ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2017; 3(10): 156-161
www.allresearchjournal.com
Received: 22-08-2017
Accepted: 23-09-2017

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

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Abstract
The present research work were conducted to estimation of carbohydrate content in haemolymph of 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 haemolymph 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/ml 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. Haemolymph exhibited higher activity while, among the breeds carbohydrate content was higher in CSR2, followed by PMXCSR2 and PM.

Keywords: *Bombyx mori* L, carbohydrates, haemolymph, pure Mysore

1. Introduction

The carbohydrates, proteins and lipids play an important role in the biochemical process underlying growth and development of insects (Ito and Horie, 1959, Wyatt, 1961 and 1967) [5]. Haemolymph proteins play an important role in insects for transport functions, as well as for their enzyme action. The synthesis and utilization of haemolymph proteins are controlled by genetic and hormonal factors (Hurliman and Chen, 1974) [4]. There is a general agreement that the fat body is one of the sources of haemolymph proteins.

In addition to transport functions, the haemolymph of insects performs several physiological functions such as immunity, transport and storage reserve (Mullins, 1985) [8]. The concentration of carbohydrates and other biochemical parameters mainly depend on the quality of mulberry leaf. The late age silkworm larvae accumulate higher carbohydrates compared to young age worms. Reducing sugars account for about 5% of the total blood sugars and the fat body. Glycogen serves as the major food reserve in insects (Kilby, 1958) [6]. Wyatt and Kalf (1956 and 1957) [10] reported that trehalose is the major blood carbohydrate in insects.

Simex and Kodrik (1986) [9] have reported that the glycogen content in the fat body, body wall and silk gland and the free carbohydrates in the haemolymph changed significantly during last larval instar and metamorphosis in silk worms. Carbohydrates are the major components in the food of all the living organisms which either directly or indirectly used as the source of energy for all vital activities. Since the mulberry leaf serves as the sole food for the silk worm Bombyx mori, the quality mulberry leaf influences the growth of the silk worm to a large extent (Benchamin and Jolly, 1986) [2].

Foliar application of nutrients is widely employed to improve the yield, quality and to correct the trace elements deficiencies. The response of plants to foliar nutrients is quick, Changes in the activities of proteins and carbohydrates in haemolymph of silkworm bombyx mori treated with greenleaf Ananda Kumar, M. D, Ann Sandhya Michael International Journal of Environmental Sciences Volume 3 No.1, 2012 120 foliar fertilization evidently activates plant metabolism and assimilation thus contributing to overcome stress.

Further, the use of recent innovation in feeding the plants with nutrients like the use of symbiotic and a-symbiotic nitrogen fixing a-microorganism, green manures, vermicompost, foliar formulations, like vipul, multiplex etc. (Ankalgi and Ansari, 1992) [1] will definitely boost up the mulberry productivity. As there is very little information pertaining to the activity of proteins and carbohydrates in the haemolymph of bivoltine variety (CSR2 X CSR4) and its hybrid with mutivoltine Mysore variety (PM X CSR2) treated with the foliar applicant greenleaf, the present work was undertaken to study the activity in the fifth instar of Bombyx mori.

In the present study, biochemical studies of carbohydrates in the haemolymph 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 haemolymph 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*)* [7]. 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 ºC and the relative humidity was in the range of 55 – 70% during the conduct of rearing.

2.1 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.

2.2 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.

2.3 Methodology

2.4 Estimation of carbohydrates

2.4.1 Collection of samples: The samples were collected from 5^{th} instar larvae of silkworm *Bombyx mori* (1-6 days) and haemolymph were collected by cutting the prolegs of the silkworm larvae in pre-chilled eppendorf tubes containing few crystals of phenylthiourea to prevent oxidation. Then preserved in deep freezer at -20^0C. Appropriate dilutions of the haemolymph were made and samples were centrifuged at 3000 rpm for 15 minutes. The supernatant was diluted appropriately and used for the estimation of carbohydrates.

