To Workout Nutrigenetic Traits for Silkworm, *Bombyx mori* L. by Absorption/Assimilation for Determining Growth and Development for Identifying Parental Breeds for Future Breeding during Spring Season under Subtropical Region of North West India

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Authors' contributions

This work was carried out in collaboration between both authors. Author SM contributed the sample collection, sample analysis and drafted the manuscript. Author SS guided all the analysis part and also helped with the drafting the manuscript and approved final manuscript.

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ABSTRACT

Study was conducted for screening and evaluation of selected breed's for 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 North Western India based on the breeds which shows less food consumption with higher efficiency conversion. The aim of this study was to identify nutritionally efficient bivoltine silkworm breeds selected from different regions of our country. Highly significant differences were found among all nutrigenetic traits of bivoltine silkworm breeds in the study. The nutritionally efficient silkworm breeds were resulted by utilizing nutrition consumption index and efficiency for conversion of ingesta/cocoon traits as the index for selection of highly promising breeds. Higher nutritional efficiency conversions were found in the bivoltine silkworm breeds on efficiency of conversion of

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ingesta to cocoon and shell were shortlisted during spring season. Furthermore, based on the overall 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_4D_2) 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.

1. INTRODUCTION

The silkworms are very important economic insect which contributes substantially to the national economy and Gross Domestic Production (GDP) of many countries like China, India, Thailand etc [1,2]. The mulberry silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) is a monophagous insect that feeds exclusively on the mulberry (*Morus* spp.) foliage for its nutrition and produces the natural proteinous silk [3]. Silkworm larvae obtain 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 [4]. The capacity of silkworm, to ingest mulberry leaf, digest, absorb, assimilate and convert it to silk fibre also differs from race to race [5]. 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 [6,7, 8,9].

Intensive and cautious domestication over centuries has apparently privileged this commercial insect the opportunity to increase in nutrition efficiency. Nutritional intake has direct impact on the overall genetic traits such as larval and cocoon weight, amount of silk production, pupation, and reproductive traits. The sericulture activity is declining due to the reduction of mulberry production area in sericulture practicing countries on silkworm rearing and silk production. 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 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 [10-16].

Nutritional ecology of insects is the prerequisite for a better knowledge on their ethobiology and physiology, which is often been neglected [17]. 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 [18]. 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 [19] and 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 [20]. As of now the production is about 40 Kg/100 Dfls with renditta of 7.50 Kg. The present project can be defined as though a number of bivoltine breeds/hybrids have been developed by different R&D institutes with high productivity. 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 [10-16]. Therefore, it is necessary to identify silkworm breeds/hybrids 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 was conducted at the Regional Sericultural Research Station, Miran Sahib, Jammu (J & K) where the selected mulberry variety (S-146) is 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/hybrid for Subtropical condition of North Western India during spring season (2018 & 2019).

2.1 Breeds

The silkworm bivoltine breeds/ germplasm breeds was selected based on commercial characteristics and geographical drift of the breeds like BHR2, BHR3, B.con1, B.con4 (CSR&TI,Berhampur), DUN6, DUN22, ATR16, ATR29,SH$_6$, NB$_4$D$_2$ (RSRS, Dehradun), CSR2, CSR6, CSR50, CSR51 (CSR&TI, Mysore), JAM2, JAM121, RSJ1 and RSJ14 (RSRS, 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 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 [21]. 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 [22-25], the equations with brief description of the nutrigenetic traits evaluated was given below.

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).

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).

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

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	Grey	W	P	O	W	H	M
6	DUN 22	Sericultural	Grey	W	M	C	W	H	M
7	ATR 16	Research Station,	Grey	W	P	O	W	H	M
8	ATR 29	Sahaspur,	Grey	W	M	C	W	H	M
9	SH_6	Dehradun	Grey	W	M	O	W	H	M
10	NB_4D_2	(Uttarakhand)	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	Grey	W	M	C	W	H	M
16	JAM 121	Sericultural	Grey	W	P	O	W	H	M
17	RSJ1	Research Station,	Grey	Y	M	C	W	H	M
18	RSJ14	Miran Sahib 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; EC- Elongated Constriction); CC- Cocoon color (W- White); B - Build (H - Hard); G - Grain (M - Medium)

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).

