Studies on Nutrigenetic Traits of Silkworm, *Bombyx mori* L. for Determining Growth and Development for Identifying Parental Breeds for Breeding under Subtropical Region of North West India

S. Murali* and Sardar Singh*1

1Regional Sericultural Research Station, Central Silk Board, Miran Sahib, Jammu – 181101, India.

Authors’ contributions

This work was carried out in collaboration between both authors. Author SM designed the objective of the study, performed the statistical analysis, wrote the methodology and wrote the draft of the manuscript. Author SS managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJECC/2021/v11i3330374

Editor(s):
(1) Dr. Arjun B.Chhetri, Dalhousie University, Canada.

Reviewer(s):
(1) Ashenafi Teklemariam Tegegn, Wolkite University, Ethiopia.
(2) Mahendran B, ICAR- Sugarcane Breeding Institute Research Centre, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/62358

ABSTRACT

Study was conducted during spring and autumn season (2018 & 2019) to screen and identify rich nutrigenetic breeds from the selected breed’s for their nutrigenetic traits in silkworm, *Bombyx mori* L.(Lepidoptera: Bombycidae) is an essential prerequisite for better understanding and development of nutritionally efficient breeds under Subtropical condition of Jammu based on the breeds which shows less food consumption with higher efficiency conversion based on leaf to cocoon and leaf to shell ratio. Highly significant variations were found among all nutrigenetic traits of bivoltine silkworm breeds in the study. The nutritionally efficient silkworm breeds were shortlisted by utilizing nutrition consumption index and efficiency for conversion of ingesta/cocoon traits as the index for selection of highly promising breeds. Furthermore, based on the average of data from both the seasons, the overall rearing nutrigenetic traits utilized as index, eight bivoltine silkworm breeds (B.con 1, B.con 4, BHR 2, ATR 16, BHR 3, CSR 50, RSJ 14 and NB*D2*) were identified as...
having the potential for nutrition efficiency conversion and can be utilized for further breeding programme. The data from the present study advances our knowledge for the development of nutritionally efficient silkworm breeds/hybrids and their effective commercial utilization in the sericulture industry.

Keywords: Nutrigenetic traits; breeds; food consumption; efficiency conversion; seasons.

1. INTRODUCTION

Silkworm larva obtains all nutrients from mulberry leaves to build its body, sustain and spin cocoons. Under different environmental, feeding and nutritional condition, it shows significant difference in its ability to ingest, digest, absorb and convert to body matter. The capacity of silkworm, to ingest mulberry leaf, digest, absorb, assimilate and convert it to silk fibre also differs from race to race. The efficiency of converting the ingested and digested food into the body, cocoon and cocoon shell varies among silkworm races under the influence of season and mulberry varieties.

Nutritional efficiency is considered important to assess the cost benefit ratio of sericulture up to the level of cocoon production. Various studies with regard to food consumed and utilization by Bombbyx mori is reviewed [1]. Food utilization studies in silkworm were well researched ever since the pioneering work was done [2] and later methodological changes were made [3] & [4]. Relationship with cocoon productivity and mulberry leaf intake has been reported [5]. Dietary efficiency of a silkworm hybrid/breed plays a major role in converting the mulberry leaves consumed by them to silk. Cocoon productivity in sericulture is dependent on breed, seed and feed and their quality. High level of heterosis for quantitative characters is observed in the crosses of highly inbreed lines and geographically divergent strains of silkworm. True hybrid/seed contribute to the cocoon productivity through substantial improvement in the important characteristics such as fecundity, larval duration, cocoon weight, cocoon yield by number and vitality.

Nutritional ecology of insects is the prerequisite for a better knowledge on their ethobiology and physiology, which is often been, neglected [6]. There are several contributing factors which determine the digestibility and conversion efficiency of a breed eco-physiological condition and morphological deviation being the major factors, which determine the efficiency. Nutritional quality as well as environmental conditions has greater impact on regulation over the quantum of ingesta, digesta, and digestibility of food among silkworm [7]. Silkworm growth is a composite result of various physiological activities of an organism, by which matter accumulates in the body as a result of balance between assimilation and dissimilation [8]. Efficiency of conversion of ingested mulberry leaves into silk or leaf silk conversion rate is a better economic index in cocoon production.

The climatic condition of the North India is suited for bivoltine sericulture but the unit production and the quality of the silk produced is much lower than the sericulturally advanced countries like Japan and China. The cocoon productivity in North India is 34.17 Kg/100 Dfls at commercial level and the average renditta is 9.50 Kg, while it is 6.50 Kg at National level [9]. As of now the production is about 40 Kg/100 Dfls with renditta of 7.50 Kg. It is very well known that, they are not able to sustain under sub-optimal conditions (high temperature and high humidity) coupled with problem encountered by environmental factors/high load of diseases prevailing in J & K region resulting decline in cocoon yield. Thus, among many factors attributed to reduction in silk production, the major one is the lack of nutrition efficiency conversion in silkworm breeds in tropical/subtropical areas. Therefore, one of the key considerations in developing hybrids for subtropical regions could be the need for studies on nutrition efficiency conversion in silkworm breeds. The recent advances in silkworm breeding and those with nutrition efficiency conversion have opened up new avenues to evolve nutritionally efficient productive silkworm hybrids [10,11,12,13,14,15] & [16]. Therefore, it is necessary to screen and identify silkworm breeds suitable for Subtropical region of North Western India and to improve the cocoon productivity, which reflects to enhance the economic returns to the farmers.

2. METHODOLOGY

The experiment trial was conducted at the Regional Sericultural Research Station, Miran Sahib, Jammu (J & K) where the selected mulberry variety (S-146) was maintained according to advocated package of practice and
the leaves were used for rearing of selected breeds from different regions. To know the nutritional efficiency conversion through absorption/assimilation among the breeds to evolve superior bivoltine breed under Subtropical condition of North Western India the study was conducted during spring and autumn season (2018 & 2019) respectively.