2.4.2 Procedure of experiment: Anthrone method was followed with slight modification for the carbohydrates estimation in the present investigation. The steps which were used for the procedure are followed by taking 1 ml of haemolymph and add 4 ml of Anthrone reagent 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.

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

3.1 Carbohydrate content in haemolymph

Among the three race/breeds selected for the present study the carbohydrate content was seen highest in CSR_2 followed by PM × CSR_2 and. PM When the feedings was given twice a day the carbohydrate content in PM × CSR_2 was 3.51mg/ml of haemolymph on 1^{st} day and 6.04mg/ml of haemolymph on 6^{th} day (increased by 41.86%), similarly when fed 4 times a day the carbohydrate content increased was 4.30mg/ml of haemolymph on 1^{st} day and on 6^{th} day it rose to 6.84mg/ml of haemolymph with V_1 variety (increased by 37.12%). When the feedings was given twice using M_5 variety the carbohydrate content recorded was 2.82mg/ml of haemolymph on 1^{st} day and 5.62mg/ml of haemolymph on 6^{th} day was seen (increased by 49.80%). Similarly the carbohydrate content of 4.16mg/ml of haemolymph was found on 1^{st} day, while it increased to 5.65 mg/ml of haemolymph on 6^{th} day with 4 feedings (increased by 26.39%).

In PM, the carbohydrate content with 2 feedings was 3.50mg/ml of haemolymph on 1^{st} day and 4.20mg/ml of haemolymph on 6^{th} day (increased by 16.57%) using V_1 variety and with 4 feedings it recorded 3.61mg/ml of haemolymph on 1^{st} day and 4.27mg/ml of haemolymph on 6^{th} day (increased by 15.38%). The feedings was given 2 times using M_5 variety of mulberry in order to estimate carbohydrate content which resulted in 2.69mg/ml of haemolymph on 1^{st} day and 4.08mg/ml of haemolymph on 6^{th} day (increased by 34.03%). With 4 feedings the carbohydrate content was 3.30mg/ml of haemolymph on 1^{st} day and 4.18mg/ml of haemolymph on 6^{th} day by using same variety (increased by 20.95%).

In CSR_2 the carbohydrate content was 3.90mg/ml of haemolymph on 1^{st} day and 6.33mg/ml of haemolymph when fed twice a day with V_1 variety (increased by 38.32%) and when fed 4 times a day the carbohydrate content increased to 4.04mg/ml of haemolymph on 1^{st} day while it was 6.43mg/ml of haemolymph on 6th day (increased by 37.14%). The CSR_2 breed fed with M_5 variety, the carbohydrate content was 3.22mg/ml of tissue on 1st day and 6.35mg/ml of haemolymph on 6th day when fed twice (increased by 49.27%). while with 4 feedings the carbohydrate content was recorded as 3.97mg/ml of haemolymph on 1st day and 6.27mg/ml of haemolymph on 6th day (increased by 36.76%).

The carbohydrate content from haemolymph showed a similar trend of increase in all the three selected strains in case of both 2 feedings and 4 feedings.