Reference ratio: An indirect expression of absorption and assimilation of food. Expresses the ingesta required per unit excreta produced: (RR= Dry weight of food ingested /Dry weight of excreta).

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).

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).

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).

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).

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).

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).

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).

Efficiency conversion of digesta to cocoon (%): It was the expression for efficiency conversion of digesta into cocoon: (ECD cocoon = Dry weight of cocoon / Dry weight of digesta × 100).

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: (ECI shell = Dry weight of shell / Dry weight of ingesta × 100).

Efficiency conversion of digesta to shell (%): The expression of efficiency conversion of digesta into shell: (ECD shell = Dry weight of shell / Dry weight of digesta × 100).

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: (I/g cocoon = Dry weight of ingesta / Dry weight of cocoon).

Digesta per gram cocoon (g): The total digesta requisite for the production of one gram of cocoon: (D/g cocoon = Dry weight of digesta / Dry weight of cocoon).

Ingesta per gram shell (g): The total ingesta requisite for the production of one gram of shell: (I/g shell = Dry weight of ingesta / Dry weight of shell).

Digesta per gram shell (g): The total digesta requisite for the production of one gram of shell: (D/g shell = Dry weight of digesta / 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 ANOVA through available statistical tools.

3. RESULTS

3.1 Performance on Nutrigenetic Traits

Considerable differences were found for 19 nutrigenetic traits among the 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.

Ingesta, digesta, excreta, AD and RR: During spring season (2018) the data was recorded for ingesta ranged between highest in CSR2 (15.01 g) and lowest in ATR16 (5.62 g) whereas the maximum digesta recorded in CSR2 (12.26 g) with minimum in ATR16 (3.77 g) whereas highest excreta recorded in CSR2 (2.74 g) and lowest in JAM2 (1.64 g). Approximate digestibility ranged between CSR2 (81.73%) to ATR16 (67.21%) and the reference ratio with maximum value in CSR2 (5.47) and minimum in ATR16 (3.05) (Table 2). All the parameters were showed statistically significant differences among them.

All the parameters were found statistically significant differences during spring season (2019), the observation recorded for ingesta ranged between maximum in CSR2 (10.98 g) and lowest in BHR3 (7.58 g). The maximum digesta recorded in CSR2& RSJ1 (8.74 g) with minimum in BHR3 (5.15 g) whereas highest excreta recorded in B.con1 (3.03 g) and lowest in JAM2 (1.64 g). Approximate digestibility ranged between RSJ1 (80.26%) to B.con1 (63.41%) and the reference ratio with maximum value in RSJ1 (5.06) and minimum in B.con1 (2.73) (Table 4).

CI, RGR, Respiration and MR: During spring season (2018) the Consumption Index (CI) found to be highest in CSR2 & CSR51 (0.62) and lowest in ATR16 (0.40). There was significant difference observed in Relative Growth Rate (RGR) with maximum in DUN22 (0.05) and minimum in JAM121 & RSJ14 (0.02). A momentous difference were recorded with respect to respiration and found relatively more in CSR2 (11.33 g) and less in ATR16 (3.06 g). There is distinguishable divergence for Metabolic Rates (MR) among bivoltine selected breeds which ranged between maximum in CSR2 (0.47) and lowest in ATR16 (0.22) (Table 2).