2.1 Breeds

The silkworm bivoltine breeds were selected based on commercial characteristics and geographical drift of the breeds like BHR2, BHR3, B.con1, B.con4 (Berhampur), DUN6, DUN22, ATR16, ATR29, SH1, NB-D2 (Dehradun), CSR2, CSR6, CSR50, CSR51 (Mysore), JAM2, JAM121, RSJ1 and RSJ14 (Jammu). These breeds, with varied phenotypic quantitative traits and hibernating nature were procured from different places and were utilized for the study during spring season (2018 & 2019) (Table 1).

2.2 Estimation of Nutritional Traits

The nutrigenetic traits estimation study was carried out for identification of suitable nutrigenetic breeds during spring and autumn season (2018 & 2019) of the year in a completely randomized block design. Silkworm rearing was conducted following the standard method under the recommended temperature and relative humidity until the 4th molt. On the 1st day of fifth instar, 50 healthy silkworm larvae per breed in three replications of 150 larvae were selected for estimation of nutritional traits analysis. Accurately weighed fresh mulberry leaves were fed 3 times a day to the experimental batch. Simultaneously, an additional batch of larvae for each breed were maintained to determine the dry weight on subsequent daily increments in larval weight was recorded separately as suggested [17]. Silkworm rearing continued using appropriate plastic trays, the healthy larvae were counted daily in each replicate, and any missed larvae were replaced from the reserved batch. Left over leaves and excreta were collected on each subsequent day, separated manually and dried in a hot air oven daily at about 100°C until they reached constant weight using an air-tight electronic balance. When the larvae finished feeding they were shifted to the mountage for spinning at normal ambient temperature of 25±2°C and RH 65±5%. Cocoons were harvested 5 days later after completion of cocoon spinning. Harvested cocoons were analyzed for quantitative traits using the equations detailed below. The dry weight of left over leaves, excreta, larvae, cocoon, and shell in each of the breed were recorded. The nutrigenetic traits interaction was obtained by utilizing standard gravimetric analysis methods for consecutive seasons.

During the silkworm nutritional study, data was collected on the biomass of larvae and cocoons for the 19 nutrigenetic traits on ingesta, digesta, excreta, approximate digestibility (AD), reference ratio (RR), consumption indices (CI), relative growth rate (RGR), respiration and metabolic rate (MR), efficiency conversion of ingesta (ECI) and digesta (ECD) for larva, cocoon, and shell. Further, the ingesta and digesta required for producing one gram of cocoon and shell (I/g and D/g) were to be collected and calculated as described by standard gravimetric methods [18] [19] [20] & [21]. The equations with brief description of the nutrigenetic traits evaluated was given below.

2.2.1 Ingesta (g)

Total intake of the dry weight (g) of mulberry leaves by silkworm larvae during the 5th stage up to spinning or ripening stage: (Dry weight of leaf fed - Dry weight of left over leaf).

2.2.2 Digesta (g)

Total assimilated dry food from the intake or ingesta of dry weight of mulberry leaves by silkworm larva during the 5th stage until spinning or ripening: (Dry weight of leaf ingested - dry weight of litter).

2.2.3 Excreta (g)

Refers to the non-utilized mulberry leaves in the form of litter from the ingested mulberry leaves of a silkworm: (Ingesta - Digesta).

2.2.4 Approximate digestibility (%)

Directly indicates the assimilation efficiency of mulberry leaves and depends on the passage rate of food through gut in silkworm: (AD = Dry weight of digesta / Dry weight of food ingested × 100).

2.2.5 Reference ratio

An indirect expression of absorption and assimilation of food. Expresses the ingestarequired per unit excreta produced: (RR= Dry weight of food ingested /Dry weight of excreta).
2.2.6 Consumption index

Relates the rate of food intake to the mean weight of the larvae during the feeding period: (CI = Ingesta / 5th stage mean fresh larval weight (g) × 5th stage larval duration in days).

2.2.7 Relative growth rate

Refers to larval gain biomass and indicates the efficiency of conversion of nutrition into larval biomass: (RGR = Weight gain of the larva during feeding period / 5th stage mean fresh larval weight (g) × 5th stage larval duration in days).

2.2.8 Respiration

A catabolic reaction in which total oxidation of the digested or assimilated food for releasing energy required for the entire biological activities by break down of macromolecules into simpler molecules: (Dry weight of food digested - Maximum dry weight of larvae).

2.2.9 Metabolic rate

Measure of total biochemical reactions involving both catabolic and anabolic reactions of an organism, associated with the degradation of macromolecules into smaller unit and vice versa: (MR = Respiration / 5th stage mean fresh larval weight (g) × 5th stage larval duration in days).

2.2.10 Efficiency conversion of ingesta to larva (%)

Associated with the efficiency conversion of ingested nutrition into biomass or body matter at different stages and expressed in percentage. ECI to larva was the efficiency of conversion of ingested food into larva: (ECI larvae = Maximum dry weight of larva / Dry weight of ingesta × 100).

2.2.11 Efficiency conversion of digesta to larva (%)

The expression of efficiency conversion of digesta into larval biomass: (ECD larvae = Maximum dry weight of larva / Dry weight of digesta × 100).

2.2.12 Efficiency conversion of ingesta to cocoon (%)

This is the most economically important trait used by the sericulture industry. It was the expression of efficiency conversion of ingesta into cocoon, also referred to as the leaf-cocoon conversion rate. This nutrigenetic trait was kept as the ultimate index for assessing the superiority of breed for nutritional efficiency in this investigation: (ECI cocoon = Dry weight of cocoon / Dry weight of ingesta × 100).

