International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 18-01-2023, Accepted: 04-02-2023, Published: 21-02-2023
Volume 8, Issue 2, 2023, Page No. 113-116

Studies on nutritional indices and digestive enzymes of new bivoltine and cross breed hybrids of silkworm *Bombyx mori* L.

Ananda Kumar M D, Maribashetty V G
Sericulture Research & Development Institute, Thalagattapura, Bangalore, Karnataka, India

Abstract
The ability of silkworm to ingest mulberry leaf, digest, absorb, assimilate and convert it to silk fiber differs from race to race. In the present study two new bivoltine silkworm double hybrids *viz,* Aditya and Kasturi, and an improved crossbreed namely Bengaluru Belli were used to analyze the leaf to silk and leaf to cocoon conversion efficiency. The dietary indices like cocoon weight, shell weight, leaf – cocoon conversion rate (LCCR), leaf – silk conversion rate (LSCR) were studied. Compared to the respective controls, the new bivoltine double hybrids (Aditya and Kasturi) and new improved crossbreed (Bengaluru Belli) exhibited significantly higher conversion efficiency. The boil – off loss ratio in new hybrids also exhibited significantly low boil-off loss ratio compared to control. Interestingly, the average filament length was also found to be significantly higher in the new bivoltine hybrids and the improved cross breed compared to their respective control. However, the activity of digestive enzyme improved is non-significant between the races studied, also neither between the instars studied. In the present study a significant difference in rearing performance was observed between the races studied even when they were reared under similar environmental conditions. The racial potential and physiological implications of feed conversion efficiency in these silkworm hybrids are discussed with respect to their suitability.

Keywords: LCCR, LSCR, Crossbreed, double hybrids, nutritive indices

Introduction

The mulberry silkworm, *Bombyx mori* L., is an important economic insect that contributes considerably to the national economy of India.The application of genetic and rearing principles in the silkworm breeding and rearing programme has increased the silk production. There is a need to recognize and incorporate the physiological and nutritional requirements of the silkworm hybrids under similar ecological conditions to know the feed conversion efficiency of breeds. Food conversion efficiency in silkworm varies according to the environmental conditions (Ito, 1978; Muniraju *et al*., 2000; Rahmathulla *et al.,* 2008) [12, 18]; food quality (Takano and Arai, 1978; Sarkar and Fujita, 1994) [25, 22] and also quantity of food (Singh and Ninagi, 1995; Mathur *et al.,* 2002) [23, 17]

The leaf to cocoon conversion rate and leaf-silk conversion rate are very important economic parameter in cocoon production. This study intends to analyze the nutritional characteristics and performance of two bivoltine double hybrids namely Aditya-(BRO2 x Nk2) X (CSR6 x CSR26) and Kasturi, (k9DMD x APS46) X (CSR2 x CSR27), and one improved crossbreed *viz,* Bengaluru Belli (IMH x CSR2) in comparison with ruling hybrids with an aim of enhancing commercial sericulture in Karnataka. The newly evolved promising bivoltine hybrids of silkworm *Bombyx mori L.* are increasingly perceived as a promising another source of income generation for rural small-scale farmers.

Materials and methods

Insect and experimental design

After hatching from the eggs, chawki worms of bivoltine Double hybrids (Aditya, Kasturi and DH of CSB as control) and Improved Crossbreed *viz,* Bengaluru Belli and Kolar gold (control) were brushed, reared up separately on fresh leaves of V1 mulberry raised under irrigated condition. The larvae were grouped into batches of four replicates each race and rearing was conducted as per the method of (Dandin & Giridhar, 2014) [7].

Estimation of nutritional indices

Nutritional indices like left over leaf, ingesta (Delvi and Pandian 1972) [8], leaf-cocoon conversion ratio (LCCR) and leaf-silk conversion ratio (LSCR) were calculated as described by Waldbaure (1968) with modified procedure of Maachi and Katagiri (1991) [15].