During spring season (2019) the Consumption Index (CI) recorded to be highest in RSJ1 (0.53) and lowest in B.con1(0.36) whereas there was significant difference observed in Relative Growth Rate (RGR) with maximum in BHR3,

CSR50, NB_4D_2 & CSR51 (0.05) and minimum in ATR29, CSR2 & SH_6 (0.02). A momentous difference were recorded with respect to respiration and found relatively more in CSR2 (7.78 g) and less in BHR3 (3.67 g). There is distinguishable divergence for Metabolic Rates (MR) among bivoltine selected breeds which ranged between maximum in RSJ1 (0.37) and lowest in B.con1 (0.17) (Table 4).

ECI and ECD to larval biomass: During spring season (2018) the data recorded 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 ATR16 (12.64%) and lowest in CSR2 (6.20%). 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 inATR16 (18.81%) and less efficient in CSR2 (7.58%).

During spring season (2019) the observation noted for efficiency of mulberry leaves ingested and converted into silkworm larval biomass varied significantly among the selected bivoltine breeds (Table 5). The highest efficiency conversion of ingesta (ECI) to larva recorded in BHR3 (19.53%) &lowest in ATR29 (7.74%). 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 BHR3 (28.74%) and less efficient in ATR29 (10.80%).

ECI and ECD to cocoon and shell: During spring season (2018) the data recorded for Efficiency Conversion of Ingesta (ECI) to cocoon revealed that higher in ATR16 (13.39%) followed by B.con 1 (9.49%) and lower in CSR2 (5.41%). Efficiency Conversion of Digesta (ECD) to cocoon varied significantly among the breeds which ranged between 19.93 *per cent* (ATR16) to 6.62 *per cent* (CSR2). ECI to shell observed to be maximum in ATR16 (5.38%) and minimum in JAM121 (2.28%). With regard to ECD for shell high conversion rate was recorded in ATR16 (8.00%) followed by B.con 1 (6.15%) and lowest in CSR 2 (3.15) (Table 3).

Table 2. Evaluation of nutrigenetic traits for selected bivoltine silkworm breeds (Spring, 2018)

Breeds	I/larva (g.)	D/larva (g.)	E/larva (g.)	AD (%)	RR	CI	RGR	R (g.)	MR
BHR 2	7.95	5.71	2.23	71.89 (57.95)	3.56	0.46	0.03	4.92	0.29
BHR 3	8.34	6.04	2.30	72.36(58.25)	3.62	0.50	0.03	5.26	0.31
B.con 1	8.28	5.63	2.65	67.96 (55.50)	3.12	0.43	0.03	4.76	0.25
B.con 4	8.10	5.67	2.43	70.02 (56.77)	3.34	0.47	0.03	4.93	0.29
ATR 16	5.62	3.77	1.84	67.21(55.04)	3.05	0.40	0.04	3.06	0.22
ATR 29	9.82	7.45	2.37	75.84(60.53)	4.14	0.55	0.03	6.69	0.38
DUN 6	8.45	6.33	2.11	75.01 (59.98)	4.00	0.56	0.04	5.56	0.37
DUN 22	8.70	6.62	2.08	76.11 (60.71)	4.19	0.56	0.05	5.78	0.37
CSR 2	15.01	12.26	2.74	81.73 (64.66)	5.47	0.62	0.03	11.33	0.47
CSR 6	9.51	7.20	2.30	75.78(60.49)	4.13	0.55	0.03	6.46	0.37
CSR 50	8.79	6.40	2.38	72.86 (58.58)	3.68	0.49	0.04	5.52	0.31
CSR 51	9.98	7.60	2.38	76.15 (60.74)	4.19	0.62	0.04	6.85	0.42
JAM 2	6.00	4.36	1.64	72.74 (58.49)	3.67	0.53	0.04	3.81	0.34
JAM 121	8.53	5.87	2.65	68.88 (56.07)	3.21	0.56	0.02	5.15	0.34
RSJ 1	6.62	4.75	1.87	71.76 (57.87)	3.54	0.53	0.03	4.29	0.34
RSJ 14	8.68	6.36	2.32	73.23 (58.81)	3.74	0.51	0.02	5.62	0.33
SH_6	8.92	6.89	2.03	77.25 (61.48)	4.40	0.54	0.03	6.32	0.38
NB_4D_2	8.65	6.30	2.35	72.79 (58.53)	3.68	0.51	0.03	5.54	0.32
CD @5%	0.07	0.01	0.01	0.01	0.01	0.02	0.06	0.02	0.02
SE.m±	0.02	0.006	0.006	0.004	0.006	0.007	0.002	0.007	0.007
CV (%)	0.39	0.14	0.38	0.010	0.23	1.92	9.03	0.17	2.98