Table 1. Showing selected breeds procured from different places with morphological features

| Sl. No. | Breeds    | Source                  | EC   | ESC | LM   | CS  | CC  | B  | G  |
|---------|-----------|-------------------------|------|-----|------|-----|-----|----|----|
| 1       | BHR 2     | Central Sericultural    | Grey | W   | M    | EC  | W  | H  | M  |
| 2       | BHR 3     | Research & Training     | Grey | W   | M    | EC  | W  | H  | M  |
| 3       | B.con 1   | Institute, Berhampur    | Grey | W   | P    | EC  | W  | H  | M  |
| 4       | B.con 4   | (West Bengal)           | Grey | W   | P    | EC  | W  | H  | M  |
| 5       | DUN 6     | Regional Sericultural   | Grey | W   | P    | O   | W  | H  | M  |
| 6       | DUN 22    | Research Station,       | Grey | W   | M    | C   | W  | H  | M  |
| 7       | ATR 16    | Sahaspur,               | Grey | W   | P    | O   | W  | H  | M  |
| 8       | ATR 29    | Dehradun                | Grey | W   | M    | C   | W  | H  | M  |
| 9       | SHs       | (Uttarakhand)           | Grey | W   | M    | O   | W  | H  | M  |
| 10      | NBJ,2     |                         | Grey | Y   | P    | C   | W  | H  | M  |
| 11      | CSR2      | Central Sericultural    | Grey | Y   | P    | O   | W  | H  | M  |
| 12      | CSR6      | Research & Training     | Grey | W   | M    | C   | W  | H  | M  |
| 13      | CSR50     | Institute, Mysore       | Grey | Y   | P    | O   | W  | H  | M  |
| 14      | CSR51     | (Karnataka)             | Grey | W   | M    | C   | W  | H  | M  |
| 15      | JAM 2     | Regional Sericultural   | Grey | W   | M    | C   | W  | H  | M  |
| 16      | JAM 121   | Research Station,       | Grey | W   | P    | O   | W  | H  | M  |
| 17      | RSJ1      | Miran Sahib             | Grey | Y   | M    | C   | W  | H  | M  |
| 18      | RSJ14     | Jammu (J & K)           | Grey | Y   | M    | O   | W  | H  | M  |

Note: EC- Egg color; ESC- Egg shell color (W - White; Y – Yellow); LM- Larval Markings (P- Plain; M - Marked); CS- Cocoon Shape (O - Oval; E- Elongated Constriction); CC- Cocoon color (W- White); B - Build (H - Hard); G - Grain (M - Medium)
2.2.13 Efficiency conversion of digesta to cocoon (%)

It was the expression for efficiency conversion of digesta into cocoon: \( \text{ECD cocoon} = \frac{\text{Dry weight of cocoon}}{\text{Dry weight of digesta}} \times 100 \).

2.2.14 Efficiency conversion of ingesta to shell (%)

This was the expression efficiency conversion of ingesta into shell. It is also referred to as the leaf-shell conversion rate and is the ultimate index to evaluate superiority of breed in nutritional efficiency: \( \text{ECI shell} = \frac{\text{Dry weight of shell}}{\text{Dry weight of ingesta}} \times 100 \).

2.2.15 Efficiency conversion of digesta to shell (%)

The expression of efficiency conversion of digesta into shell: \( \text{ECD shell} = \frac{\text{Dry weight of shell}}{\text{Dry weight of digesta}} \times 100 \).

2.2.16 Ingesta per gram cocoon (g)

This was another important trait of economical significance to assess silkworm breed performance in nutrigenetic analysis. It was the expression of total ingesta required for the production of one gram of cocoon: \( \text{I/g cocoon} = \frac{\text{Dry weight of ingesta}}{\text{Dry weight of cocoon}} \).

2.2.17 Digesta per gram cocoon (g)

The total digesta requisite for the production of one gram of cocoon: \( \text{D/g cocoon} = \frac{\text{Dry weight of digesta}}{\text{Dry weight of cocoon}} \).

2.2.18 Ingesta per gram shell (g)

The total ingesta requisite for the production of one gram of shell: \( \text{I/g shell} = \frac{\text{Dry weight of ingesta}}{\text{Dry weight of shell}} \).

2.2.19 Digesta per gram shell (g)

The total digesta requisite for the production of one gram of shell: \( \text{D/g shell} = \frac{\text{Dry weight of digesta}}{\text{Dry weight of shell}} \).

The data on nutritional traits of the experimental breeds were recorded on 19 nutrigenetic traits for each replicate were subjected to suitable statistical analysis by ANOVA (one way) through available software (SPSS). The data for transformation, arc sign and square root transformation was used.

3. RESULTS

3.1 Performance on Nutrigenetic Traits

Considerable variations were noticed for 19 nutrigenetic traits among the selected bivoltine breeds on nutritional parameters. Data were obtained for ingesta, digesta, excreta, AD, RR, CI, RGR, respiration, MR, ECI, and ECD to larval biomass, ECI and ECD to cocoon and shell, I/g and D/g to cocoon and shell for eighteen bivoltine breeds under standard nutritional traits estimation. There was evidence of significant differences in consumption of mulberry leaf and food conversion to biomass for major nutrigenetic traits in all experimental bivoltine breeds during spring and autumn season (2018 & 2019). The average results of the overall rearing of the two years data were presented below here under.

3.1.1 Ingesta, digesta, excreta, AD and RR

From the overall rearing (2018 & 2019), all the parameters were showed statistically significant differences among them. The observation recorded for ingesta ranged between maximum in CSR2 (10.51 g) and lowest in ATR16 (6.94 g). The maximum digesta recorded in CSR2 (8.06 g) with minimum in ATR16 (4.88 g) whereas highest excreta recorded in B.con 1 (2.51 g) and lowest in JAM2 (1.59 g). Approximate digestibility ranged between DUN6 (78.59%) to B.con 4 (69.09%) and the reference ratio with maximum value in DUN6 (5.17) and minimum in B.con 1 (3.19) (Table 2).