The productive index during fifth instar and boil-off loss were studied as per the methodology of Basavaraj *et al.,* (2000) [5]. The activity of digestive enzymes *viz,* Amylase was analyzed by following the method of Noelting and Bernfeld (1948) [19] and Baker (1991) [3] using the 3-5 dinitro salicylic acid reagent as modified by Ishaaya and Swirski (1976). Protease activity was analyzed by following the method of Bernfeld (1955) [6].

The pooled data was subjected to statistical analysis (ANOVA) to determine the significance among the hybrids with regard to nutritional efficiency as described by Kegan and Parra (1981) [14].

Results and discussion

The data pertaining to food intake and utilization during fifth instar in the larvae of new bivoltine hybrids Aditya, Kasturi and Bengaluru Belli followed by DH (FC1xFC2) and Kolar Gold as control are depicted in table-1 (ANOVA-one-way CRD). The total cocoon weight (193.5g), total shell weight (27.5 g) and leaf to silk conversion rate (3.48%) are significantly higher ($P<0.05$) in Bengaluru

Belli, followed by Kolar Gold which exhibited on-par values for the parameters considered. Further, the amount of dry food consumed (793.87 g) was also significantly higher in comparison with Kolar gold (c).

Further, the leaf consumed (778.92 g), total cocoon weight (212.5 g) and shell weight (38.55 g) are significantly higher in Aditya followed by 'Kasturi' and control (table1) In addition, Leaf-cocoon conversion rate (18.24 and 19.90) and leaf-silk conversion rate (3.48% and 4.84%) are significantly higher in Bengaluru belli (ICB) and Aditya (BV-DH) respectively. However, the values for CSR% are non-significant in all the hybrids. Further, the value of interaction between the hybrids was on-par with control for the parameters studied.

Table 1: Feed conversion efficiency of new silkworm races of *Bombyx mori* L. Var. V1; Moisture % = 67.8; (V instar -100 L/Rep. X 4 rep./race)

Races	Leaf Cons. (Dry Wt.-g)	Total Cocoon Wt. green (g)	Total Shell Wt. (g)	CSR (%)	LCCR	LSCR
Beng. Belli (IMHxCSR2)	793.87	193.5	27.5	19.20	18.24	3.48
Kolar Gold(C)(PMxCSR2)	889.07	188.7	25.15	18.75	16.74	3.12
Aditya-DH (BRO2xNk2) X(CSR6xCSR26)	778.92	212.5	38.55	22.75	19.90	4.84
Kasturi- DH (K9DMDxAPS46) X(CSR2xCSR27)	784.67	198.5	37.32	21.37	19.69	4.38
DH (C) (FC1xFC2)	779.7	201.1	38.12	22.21	19.13	4.67
Mean	804.55	198.46	33.30	20.84	18.65	4.08
SD±	43.99	8.46	6.00	1.64	1.23	0.70
F-value	3237.55	490.30	4784.86	2588.2	112.5	3722.3
CD @ 5%	3.12	1.54	0.11	0.13	0.46	0.05
Significance	HS**	HS**	HS**	NS	HS**	HS**

The values of boil-off loss% and filament length in Bengaluru-belli (27.26% and 959 m) are significantly better than Kolar Gold (control) respectively. Similarly, these values are significantly better in BV-DH in 'Aditya' when compared to 'Kasturi' and FC1 x FC2 compared to control. (Table-2).

Table 2: Boil-off loss ratio of new silkworm hybrids of *Bombyx mori* L.