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 in selected bivoltine silkworm breeds (Spring, 2018)

Breeds	Larva (%)		Cocoon (%)		Shell (%)		Cocoon (g.)		Shell (g.)	
	ECI	ECD	ECI	ECD	ECI	ECD	I/g	D/g	I/g	D/g
BHR 2	9.94 (18.37)	13.83 (21.81)	8.91 (17.36)	12.39 (20.59)	3.88 (11.35)	5.39 (13.41)	11.22	8.07	25.80	18.55
BHR 3	9.35 (17.79)	12.92 (21.05)	8.92 (17.37)	12.33 (20.54)	3.96 (11.47)	5.47 (13.52)	11.21	8.11	25.27	18.29
B.con 1	10.51 (18.90)	15.46 (23.14)	9.49 (17.93)	13.97 (21.93)	4.18 (11.79)	6.15 (14.35)	10.53	7.16	23.93	16.26
B.con 4	9.14 (17.59)	13.06 (21.17)	8.08 (16.50)	11.54 (19.85)	3.48 (10.73)	4.98 (12.88)	12.38	8.67	28.71	20.10
ATR 16	12.64 (20.81)	18.81 (25.68)	13.39 (21.45)	19.93 (26.50)	5.38 (13.40)	8.00 (16.42)	7.47	5.02	18.59	12.50
ATR 29	7.74 (16.14)	10.20 (18.61)	6.99 (15.31)	9.21 (17.66)	3.18 (10.26)	4.19 (11.80)	14.31	10.86	31.47	23.87
DUN 6	9.12 (17.57)	12.16 (20.40)	7.41 (15.78)	9.88 (18.31)	3.29 (10.44)	4.39 (12.09)	13.49	10.12	30.38	22.79
DUN 22	9.66 (18.10)	12.69 (20.85)	7.15 (15.49)	9.40 (17.84)	3.22 (10.32)	4.23 (11.86)	13.98	10.64	31.05	23.63
CSR 2	6.20 (14.41)	7.58 (15.97)	5.41 (13.44)	6.62 (14.90)	2.57 (9.22)	3.15 (10.21)	18.48	15.10	38.87	31.77
CSR 6	7.79 (16.20)	10.27 (18.68)	6.71 (15.00)	8.86 (17.31)	3.28 (10.43)	4.33 (12.00)	14.90	11.29	30.46	23.09
CSR 50	10.02 (18.44)	13.75 (21.75)	7.97 (16.38)	10.94 (19.30)	3.73 (11.13)	5.12 (13.07)	12.55	9.14	26.78	19.51
CSR 51	7.52 (15.90)	9.87 (18.30)	6.52 (14.78)	8.56 (17.00)	3.19 (10.28)	4.19 (11.80)	15.35	11.69	31.37	23.89
JAM 2	9.17 (17.61)	12.60 (20.77)	6.87 (15.18)	9.44 (17.88)	2.90 (9.79)	3.99 (11.51)	14.56	10.59	34.48	25.08
JAM 121	8.45 (16.88)	12.26 (20.48)	5.44 (13.48)	7.90 (16.31)	2.28 (8.67)	3.30 (10.45)	18.37	12.66	43.94	30.27
RSJ 1	6.95 (15.27)	9.69 (18.13)	6.89 (15.20)	9.61 (18.05)	2.69 (9.42)	3.75 (11.15)	14.51	10.41	37.16	26.67
RSJ 14	8.53 (16.96)	11.64 (19.93)	7.83 (16.23)	10.70 (19.08)	3.32 (10.49)	4.53 (12.28)	12.76	9.35	30.14	22.07
SH_6	6.39 (14.63)	8.27 (16.70)	7.58 (15.96)	9.81 (18.24)	3.07 (10.08)	3.98 (11.49)	13.20	10.19	32.55	25.15
NB_4D_2	8.79 (17.23)	12.07 (20.31)	7.79 (16.19)	10.70 (19.08)	3.38 (10.58)	4.64 (12.42)	12.83	9.34	29.62	21.56
CD @5%	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.02	0.01
SE.m±	0.005	0.005	0.006	0.005	0.008	0.007	0.006	0.005	0.008	0.005
CV (%)	0.04	0.03	0.05	0.03	0.10	0.07	0.06	0.07	0.03	0.03