3.1.2 CI, RGR, respiration and MR

From the overall rearing (2018 & 2019), the Consumption Index (CI) recorded to be highest in CSR6 (0.60) and lowest in B.con1 (0.42) whereas there was significant difference observed in Relative Growth Rate (RGR) among all of them. A significant differences were recorded with respect to respiration and found relatively more in CSR2 (7.21 g) and less in B.con 1 & ATR16 (3.98 g). There is distinguishable divergence for Metabolic Rates (MR) among bivoltine selected breeds which ranged between maximum in CSR6, CSR51, RSJ 1 & SH 6 (0.39) and lowest in B.con1 (0.23) respectively (Table 2).

3.1.3 ECI and ECD to larval biomass

From the overall rearing (2018 & 2019), the observation noted for efficiency of mulberry leaves ingested and converted into silkworm
larval biomass varied significantly among the selected bivoltine breeds (Table 3). The highest efficiency conversion of ingesta (ECI) to larva recorded in B.con 4 (14.44%) & lowest in SH6 (8.21%). With regard to Efficiency Conversion of Digesta (ECD) to larva significant variation was observed and more efficient conversion of digested food into larval biomass is recorded in B.con 1 (21.12%) and less efficient in SH6 (10.68%).

3.1.4 ECI and ECD to cocoon and shell

Efficiency Conversion of Ingesta (ECI) to cocoon revealed that higher in ATR16 (10.36%) followed by BHR2 (9.96%) and lower in JAM121 (6.68%). Efficiency Conversion of Digesta (ECD) to cocoon varied significantly among the breeds which ranged between 15.03 per cent (B.con1) to 7.63 per cent (RSJ1). ECI to shell observed to be maximum in ATR16 (4.37%) and minimum in SH6 (2.64%). With regard to ECD for shell high conversion rate was recorded in ATR16 (6.22%) followed by BHR3 (5.78%) and lowest in RSJ1 (3.31) from the overall rearing (2018 & 2019)(Table 3).

3.1.5 I/g and D/g to cocoon and shell

All the parameters were showed statistically significant differences among them from the overall rearing (2018 & 2019).The data for ingesta per gram cocoon (I/g) was observed it ranged between maximum in DUN6 (17.11 g) and minimum in ATR16 (10.00 g). The amount of digesta per gram cocoon (D/g) observed was highest in DUN6 (13.45 g) and lowest in B.con1 (6.92 g). With regard to ingesta per gram (I/g) shell noticed to be ranged between RSJ1 (39.53 g) and lowest in ATR16 (23.46 g). The digesta required to one gram (D/g) per shell revealed it ranged between maximum in RSJ1 (30.54 g) and minimum in ATR16 (16.51 g) (Table 3).

4. DISCUSSIONS

The physiology of growth manifested by the accumulation of organic matter resulting from the balance between anabolic and catabolic reactions fuelled by the nutritive substances digested in any animal. In silkworm, the food consumption has direct relevance to the weight of larva, cocoon, pupa and shell. However, these parameters of consumption and productivity will vary depending on the season, breeds and instars. Accumulation of nutrients in insect is greatly influenced by the nutritional richness of the host plant or diet fed and this storage function as the reservoirs for the supply both at the time of larval moult and during metamorphosis. Variation in the quantity or quality of nutrition can have profound effect on insect development [22] [23] [21] & [24]. The silkworm feeds voraciously at larval stages only and such abundant dietary intake serve as reserve matter during non-feeding phase of the development in the life cycle.

The fundamental understanding on the nutrition-gene interactions and its effect on economic traits in silkworm essential for evaluation of nutritionally efficient silkworm breeds. As dietary or nutrient factors and related metabolic interactions has direct and indirect influence on specific gene regulation and expression [25] & [26]. Such interactions and variations in the field of nutrigenetics could be applied to choose the silkworm breeds based on their nutritional efficiency parameters as biomarkers.

Development and utilization of artificial diet in Japan became possible only through the associated work of breeding, genetics and physiology revealed association with gene - interaction of an individual. The majority of silkworm germplasm breeds were evaluated based on the feeding habit and adaptability for the commercial rearing on artificial diet that can feed on low cost artificial diet lacking mulberry [11] & [14]. Further, it was established that silkworm derives over 70 per cent of the protein from the mulberry leaves and in 5th instar up to 96 per cent of ingested protein is used for silk protein synthesis and variation in the quantity or quality of nutrition have profound effect on insect development [27]. In sericulture, nutritional requirement and its conversion efficiency contribute directly or indirectly on the cost benefit ratio of silkworm rearing. It was considered as an important physiological criterion for evaluating the superiority of silkworm breeds In silkworm, 97 per cent of the total food intake during the last two instars and the feed utilization study confined to 5th instar larvae as 80-85 per cent of the total leaves consumed in this instar as silkworm very active metabolically at this stage [28]. Hence, the present study was chosen to conduct experiment to confine to 5th instar or stage of the silkworm rearing only. The feed efficiency or nutrigenetic traits, its genetic expression and its inheritance prototype in silkworm comprehensively discovered [12] [29] & [30].
### Table 2. Evaluation of nutrigenetic traits for overall rearing of selected bivoltine silkworm breeds