Races	Boil-off loss (%)	Avg. Filament Length (M)
Beng. Belli (IMHxCSR2)	27.26	959
Kolar Gold(C)(PMxCSR2)	29.24	837
Aditya-DH (BRO2xNk2) X(CSR6xCSR26)	26.8	1221
Kasturi- DH (K9DMDxAPS46) X(CSR2xCSR27)	26.4	1179
DH (C) (FC1xFC2)	26.7	1213
Mean	27.28	1082.20
SD±	0.91	157.11
F- value	33.76	586.46
CD @ 5%	0.60	26.13
Significance	HS**	HS**

The midgut enzyme assay was carried out to understand the leaf physiological intricacies of leaf to cocoon conversion and leaf to silk conversion ratio of new silkworm races. Midgut protease activity in the new silkworm races in 4^{th} and 5^{th} day of fifth instar was found to be on-par with the control silkworm race studied. The amylase enzyme activity also showed a similar trend, and the significant difference was not observed between the silkworm races neither between the 4^{th} and 5^{th} day of V instar as depicted in table-3 and 4 respectively.

Table 3: Midgut protease enzyme activity (µ mole tyrosine liberated/min/g tissue) in new silkworm hydrids of *Bombyx mori* L.

Races	5^{th}Instar fourth Day	5^{th}Instar fifth Day
Beng. Belli (IMHxCSR2)	0.688 ±0.002	0.612 ±0.003
Kolar Gold(C)(PMxCSR2)	0.681 ±0.001	0.606 ±0.002
Aditya-DH (BRO2xNk2) X(CSR6xCSR26)	0.725 ±0.002	0.681 ±0.001
Kasturi- DH (K9DMDxAPS46) X(CSR2xCSR27)	0.719 ±0.004	0.678 ±0.002
DH (C) (FC1xFC2)	0.724 ±0.003	0.680 ±0.001
SD±	±0.002	±0.002
F-test	3.46	3.45
CD @ 5%	0.002	0.002
Significance	NS	NS

Table 4: Midgut amylase enzyme activity (mg glucose liberated/min/g tissue) in new silkworm hydrids of *Bombyx mori* L.

Races	5thInstar Fourth Day	5thInstar Fifth Day
Beng. Belli (IMHxCSR2)	0.442 ±0.010	0.381 ±0.010
Kolar Gold(C)(PMxCSR2)	0.41 ±0.012	0.352 ±0.020
Aditya-DH (BRO2xNk2) X(CSR6xCSR26)	0.569 ±0.021	0.506 ±0.012
Kasturi- DH (K9DMDxAPS46) X(CSR2xCSR27)	0.537 ±0.030	0.483 ±0.024
DH (C) (FC1xFC2)	0.551 ±0.032	0.496 ±0.015
SD±	±0.003	±0.003
F-test	4.27	4.27
CD @ 5%	0.004	0.004
Significance	NS	NS

The major ecological factors like relative humidity, temperature, in addition to other physiological conditionsalso influencesthe feeding activity of silkworm. The accumulation of organic matterfueled by the nutritive substances absorbed after digestion of fooddue to the balance between anabolic and catabolic reactions manifest the silkworm growth.

In silkworm *Bombyx mori*, the food consumption has a direct relevance on weight of larvae, cocoon and shell weights. However, these inter dependent parameters of consumption and productivity varies depending on the season and silkworm breeds (Maribashetty *et al*., 1999) [16]. Mulberry leaves provides silkworm larva all nutrition required tobuild its body, sustain and spin cocoons. Such nutritional requirement in food consumption has direct impact on all genetic traits such as larval and cocoon weight, quantity of silk production, pupation and reproductive traits (Ramesha *et al*., 2010) [21]. Silkworm nutrition refers to the substances required by silkworm for its growth and metabolic functions, which are obtained from ingested food of mulberry/artificial diet and the other remaining nutritional components are synthesized through various biochemical pathways (Takano and Arai, 1978; Hamano *et al*., 1986; Zhang *et al*., 2002) [25, 9, 27].

