central Silk Board, Bangalore, 1978, 23.
5. Miyashita Y. A report on mulberry cultivation and training methods suitable to bivoltine rearing in Karnataka, 1986, 1-7.
6. Murugan K, George SrA. Feeding and nutritional influence on the growth and reproduction of *Daphnis nerii* (L.). J Insect Physiol. 1992; 38:961-968.
7. Patil RR, Kusugal SN, Banakar BR, Hipparagi GD, Kazi MM, Srinivasan R *et al*. Performance of mulberry silkworm, *Bombyx mori* L. on few varieties of mulberry. Proc. Natl. Sem. Mulb. Seri. Res. India, 2001, 620-626.
8. Yakayama T. sericulture. Annual Review of Entomology. 1963; 8:287-298.