| Breeds   | I/larva (g.) | D/larva (g.) | E/larva (g.) | AD (%) | RR  | CI  | RGR | R (g.) | MR  |
|----------|--------------|--------------|--------------|--------|-----|-----|-----|--------|-----|
| BHR 2    | 7.53         | 5.23         | 2.30         | 69.73 (56.59) | 3.37 | 0.44 | 0.03 | 4.24   | 0.25 |
| BHR 3    | 7.42         | 5.15         | 2.26         | 69.89 (56.69) | 3.42 | 0.45 | 0.04 | 4.16   | 0.25 |
| B.con 1  | 7.56         | 5.05         | 2.51         | 67.30 (55.09) | 3.19 | 0.42 | 0.04 | 3.98   | 0.23 |
| B.con 4  | 7.56         | 5.19         | 2.37         | 69.09 (56.19) | 3.33 | 0.44 | 0.04 | 4.14   | 0.24 |
| ATR 16   | 6.94         | 4.88         | 2.06         | 70.58 (57.12) | 3.54 | 0.44 | 0.04 | 3.98   | 0.25 |
| ATR 29   | 8.90         | 6.62         | 2.28         | 75.50 (60.30) | 4.55 | 0.53 | 0.03 | 5.84   | 0.35 |
| DUN 6    | 8.74         | 6.82         | 1.92         | 78.59 (62.40) | 5.17 | 0.54 | 0.04 | 5.78   | 0.36 |
| DUN 22   | 8.47         | 6.38         | 2.08         | 75.76 (60.47) | 4.19 | 0.55 | 0.04 | 5.47   | 0.35 |
| CSR 2    | 10.51        | 8.06         | 2.45         | 75.37 (60.21) | 4.27 | 0.53 | 0.03 | 7.21   | 0.36 |
| CSR 6    | 8.91         | 6.80         | 2.11         | 76.30 (60.84) | 4.23 | 0.60 | 0.04 | 5.87   | 0.39 |
| CSR 50   | 7.89         | 5.54         | 2.34         | 69.98 (56.75) | 3.38 | 0.46 | 0.04 | 4.57   | 0.26 |
| CSR 51   | 9.18         | 6.97         | 2.22         | 75.66 (60.41) | 4.12 | 0.59 | 0.04 | 6.07   | 0.39 |
| JAM 2    | 7.21         | 5.61         | 1.59         | 78.05 (62.03) | 4.69 | 0.52 | 0.04 | 4.79   | 0.34 |
| JAM 121  | 7.85         | 5.77         | 2.08         | 74.11 (59.38) | 3.99 | 0.58 | 0.04 | 4.90   | 0.37 |
| RSJ 1    | 8.58         | 6.67         | 1.91         | 77.08 (61.36) | 4.44 | 0.58 | 0.04 | 5.88   | 0.39 |
| RSJ 14   | 8.01         | 5.87         | 2.14         | 74.09 (59.37) | 4.16 | 0.46 | 0.03 | 4.89   | 0.28 |
| SH6      | 8.47         | 6.54         | 1.93         | 76.92 (61.25) | 4.37 | 0.57 | 0.03 | 5.85   | 0.39 |
| NBxD2    | 7.83         | 5.63         | 2.20         | 72.50 (58.34) | 3.86 | 0.47 | 0.04 | 4.67   | 0.28 |
| CD @ 5%  | 0.01         | 0.01         | 0.01         | 0.01    | 0.01 | 0.01 | 0.01 | 0.007  | 0.01 |
| Sem±     | 0.005        | 0.005        | 0.005        | 0.003   | 0.005| 0.005| 0.002| 0.005  | 0.005|
| CV (%)   | 0.09         | 0.11         | 0.35         | 0.008   | 0.17 | 1.40 | 9.23 | 0.13   | 2.25 |

Note: I – Ingesta, D – Digesta, E – Excreta, AD – Approximate Digestibility, RR – Reference ratio, CI – Consumption Index, RGR – Relative Growth Rate, R – Respiration, MR – Metabolic rate; CD – Critical Difference, Se.m – Standard Error of Mean, CV – Coefficient of Variation; Values in parentheses are statistically transformed values.
Table 3. Nutritional efficiency conversion parameters for overall rearing of selected bivoltine silkworm breeds

| Breeds   | Larva (%) | Cocoon (%) | Shell (%) | Cocoon (g.) | Shell (g.) |
|----------|-----------|------------|-----------|-------------|------------|
|          | ECI       | ECD        | ECI       | ECD         | I/g        | D/g        | I/g        | D/g        |
| BHR 2    | 13.23 (21.32) | 18.96 (25.80) | 9.96 (18.39) | 14.46 (22.34) | 3.83 (11.27) | 5.50 (13.56) | 10.35 | 7.29 | 26.22 | 18.21 |
| BHR 3    | 13.67 (21.69) | 19.54 (26.22) | 9.60 (18.04) | 13.86 (21.85) | 4.04 (11.58) | 5.78 (13.90) | 10.71 | 7.52 | 24.90 | 17.31 |
| B.con 1  | 14.35 (22.25) | 21.12 (27.34) | 9.95 (18.38) | 15.03 (22.80) | 3.83 (11.27) | 5.71 (13.81) | 10.18 | 6.92 | 26.26 | 17.58 |
| B.con 4  | 14.44 (22.32) | 20.54 (26.94) | 9.45 (17.89) | 13.76 (21.77) | 3.68 (11.04) | 5.34 (13.34) | 10.83 | 7.48 | 27.32 | 18.79 |
| ATR 16   | 13.08 (21.19) | 18.38 (25.37) | 10.36 (18.76) | 14.87 (22.67) | 4.37 (12.05) | 6.22 (14.43) | 11.11 | 7.29 | 26.22 | 18.21 |
| ATR 29   | 9.20 (17.64) | 12.00 (20.26) | 7.16 (15.54) | 9.57 (18.00) | 3.04 (10.02) | 4.04 (11.58) | 13.98 | 10.57 | 33.03 | 24.83 |
| DUN 6    | 12.01 (20.27) | 15.12 (22.87) | 5.96 (14.11) | 7.63 (16.02) | 3.34 (10.52) | 4.23 (11.85) | 17.11 | 13.45 | 30.77 | 23.97 |
| DUN 22   | 10.97 (19.33) | 14.38 (22.27) | 7.19 (15.54) | 9.53 (17.97) | 3.39 (10.60) | 4.66 (12.18) | 13.97 | 10.60 | 30.03 | 22.65 |
| CSR 2    | 8.64 (17.08) | 11.64 (19.94) | 6.85 (15.16) | 9.23 (17.68) | 3.18 (10.26) | 4.27 (11.91) | 15.00 | 11.43 | 32.49 | 24.66 |
| CSR 6    | 10.78 (19.16) | 14.11 (22.06) | 7.47 (15.85) | 9.76 (18.19) | 3.17 (10.24) | 4.15 (11.74) | 13.78 | 10.49 | 31.74 | 24.20 |
| CSR 50   | 12.45 (20.65) | 17.85 (24.98) | 8.94 (17.38) | 12.80 (20.96) | 4.20 (11.82) | 6.04 (14.21) | 11.29 | 7.90 | 24.12 | 16.94 |
| CSR 51   | 9.73 (18.16) | 12.85 (20.99) | 7.38 (15.75) | 9.75 (18.18) | 3.19 (10.28) | 4.22 (11.84) | 13.87 | 10.48 | 31.58 | 23.92 |
| JAM 2    | 11.88 (20.14) | 15.09 (22.84) | 7.44 (15.82) | 9.52 (17.96) | 3.05 (10.04) | 3.90 (11.38) | 13.79 | 10.72 | 33.19 | 25.81 |
| JAM 121  | 11.46 (19.77) | 15.24 (22.96) | 6.68 (14.97) | 8.97 (17.42) | 2.86 (9.72) | 3.84 (11.29) | 15.71 | 11.56 | 35.85 | 26.32 |
| RSJ 1    | 9.72 (18.15) | 12.59 (20.77) | 6.43 (14.97) | 8.70 (17.14) | 2.54 (9.15) | 3.31 (10.47) | 15.33 | 11.84 | 39.53 | 30.54 |
| RSJ14    | 12.53 (20.72) | 16.71 (24.11) | 8.82 (17.27) | 12.08 (20.32) | 3.91 (11.39) | 5.28 (13.27) | 11.48 | 8.56 | 26.06 | 19.16 |
| SH 6     | 8.21 (16.64) | 10.68 (19.06) | 7.05 (15.39) | 9.15 (17.59) | 2.64 (9.33) | 3.44 (10.68) | 14.43 | 11.06 | 38.87 | 29.90 |
| NBX2     | 12.46 (20.66) | 17.07 (24.39) | 8.84 (17.29) | 12.38 (20.59) | 3.65 (11.01) | 5.05 (12.97) | 11.49 | 8.39 | 27.56 | 19.89 |