Further, dietary or nutritional efficiency considerably contributes to the cost-benefit ratio in cocoon production. In addition, leaf cocoon ratio can vary from rearer to rearer, since a rigid guideline is not followed as to the quantum of leaf fed. But on the other hand, the basis of calculation of the leaf-cocoon conversion ratio (LCCR) or for that matter leaf-silk conversion ratio (LSCR) is the precise amount of leaf ingested by larvae and the amount of cocoon or silk produced from that amount of leaf (Maachi and Katagiri, 1991; Trivedy and Nair, 2003; Anilkumar, 2018) [15, 13, 2].

In the present investigation this difference between breeds is clearly observed as the consumption and conversion efficiencies are higher in new bivoltine and multi x bi races compared to their respective controls, when they are reared under similar environmental conditions. These results corroborate the findings of Anantharaman *et al*., (1995) [1]; Horie and Watanabe, (1983) [10]. In the present study all the treatments as compared to the control, greatly increased assimilation in the fifth instar. According to Trivedi *et al.*, (2003), a higher absorption rate is the result of better digestion and assimilation.

Balamani *et al*., (1995) [4] have reported that the growth of the larvae in turn depends on the nutrient content of mulberry leaf and their ability to feed, digest, absorb and re-synthesize the body proteins. Therefore, though the nutritional quality of mulberry leaf of rain fed area is inferior to irrigated garden, the multivoltine hybrid *viz*, PM X C_{110} has exhibited significantly higher values for LCCR and LSCR. This corroborates the report of Slansky and Feeny (1977) that the larvae under low input of nutrients manage to accumulate required quantity of the food in order to maintain the critical level of growth limiting nutrients in the body.

This study indicates that Aditya and Bengaluru Belli (ICB) were superior to the control hybrids in feed conversion with regard to majority of the indices considered and showed their supremacy over the existing control hybrids. Hence, the hybrids identified here consume less/on-par amount of leaf as compared to the existing popular ones and at the same time remain more productive. In conclusion the hybrids *viz*, Aditya and Bengaluru Belli (ICB) are comparatively better convertors of food to cocoon/silk.

Acknowledgement

The authors thank the Director, Karnataka state sericulture research and development institute and commissioner for Seri development (Gok) for facilities provided and financial assistance as Puna Chethana project. The help and co-operation rendered by Section head of Silkworm Breeding and Genetics section, KSSRDI Thalaghttapura is duly acknowledged.

References

1. Anantharaman KV, Magadum SB, Datta RK. Feed efficiency of silkworm, Bombyx mori L. hybrid (NB4D2 × KA). Insect Sci. Applic,1995, 111-116.
2. Anilkumar MN. Analysis of boil-off loss in the cocoon shells of the silkworm (Bombyx mori L.) bivoltine breeds and their hybrids. Int. J. Res. In Biosc. Agr. & Tech. I, 2018, 159-168.
3. Baker JE. Purification and partial characterization of amylase enzymes from the lesser grain borer, Rhyzoptheradominica. Insect Biochem,1991:21:303-311
4. Balamani R, Prince SPM, Subbbram WV. Effect of zinc on the nutritional indices, economic characters of cocoon and quality of silk in Bombyx mori L, Indian. J. Sericulture,1995:34:69-71.
5. Basavaraja HK, Suresh Kumar N, Mal Reddy N, Datta RK. Studies on the boil-off loss ratio with reference to the cocoon shell in bivoltine silkworm, Bombyx mori L. Indian Journal Sericulture,2000:31(1):60-65.
6. Bernfeld P. Amylases α, β. In: Methods in Enzymology, Colowick, S. P. and Kaplan, N. O. (Eds.). Vol. I. Academic press. Newyork, 1955, 149-158.