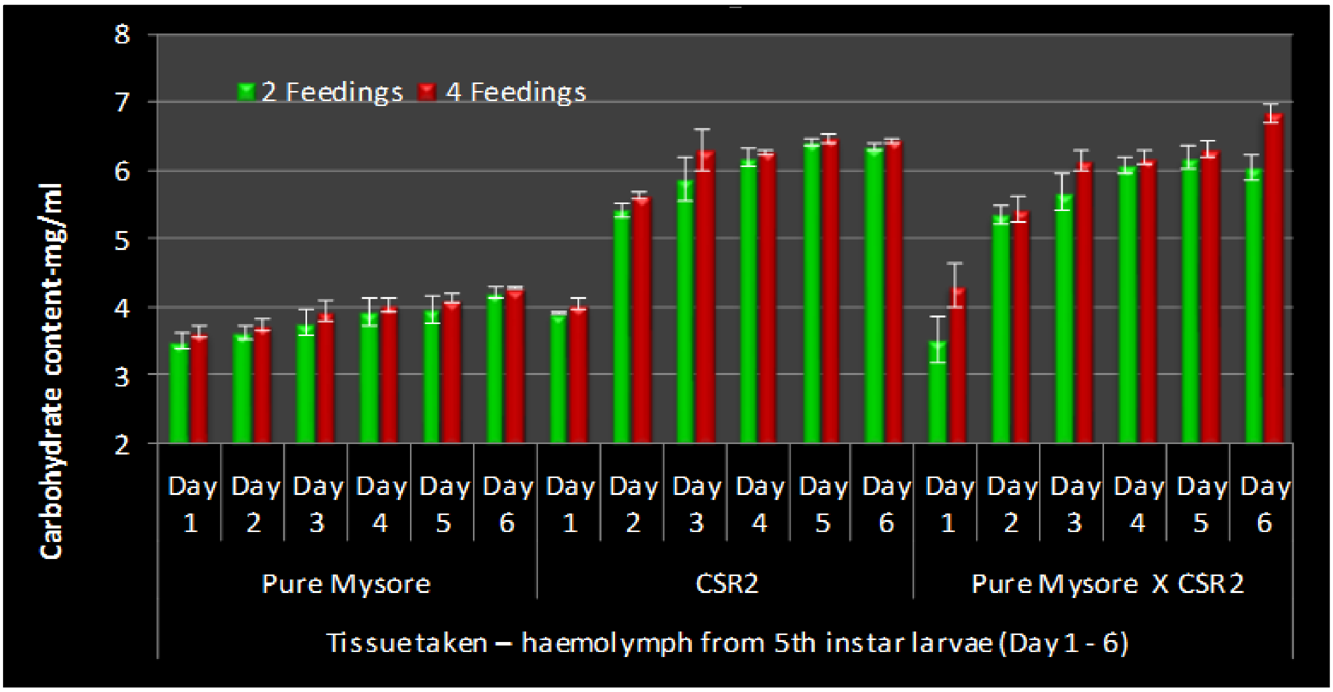

Fig 1: Carbohydrates content in the haemolymph of multivoltine, bivoltine and multi × bi hybrid of the silkworm *Bombyx mori* fed with V_1 variety