Note: ECI – Efficiency conversion for Ingesta, ECD - Efficiency conversion for Digesta; I – Ingesta; D -Digesta; Values in parentheses are statistically transformed values
Therefore, it was very much essential to analyze the nutrigenetic traits in growing larvae and useful in understanding the racial difference among the germplasm. However, even silkworm from the same genetic stock found to exhibit varied response when fed on the mulberry leaves of different nutritional quality, its growth being dependent on the efficient utilization and conversion of nutrition into silk substance [10]. In order to achieve greater success in this regard, it is important to understand the level of nutrition efficiency in bivoltine silkworm breeds. The main objective of this study was to identify bivoltine silkworm breeds with nutrition efficiency among eighteen bivoltine breeds evaluated for 19 important nutrigenetic traits. The results obtained for conversion index and efficiency of conversion of ingesta to biomass through standard gravimetric method during spring season (2018 & 2019) is supported by earlier observations [31] [32] [33] [34] & [35]. Our emphasis was on the phenotypic manifestation of 19 nutrigenetic traits. The results revealed highly significant ($p \leq 0.001$) variability among the bivoltine breeds with respect to nutrigenetic traits for the experimental selected breeds from different regions.

The average data for overall rearing for the year (2018 & 2019)spring and autumn seasons revealed that, the consumption index was very less in breeds viz., B.con 1 (0.42), B.con 4 (0.44), BHR 2 (0.44), ATR 16 (0.44), BHR 3 (0.45), CSR 50 (0.46), RSJ 14 (0.46) and NBxD2 (0.47) compared to other breeds. The efficiency conversion for ingesta to cocoon was highest in the breeds viz., ATR 16 (10.36), BHR 2 (9.96), B.con 1 (9.95), BHR 3 (9.60), B.con 4 (9.45), CSR 50 (8.94), NBxD2 (8.84) and RSJ 14 (8.82) and efficiency conversion for ingesta to shell was highest in the breeds viz., ATR 16 (4.37), CSR 50 (4.20), BHR 3 (4.04), RSJ 14 (3.91), BHR 2 (3.83), B.con 1 (3.83), B.con 4 (3.68) and NBxD2 (3.65). The ingesta per gram of the cocoon is very less compared to other breeds viz., ATR 16 (10.00), B.con 1 (10.18), BHR 2 (10.35), BHR 3 (10.71), B.con 4 (10.83), CSR 50 (11.29), RSJ 14 (11.48), NBxD2 (11.49) and the ingesta per gram of the shell is very less compared to other breeds viz., ATR 16 (23.46), CSR 50 (24.12), BHR 3 (24.90), RSJ 14 (26.06), BHR 2 (26.22), B.con 1 (26.26), B.con 4 (27.32), NBxD2 (27.56) and all the parameters taken for the study showed stastically significantly different from each other. Similar study was investigated through systematic nutrigenetic traits analysis for bivoltine silkworm germplasm breeds achieved as first report of this kind and utilized as biomarkers to identify nutritionally efficient breed, which opt the lower consumption and higher conversion of nutrition into cocoon and shell, would attract the attention of the sericulturists. Henceforth, the study sturdily suggested the four bivoltine silkworm germplasm breeds viz., RBD1, RBD4 (peanut type cocoon), RBO2, RBO3 (oval type cocoon) as nutritionally efficient breeds were shortlisted based on the nutrigenetic traits analysis for future synthesis of nutritionally efficient hybrids in hybridization program [16]. Similar study was reported for polyvoltine breeds concluded that, polyvoltine breeds with minimum consumption index and maximum efficiency of conversion of ingesta/cocoon identified strains Rmg4, RMW2, and RMW3 as potential polyvoltine breeding resource material for the development of nutritionally efficient breeds/hybrids in Asia and Pacific regions [36]. Similar results were observed from the present study that bivoltine breeds with minimum consumption index and maximum efficiency of conversion of ingesta/cocoon identified/shortlisted as breeds viz., BHR 2, BHR 3, B.con 1, B.con 4, CSR 50, ATR 16, NBxD2 and RSJ 14 as potential bivoltine breeding resource material for the development of nutritionally efficient breeds under North Western India during spring and autumn season (2018 & 2019) respectively.