Note: ECI – Efficiency conversion for Ingesta, ECD - Efficiency conversion for Digesta; Values in parentheses are statistically transformed values

Efficiency Conversion of Ingesta (ECI) to cocoon revealed that higher in BHR3 (12.53%) followed by BHR2 (12.34%) and lower in CSR2 (6.28%). Efficiency Conversion of Digesta (ECD) to cocoon varied significantly among the breeds which ranged between 18.45 *per cent* (BHR3) to 6.62 *per cent* (DUN6). ECI to shell observed to be maximum in BHR3 (3.93%) and minimum in SH_6 (2.14%). With regard to ECD for shell high conversion rate was recorded in BHR3 (5.79%) followed by B.con 1 (5.71%) and lowest in SH_6 (2.69) during spring season (2019**)** (Table 5).

Table 4. Evaluation of nutrigenetic traits for selected bivoltine silkworm breeds (Spring, 2019)

Breeds	I/larva (g.)	D/larva (g.)	E/larva (g.)	AD (%)	RR	CI	RGR	R (g.)	MR
BHR 2	7.94	5.37	2.57	67.67 (55.33)	3.09	0.38	0.03	3.94	0.19
BHR 3	7.58	5.15	2.43	67.94 (55.49)	3.12	0.40	0.05	3.67	0.19
B.con 1	8.28	5.25	3.03	63.41 (52.75)	2.73	0.36	0.04	3.86	0.17
B.con 4	8.09	5.77	2.32	71.28 (57.57)	3.48	0.40	0.04	4.37	0.22
ATR 16	8.28	6.07	2.21	73.28 (58.85)	3.74	0.41	0.03	4.79	0.24
ATR 29	9.82	7.04	2.78	71.65 (57.80)	3.53	0.48	0.02	6.28	0.30
DUN 6	9.98	7.70	2.28	77.15 (61.41)	4.38	0.47	0.04	6.14	0.29
DUN 22	9.88	7.64	2.24	77.35 (61.54)	4.41	0.47	0.03	6.30	0.30
CSR 2	10.98	8.74	2.24	79.64 (63.15)	4.91	0.47	0.02	7.78	0.33
CSR6	9.5	7.43	2.07	78.18 (62.12)	4.58	0.49	0.03	6.24	0.32
CSR 50	8.78	6.50	2.28	74.02 (59.33)	3.85	0.39	0.05	5.29	0.24
CSR 51	9.97	7.67	2.30	76.93 (61.27)	4.33	0.45	0.05	6.22	0.28
JAM 2	7.90	6.26	1.64	79.19 (62.83)	4.80	0.46	0.04	5.07	0.29
JAM 121	7.96	6.26	1.70	78.64 (62.44)	4.68	0.48	0.04	5.03	0.30
RSJ 1	10.89	8.74	2.15	80.26 (63.59)	5.06	0.53	0.03	7.71	0.37
RSJ14	8.68	6.50	2.18	74.85 (59.87)	3.98	0.41	0.04	4.99	0.23
SH_6	9.92	7.88	2.04	79.41 (62.99)	4.86	0.47	0.02	6.94	0.33
NB_4D_2	8.65	6.31	2.34	72.97 (58.65)	3.70	0.41	0.05	4.92	0.23
CD@5%	0.14	0.23	0.04	0.09	0.70	0.09	0.004	0.20	0.01
Sem±	0.04	0.08	0.01	0.03	0.23	0.03	0.001	0.06	0.005
CV (%)	0.76	1.65	1.03	0.07	8.22	9.70	4.65	1.73	1.99