7. Dandin SB, Giridhar K. (Eds.) Handbook of Sericulture Technologies. Central Silk Board, Bangalore, 2014.
8. Delvi MR, Pandian TJ. Rates of feeding and assimilation in the grasshopper Poecilocerus pictus. Journal of Insect Physiology,1972:18:1829-1843.
9. Hamano K, Miyazawa K, Mukiyama F. Racial difference in the feeding habit of the silkworm, Bombyx mori, J. Sericl. Sci. Jap,1986:55:68-72.
10. Horie Y, Watanabe K. Daily utilization and consumption of dry matter in food by the silkworm Bombyx mori (Lepidoptera; Bombycidae). Appl. Entomol. Zool,1983:18:70-80.
11. Ishaaya I, Swirski E. Trehalase, invertase and amylase activities in black scale, Saissetiaoleae and their relation to host adaptability. J. Insect Physiol.,1976:22:1025-1029.
12. Ito T. Silkworm nutrition In Silkworm an important laboratory tool. Tazima Y. (Ed) Ko ltd., Tokyo,1978, 121-157.
13. Karnika Trivedy, Nair KS, Begum AN. Digestibility in the newly developed bivoltine hybrids of silkworm Bombyx mori L. Indian J. Seri.,2003:42(2):142-145.
14. Kegan M, Parra JRP. Techniques and applications of measurements of consumption and utilization of feed by phytophagous insects. In: Current topics in Insect Endocinology and Nutrtion, Bhaskaran, G., Friedman, S. and Rodrigues, J. G. (Eds.), Plenum Press, New York, 1981, 337-352.
15. Maachi H, Katagiri K. Varietal differences in nutritivevalues of mulberry leaves for rearing silkworms. Japan Agricultural Research,1991:25:202-208.
16. Maribashetty VG, Chandrakala MV, Aftab Ahmad CA, Krishna Roa S. Food and water utilization patters in new bivoltine races of silkworm Bombyx mori L. Bull. Indian Acad. Seric1999:3:83-90.
17. Mathur VB, Rahmathulla VK, Vijaya BO. Consumption and Conversion Efficiency of Food in New Elite Bivoltine Hybrid of Silkworm, Bombyx mori L. under Restricted Feeding Levels, Int. J. Indl. Entom,2002:5:213- 216.
18. Muniraju E, Sekharappa BM, Raghuraman R. Role of temperature on leaf to silk conversion in silkworm Bombyx mori L. Sericologia,2000:39:225-231.
19. Noelting G, Bernfeld P. Surles enzymes Amylolyteques-III La B-amylase dosage. D. activite’ et controle de L’ aberence d’ L-amylase”, *Helv-Chim*,1948:31:290-296.
20. Rahamathulla VK, Suresh HM. Feed consumption and conversion efficiency in male and female BV silkworms (Bombyx mori L.) – A comparative study, J. Entoml. Res. Soc.,2008:10(1):59-65.
21. Ramesha C, Kumari SS, Anuradha CM, Lakshmi H, Kumar CS. Nutrigenomic analysis of mulberry silkworm (Bombyx mori L.) strains using Polymerase Chain Reaction - Simple Sequence Repeats (PCR-SSR), Intl. J. Biotech. Mol. Bio. Res,2010:1:92-100.
22. Sarkar A, Fujita H. Better technique for nutritive evaluation of mulberry leaves for silkworm, Bombyx mori L. Indian Journal of Sericulture,1994:33:19-22.
23. Singh GB, Ninagi O. Comparative studies on food utilization efficiency in some silkworm strains under different feeding levels. Sericologia, 1995:354:667-675
24. Slansky FJ, Fenny P. Stabilization of rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. Ecol. Monog,1977:47:209-228
25. Takano K, Arai N. Studies on the food value on the basis of feeding and cocoon productivity in the silkworm Bombyx mori L. treatment of food intake and cocoon productivity, J. Seri. Sci. Jap. 1978:47:134-142.
26. Waldbauer GP. The consumption and the utilization of food by insects. Adv. Insect Physiol,1968:5:229-288.
27. Zhang YH, Xu AY, Wei YD, Li MW, Hou CX, Zhang GZ. Studieson feeding habits of silkworm germplasm resources for artificial diet without mulberry,2002:28:333-336.