The result obtained from the study revealed a highly significant variance on nutritional traits between the breeds and within the breed during different seasons as reported [37] & [38]. Comparatively quite variable digesta among all the breeds might be due to variation in genetic components and agreement on degree of food digestion in silkworm differs from one race to another when fed on same variety of mulberry leaves [31]. Accumulation of nutrients in insect greatly influenced by the nutritional richness of the host plant or diet fed and this storage nutrition function as the reservoirs for the supply both at the time of larval moult and during metamorphosis. The silkworm feeds voraciously at larval stages only and such abundant dietary intake acts as reserve during non feeding phase of development in the life cycle. Further, it was also observed that increase in ingestion and digestion suggests the possibility of increase in the accumulation of organic constituents in the body tissue of the silkworm as biomass but it varied among breeds in this study. Maximum excreta obtained in breeds than those of other silkworm breeds indicate that breeds were more efficient on biomass conversion of ingested and
digested food. The Approximate Digestibility (AD) analyzed in the study stated that it was a racial trait as higher food intake does not necessarily result in higher digestibility. Reference Ratio (RR) as indicative of the retention efficiency of food reported between 1.56-1.59 in silkworm larvae except in one breed in concord with the studies [39] & [40]. The passage of food through gut became slow when consumption index decreases to facilitate increased digestion and assimilation with ultimate result of improved approximate digestibility and corresponding traits. It was registered that the consumption index values of polyvoltine was slightly higher when compared to the bivoltine breeds may be due to less 5th stage larval duration [28]. A slant variation observed in relative growth rate between the breeds was due to less difference in larval duration among bivoltine germplasm breeds. It also noted firmly that bivoltine breeds of semi exotic races found to be better converter than the polyvoltine breeds of indigenous races in harmony to prior observation [41].

5. CONCLUSION

From the present study, eighteen bivoltine silkworm breeds were procured and subjected for investigation on ingestion, digestion and utilization of dry food matter in single silkworm larva and it’s sharing on economically important stages of larva, cocoon and shell weight in 5th stage. It was resolved that low consumption with high conversion efficiency of food ingested in silkworm breeds on nineteen nutrigenetic traits was analyzed. The screening of selected 18 breeds from different institutes for nutrigenetic traits were conducted by rearing during spring and autumn season (2018 & 2019) respectively and the average data from the two years, 8 breeds were shortlisted viz., BHR 2, BHR 3, B.con 1, B.con 4, CSR 50, ATR 16, NB.D2 and RSJ 14 based on desired nutrigenetic traits i.e. Consumption Index, Efficiency conversion for Ingesta to Larva, Efficiency conversion for Ingesta to Cocoon & Efficiency conversion for Ingesta to Shell and Ingesta per gram of cocoon & Shell was having better results compared to other breeds. The availability of these shortlisted nutrigenetic breeds can be utilized for development of nutrigenetic specific hybrids through hybridization programme will increase the quality cocoon production under Subtropical condition of North West India.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Legay JM. Recent advances in silkworm nutrition. Ann. Rev. Ent. 1958;3:75-86.
2. Hiratsuka E. Researches on the nutrition of silkworm. Bulletin of the Sericultural Experiment Station.1920;1:257-315.
3. Nakano, Monsi. An experimental approach to some quantitative aspects of grazing by silkworm (B. mori). Jap. J. Ecol.1968;18:212-229.
4. Horie Y, Inokuchi T, Watanabe K, Nakasone S, Yanagaira H. Quantitative study on food utilization by the silkworm, Bombyx mori through its life cycle, 1: Economy of dry matter, energy and carbon. Bull. Sencult. Exp. Sta. 1976;26:411-442.
5. Takano K, Arai N. Studies on the food values on the basis of feeding and cocoon productivity in the silkworm, Bombyx mori. Seric. Sci. Jap. 1978;47:134-142.
6. Scriber JM, Slansky F. The nutritional ecology of immature insects. Annual Review of Entomology. 1981;26:183-211.
7. Ito T. Amino acid nutrition of the silkworm, Bombyx mori. Proc. Jap. Acad. 1972;48:613-617.
8. Ueda S. Theory of the growth of silkworm larvae and its application. JARQ. 1982; 15(3):180-184.
9. Dhar A, Chauhan TPS, Sardar Singh, Ahmad MN, Khan MA. Strategies for development of bivoltine sericulture in North India. Asian textile J.2009;18(1):75-77.
10. Hamano K, Miyazawa K, Mukiayama F. Racial difference in the feeding habit of the silkworm, Bombyx mori. J. Sericol. Sci. Jap. 1986;55:68-72.
11. Mano Y, Ashoka K, Ihara O, Nakagawa H, Hirabayashi T, Murakami M, Nagayashu K. Breeding and evaluation of adaptability of silkworm, Bombyx mori to new low cost artificial diet, LPY lacking mulberry leaf powder. Bull. Nat. Inst. Seric. Entomol. Sci. 1991;3:31-56.
12. Ding N, Zhang XM, Jiang MO, Xu WH, Wang ZE, Xu MK. Genetical studies on the dietary efficiency of the silkworm, Bombyx mori L. CanveKexue. 1992;18:71-76.
13. Tzenov P, Petkov N, Natcheva, Y. Study on the inheritance of food ingestion and digestion in hybrids between univoltine and multivoltine silkworm, *Bombyx mori* L. races. Sericologia. 1999; 39:171-177.

14. Zhang YH, Xu YW, Wei YD, Li MW, Hou CX, Zhang GZ. Studies on feeding habits of silkworm germsplasm resources for artificial diet without mulberry. Acta Sericologia Sinica. 2002; 28:333-336.

15. Rahmathulla VK, Mathur VB and Geethadevi RG. Growth and dietary efficiency of mulberry silkworm (*Bombyx mori* L) under various nutritional and environmental stress conditions. Philippines Journal of Science. 2004;33: 39-43.