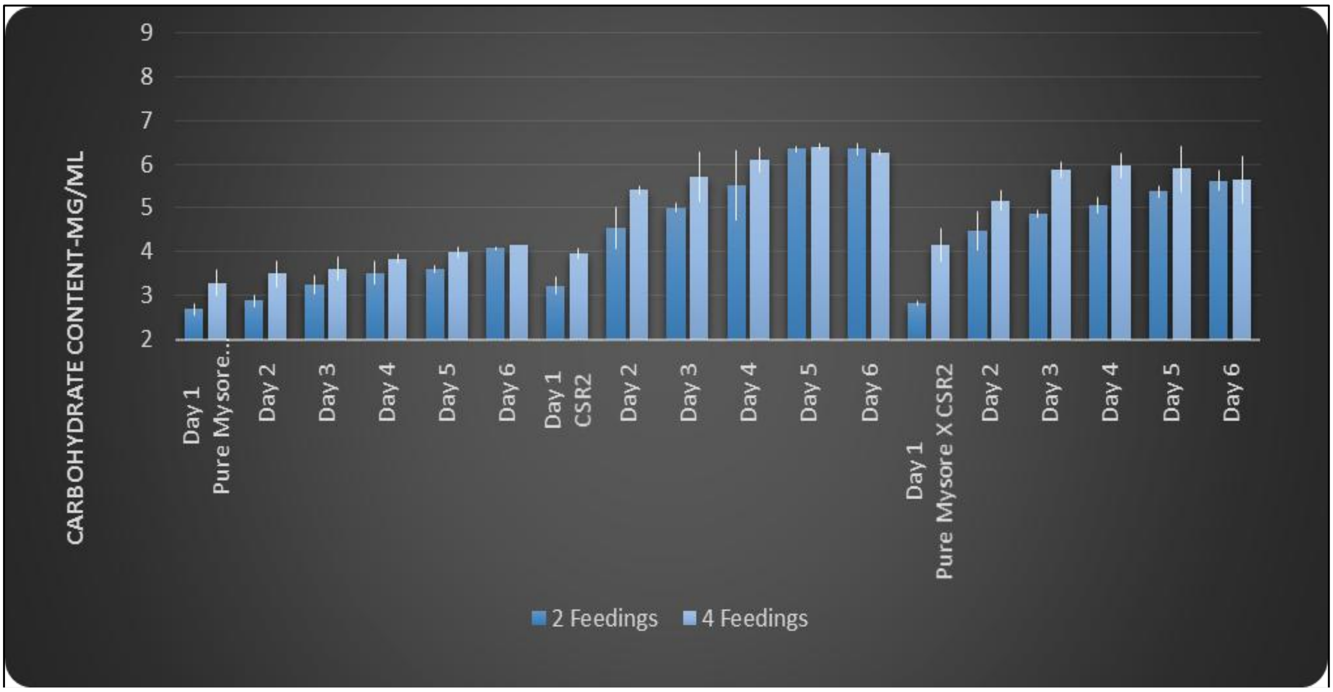

Fig 2: Carbohydrates content in the haemolymph of multivoltine, bivoltine and Multi × bi hybrid fed of the silkworm *Bombyx mori* with M_5 varie

Table 1: Carbohydrate content in multivoltine, bivoltine and multi × bi hybrid of the silkworm *Bombyx mori* fed with V_1 variety

V_1 variety	**Tissue taken – haemolymph from 5^{th} instar larvae (Day 1 - 6) (carbohydrates content - mg/ml of sample)**																	
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	3.504 ± 0.114	3.613 ± 0.107	3.761 ± 0.196	3.917 ± 0.219	3.961 ± 0.203	4.200± 0.094	3.904 ± 0.020	5.409 ± 0.105	5.857 ± 0.319	6.174 ± 0.134	6.417 ± 0.047	6.330± 0.053	3.513 ± 0.347	5.348 ± 0.132	5.678 ± 0.266	6.083 ± 0.117	6.183 ± 0.181	6.043± 0.183
4 Feedings	3.613 ± 0.086	3.739 ± 0.089	3.930 ± 0.144	4.022 ± 0.114	4.117 ± 0.066	4.27 ± 0.027	4.039 ± 0.092	5.639± 0.054	6.296 ± 0.296	6.270 ± 0.033	6.470 ± 0.065	6.426± 0.038	4.300 ± 0.313	5.422 ± 0.174	6.122 ± 0.151	6.187 ± 0.101	6.304 ± 0.121	6.839 ± 0.138

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 – haemolymph from 5^{th} instar larvae (Day 1 - 6) (carbohydrates content - mg/ml of sample)**																	
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	2.690 ± 0.139	2.887 ± 0.137	3.257 ± 0.202	3.526 ± 0.261	3.600 ± 0.081	4.078± 0.027	3.222 ± 0.197	4.552 ± 0.470	5.004 ± 0.096	5.504 ± 0.793	6.352 ± 0.069	6.352± 0.132	2.822 ± 0.054	4.483 ± 0.437	4.870 ± 0.089	5.052 ± 0.181	5.374 ± 0.132	5.622 ± 0.226
4 Feedings	3.296 ± 0.294	3.496 ± 0.301	3.622 ± 0.247	3.839 ± 0.100	3.991 ± 0.114	4.170± 0.008	3.965 ± 0.102	5.409 ± 0.105	5.717 ± 0.561	6.083 ± 0.275	6.400 ± 0.061	6.270± 0.062	4.157 ± 0.366	5.174 ± 0.228	5.878 ± 0.178	5.965 ± 0.287	5.891 ± 0.523	5.648 ± 0.530