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

I/g and D/g to cocoon and shell: During spring season (2018) the ingesta per gram cocoon (I/g) was observed it ranged between maximum in CSR2 (18.48 g) and minimum in ATR16 (7.47 g). The amount of digesta per gram cocoon (D/g) observed was highest in CSR2 (15.10 g) and lowest in ATR16 (5.02 g). With regard to ingesta per gram (I/g) shell was noticed to be ranged between JAM121 (43.94 g) and lowest in ATR16 (18.59 g). The digesta required to one gram (D/g) shell revealed it ranged between maximum in CSR2 (31.77 g) and minimum in ATR16 (12.50 g) (Table 3). All the parameters were showed statistically significant differences among them.

Table 5. Nutritional efficiency conversion parameters in selected bivoltine silkworm breeds (Spring, 2019)

Breeds	Larva (%)		Cocoon (%)		Shell (%)		Cocoon (g.)		Shell (g.)	
	ECI	ECD	ECI	ECD	ECI	ECD	I/g	D/g	I/g	D/g
BHR 2	18.01 (25.10)	26.62 (31.04)	12.34 (20.55)	18.24 (25.27)	3.65 (11.01)	5.4 (13.42)	8.10	5.48	27.38	18.53
BHR 3	19.53 (26.21)	28.74 (32.40)	12.53 (20.72)	18.45 (25.42)	3.93 (11.42)	5.79 (13.91)	7.98	5.42	25.44	17.28
B.con 1	16.79 (24.18)	26.47 (30.95)	11.47 (19.78)	18.09 (25.16)	3.62 (10.96)	5.71 (13.81)	8.72	5.53	27.60	17.50
B.con 4	17.31 (24.57)	24.28 (29.50)	11.99 (20.25)	16.82 (24.20)	3.58 (10.90)	5.03 (12.95)	8.34	5.94	27.9	19.88
ATR 16	15.46 (23.14)	21.09 (27.32)	10.63 (19.02)	14.50 (22.37)	3.74 (11.13)	5.11 (13.05)	9.41	6.90	26.71	19.56
ATR 29	7.74 (16.14)	10.80 (19.17)	6.95 (15.27)	9.69 (18.12)	2.77 (9.56)	3.87 (11.34)	14.4	10.32	36.10	25.87
DUN 6	15.63 (23.27)	20.26 (26.74)	5.11 (13.05)	6.62 (14.90)	3.57 (10.87)	4.62 (12.40)	19.57	15.10	28.03	21.63
DUN 22	13.56 (21.59)	17.53 (24.74)	6.38 (14.62)	8.24 (16.67)	3.32 (10.49)	4.29 (11.94)	15.68	12.13	30.12	23.30
CSR 2	8.74 (17.18)	10.98 (19.34)	6.28 (14.50)	7.89 (16.30)	3.11 (10.15)	3.91 (11.39)	15.91	12.67	32.11	25.57
CSR 6	12.53 (20.72)	16.02 (23.58)	9.85 (18.28)	12.60 (20.78)	3.43 (10.66)	4.39 (12.08)	10.15	7.94	29.14	22.78
CSR 50	13.78 (21.78)	18.62 (25.55)	10.18 (18.59)	13.76 (21.76)	3.85 (11.31)	5.20 (13.17)	9.82	7.27	25.98	19.23
CSR 51	14.54 (22.40)	18.91 (25.76)	9.45 (17.76)	12.28 (20.50)	3.23 (10.34)	4.20 (11.82)	10.58	8.14	30.96	23.82
JAM 2	15.06 (22.82)	19.02 (25.84)	8.99 (17.44)	11.35 (19.68)	2.99 (9.95)	3.77 (11.19)	11.13	8.81	33.47	26.51
JAM 121	15.45 (23.13)	19.65 (26.30)	9.42 (17.86)	11.98 (20.24)	3.42 (10.65)	4.35 (12.03)	10.61	8.35	29.26	23.01
RSJ 1	9.46 (17.90)	11.79 (20.07)	6.52 (14.78)	8.12 (16.55)	2.30 (8.71)	2.86 (9.73)	15.34	12.31	43.56	34.96
RSJ14	17.40 (24.64)	23.24 (28.81)	9.79 (18.22)	13.08 (21.19)	3.69 (11.10)	4.93 (12.81)	10.21	7.64	27.13	20.30
SH$_6$	9.48 (17.92)	11.93 (20.19)	7.48 (15.86)	9.42 (17.86)	2.14 (8.40)	2.69 (9.43)	13.37	10.62	46.79	37.16
NB$_4$D$_2$	16.07 (23.62)	22.02 (27.97)	10.29 (18.70)	14.1 (22.11)	3.70 (11.10)	5.07 (13.00)	9.72	7.09	27.03	19.73
CD @ 5%	0.07	0.03	0.19	0.12	0.05	0.37	019	0.27	0.08	0.04
Sem±	0.02	0.01	0.06	0.04	0.01	0.12	0.06	0.09	0.02	0.01
CV (%)	0.15	0.05	0.51	0.27	0.26	1.44	0.79	1.47	0.12	0.09