16. Ramesh C, Anuradha CM, Lakshmi H, Sujana Kumari S, Seshagiri SV, Goel AK, Suresh Kumar C. Nutrigenetic traits analysis for identification of nutritionally efficient silkworm germplasm breeds. Biotechnology. 2010; 9:131-140.

17. Maynard AL, Loosli KJ. Animal Nutrition, 5th Edition. McGraw Hill; 1962.

18. Waldbauer GP. The consumption and utilization rate of food by insects. Advanced Insect Physiology. 1968; 5:229-288.

19. Slicher JM, Feeny P. (1979). Growth of herbivorous caterpillars in relation to feeding specialization and to the growth form of their food plant. Ecology, 1979; 60: 829-850.

20. Kogan M, Parra JR P. Techniques and applications of measurements of consumption and utilization of feed by phytophagous insects. In: Bhaskaran G, Friedman S, Rodrigues JG, Editors. Current Topics in Insect Endocrinology and Nutrition. Plenum Press.1981;337-352.

21. Slansky F, Slicher JM. Food consumption and utilization. In: Kerkut, A. A., Gilbert, L. I., Editors. Comprehensive Insect Physiology, Biochemistry and Pharmacology. Pergamon Press. 1985;87-163.

22. Horie Y, Watanabe K. Effect of various kinds of dietary protein and supplementation with limiting amino acids on growth, haemolymph components and uric acid excretion in the silkworm, *Bombyx mori*. J. Insect Physiol. 1983a; 29:187-199.

23. Horie Y, Watanabe K. Effects of dietary pyridoxine on larval growth, free amino acid pattern in haemolymph and uric acid excretion in the silkworm, *Bombyx mori*. Insect Biochem. 1983b;13:205-212.

24. Chapman RF. The insects: Structure and function. 4th ed. Cambridge, UK; New York: Cambridge University Press. 1998;17:770.

25. Iftikhar T, Hussain A. Effect of nutrients on the extracellular lipase production by mutant strain of *Rhizopus ologosporous*TU-31. Biotechnology. 2002; 1:15-20.

26. Phillips CN, Tierney AC, Roche HM. Gene-nutrient interactions in the metabolic syndrome. J. Nutrigenet. Nutrigenomics. 2008;1:136-151.

27. Fukuda T, Kamegame T, Matsuda MA. correlation between the mulberry leaves consumed by the mulberry silkworm larvae in different ages of the larval growth and production of cocoon fiber spun by silkworm larvae and the eggs laid by the silkworm. Bull. Sericol. Exp. Stn.1963;8: 165-171.

28. Rahmathulla VK, HaqueRufaie SZ, Himanthraj MT, Vindhya GS, Rajan RK. Food ingestion, assimilation and conversion efficiency of mulberry silkworm, *Bombyx mori* L. International Journal of Industrial Entomology. 2005;11:1-12.

29. Tzenov P, Natcheva Y, Petkov N. Phenotypic correlations between the traits characterizing the food ingestion, digestion and utilization and the most important quantitative feeding traits in silkworm, *Bombyx mori* L. Genet. Breed. 1995;27: 50-50.

30. Tzenov P, Petkov N, Natcheva Y. A study on the amount of consumed and digested mulberry leaf and inheritance in F generation for different breeds of silkworm (*Bombyx mori* L). Anim. Sci. 1997; 34:129-131.

31. Hassanein MH, El-Shaaraway MF, El-Garthy AT. Food assimilation and output of the silk in the different races of the silkworm, *Bombyx mori*. Bull. Soc. Environ. Egypt. 1972;56:333-337.

32. Sumioka HS, Kuroda H, Yoshitake N. Feed efficiency and expression of several characters of the silkworm, *Bombyx mori*, under restricted feeding. Journal of Sericultural Science of Japan. 1982;51: 415-419.

33. Anantharaman KV, Magadum SB, Datta RK. Feed efficiency of silkworm, *Bombyx mori* L. hybrid (NB×KA). Insect ScienceApplication. 1994;15:111-116.
34. Trivedy K, Nair, KS. Feed conversion efficiency of improved multi × bivoltine hybrids of silkworm, *Bombyx mori* L. Indian Journal of Sericulture. 1999;38:30-34.

35. Kumaresan P, Sinha RK, Sahni NK, Sekar S. Genetic variability and selection indices for economic quantitative traits of multivoltine mulberry silkworm, *Bombyx mori* L. genotypes. Sericologia. 2000;40:595-605.

36. Ramesha C, Lakshmi H, Kumari SS, Anuradha CM, Kumar CS. Nutrigenetic screening strains of the mulberry silkworm, *Bombyx mori*, for nutritional efficiency. Journal of Insect Science. 2012;12(3):1-18.

37. Magadum SB, Ramadevi OK, Shivashankar N, Datta RK. Nutritional indices in some bivoltine breed of silkworm, *Bombyx mori* L. Indian J. Sericol. 1996;35:95-98.

38. Gokulamma K, Reddy YS. Role of nutrition and environment on the consumption, growth and utilization indices of selected silkworm races of *Bombyx mori* L. Indian Journal of Sericulture. 2005;44:165-170.

39. Anantharaman KV, Mala VR, Magadum SB, Datta RK. Effect of season and mulberry varieties on the feed conversion efficiencies of different silkworm hybrids of *Bombyx mori* L. Uttar Pradesh J. Zool. 1995;15:157-161.

40. Rahmathulla VK, Vindy GS, Sreenivasa G, Geethadevi RG. Evaluation of the consumption and nutritional efficiency in three new bivoltine hybrids (CSR series) of silkworm *Bombyx mori* L. J. Exp. Zool. 2003;6:157-161.

41. Periaswamy K, Prakash R, Radhakrishnan S. Food utilization in exotic and indigenous races of *Bombyx mori* L. (Lepidoptera: Bombycidae). Sericologia. 1984;24:43-50.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/62358