Table 3: Day-wise changes in carbohydrate content in haemolymph of 5^{th} instar larvae showing percentage level fed with V_1 variety

V_1 variety	**Day-wise changes in carbohydrate content in haemolymph of 5^{th} 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	0.30	6.83	10.54	11.53	16.57	9.15	27.82	33.34	36.76	39.16	38.32	35.08	5.22	38.12	42.24	43.18	41.86	34.12
4 Feedings	3.36	8.06	10.16	12.24	15.38	9.84	28.37	35.84	35.58	37.57	37.14	34.9	20.69	29.76	30.49	31.78	38.12	29.96
2 × 4 Feedings	3.01	4.33	2.61	3.79	1.63	--	4.07	6.98	1.53	0.82	1.45	--	1.37	7.25	1.68	1.91	11.63	--

Table 4: Day-wise changes in carbohydrate content in haemolymph of 5^{th} instar larvae showing percentage level fed with M_5 variety

M_5 variety	**Day-wise changes in carbohydrate content in haemolymph of 5^{th} instar larvae (Day 1 - 6) expressed in percentage level**																	
Treatments	**Pure Mysore**						**CSR_2**						**Pure Mysore × CSR_2**					
	Day 2 × Day 1	**Day 3 × Day 1**	**Day 4 × Day 1**	**Day 5 × Day 1**	**Day 6 × Day 1**	**Mean change**	**Day 2 × Day 1**	**Day 3 × Day 1**	**Day 4 × Day 1**	**Day 5 × Day 1**	**Day 6 × Day 1**	**Mean change**	**Day 2 × Day 1**	**Day 3 × Day 1**	**Day 4 × Day 1**	**Day 5 × Day1**	**Day 6 × Day 1**	**Mean change**
2 Feedings	6.82	17.40	23.70	25.27	34.03	21.44	29.21	35.62	41.46	49.27	49.27	40.96	37.05	42.05	44.14	47.48	49.80	44.10
4 Feedings	5.72	9.00	14.14	17.41	20.95	13.44	25.69	30.64	34.81	38.84	36.76	33.38	19.65	29.27	30.31	29.43	26.39	27.01
2 × 4 Feedings	17.42	10.07	8.15	9.80	2.20	--	15.84	12.47	9.51	0.75	-1.30	--	13.35	17.15	15.30	8.78	0.46	--

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.

Genotypic factors and nutritional factors have a clear bearing on the economic characters of this insect. In this context, the differential performance of the genotypes based on the nutritional factors is of high practical significance. Perusal of literature demonstrates that effect of different quality mulberry leaves and differential feeding frequencies is important for the expression of productive and viability traits.

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.

V_1 variety is popular among the framers since it is highly rich in nutrition when compared to other mulberry varieties haemolymph store the carbohydrates assimilated by the silkworm from the mulberry leaves. In this context, the study investigated the carbohydrate content in the haemolymph of PM, CSR_2, and PM × CSR_2 genotypes when fed with V_1 and M_5 mulberry leaves and at different feeding frequencies.

The data presented in table 1 pertaining to carbohydrates content in the haemolymph of 5th instar larvae fed withV_1 variety of mulberry leaves, the results clearly indicate that the carbohydrate content in the haemolymph 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 haemolymph 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 haemolymph 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 in haemolymph 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 haemolymph were observed to be maximum in CSR_2 with four feedings. On the other hand, the carbohydrate content in haemolymph showed decreased trend in PM and PM × CSR_2. Irrespective of the silkworm race/breed/hybrid the carbohydrate activity in haemolymph 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 both 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 haemolymph.
- The carbohydrate content was high in the haemolymph in all the three race/breed/hybrid viz., CSR_2, PM and PM × CSR.
- 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 haemolymph 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.

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