Note: ECI – Efficiency conversion for Ingesta, ECD - Efficiency conversion for Digesta; I - Ingesta; D -Digesta; Values in parentheses are statistically transformed values

The data for ingesta per gram cocoon (I/g) was observed it ranged between maximum in DUN6 (19.57 g) and minimum in BHR3 (7.98 g). The amount of digesta per gram cocoon (D/g) observed was highest in CSR2 (12.67 g) and lowest in BHR3 (5.42 g). With regard to ingesta per gram (I/g) shell noticed to be ranged between SH_6 (46.79 g) and lowest in BHR3 (25.44 g). The digesta required to one gram (D/g) shell revealed it ranged between maximum in SH_6 (37.16 g) and minimum in BHR3 (17.28 g) (Table 5). All the parameters were showed statistically significant differences among them during spring season (2019**)**.

4. DISCUSSION

The physiology of growth is 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 consummation 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 nutrition 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 [26,27,25,28]. 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 feeding activity of silkworm is influenced by major ecological factors like temperature, relative humidity in addition to other physiological conditions. The silkworm growth is manifested by the accumulation of organic matter resulting from the balance between anabolic and catabolic reactions fuelled by the nutritive substances absorbed after digestion of food. The silkworms from the same genetic stock responded variedly when fed on the leaves of different nutritional quality, which is an indicator of efficient utilization and conversion of food into silk substance. Variation of the ingesta and digesta values among the different breeds and same breed in different seasons have been reported [29]. The rate of food consumption and leaf quality influence significantly larval growth, weight and probability of survival [30]. Analysis of the nutritional indices like the rates of ingestion, digestion, assimilation and conversion in the growing larvae would be useful in understanding the racial differences in the digestive and assimilation abilities of the silkworm.

The fundamental understanding on the nutrition-gene interactions and its effect on economic traits in silkworm is 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 [31] & [32]. 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 [33]. In sericulture, nutritional requirement and its conversion efficiency contribute directly or indirectly on the cost benefit ratio of silkworm rearing and 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 [34]. 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 [12,35] & [36].

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 [37,38,7,39,40]. 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 19 nutrigenetic traits for the experimental germplasm breeds from different regions.

From the present study, the results revealed that, the screening and evaluation of nutrigenetic traits of bivoltine silkworm breeds was done during spring (2018), the consumption index was very less in breeds *viz*., ATR-16 (0.40) followed by B.con1 (0.43), BHR 2 (0.46), B.con 4 (0.47), CSR 50 (0.49), BHR 3 (0.50), RSJ 14 (0.51) and NB_4D_2 (0.51) compared to other breeds respectively. The efficiency conversion for ingesta to cocoon was highest in the breeds *viz*., ATR-16 (13.39) followed by B.con1 (9.49), BHR 3 (8.92), BHR 2 (8.91) B.con 4 (8.08), CSR 50 (7.97), RSJ 14 (7.83) and NB_4D_2(7.79) whereas efficiency conversion for ingesta to shell was highest in the breeds *viz*., ATR-16 (5.38) followed by B.con1 (4.18), BHR 3 (3.96), BHR 2 (3.88), CSR 50 (3.73), B.con 4 (3.48), NB_4D_2 (3.38), RSJ 14 (3.32). The ingesta per gram of the cocoon is very less compared to other breeds *viz*., ATR-16 (7.47) followed by B.con1 (10.53), BHR 3 (11.21), BHR 2 (11.22), B.con 4 (12.38), CSR 50 (12.55), RSJ 14 (12.76) and NB_4D_2 (12.83) whereas the ingesta per gram of the shell is very less compared to other breeds *viz*., ATR-16 (18.59) followed by B.con1 (23.93), BHR 3 (25.27), BHR 2 (25.80), B.con 4 (28.71), CSR 50 (26.78), NB_4D_2 (29.62), RSJ 14 (30.14). All the parameters taken for the study showed statistically significantly different from each other.

Ramesha et al. (2010) investigated 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. Similar results reported for polyvoltine breeds [3] by 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. 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*., B.con 1, B.con 4, BHR 2, ATR 16, BHR 3, CSR 50, RSJ 14 and NB_4D_2 as potential bivoltine breeding resource material for the development of nutritionally efficient breeds/hybrids under North Western India during spring season (2018).

Similar results were obtained during spring (2019), the data revealed that, the consumption index was very less in breeds *viz*., B.con 1 (0.36), BHR 2 (0.38), CSR50 (0.39), B.con 4 (0.40), BHR3 (0.40), ATR 16 (0.41), RSJ 14 (0.41) and NB_4D_2 (0.41) compared to other breeds. The efficiency conversion for ingesta to cocoon was highest in the breeds *viz*., BHR 3 (12.53), BHR 2 (12.34), B.con 4 (11.99), B.con 1 (11.47), ATR 16 (10.63), NB_4D_2 (10.29), CSR 50 (10.18) and RSJ 14 (9.79) and efficiency conversion for ingesta to shell was highest in the breeds *viz*., BHR 3 (3.93), CSR 50 (3.85), BHR 2 (3.65), B.con 1 (3.62), B.con 4 (3.51), NB_4D_2 (3.42), ATR 16 (3.14) and RSJ 14 (2.93). The ingesta per gram of the cocoon was very less compared to other breeds *viz*., BHR 3 (7.98), BHR 2 (8.10), B.con 4 (8.34), B.con 1 (8.72), ATR 16 (9.41), NB_4D_2 (9.72), CSR 50 (9.82), RSJ 14 (10.21) and the ingesta per gram of the shell was very less compared to other breeds *viz*., BHR3 (25.44), CSR50 (25.98), ATR16 (26.71), NB_4D_2 (27.03), RSJ 14 (27.13), BHR 2 (27.38), B.con 1 (27.60), B.con 4 (27.90).

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 [41,42]. 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 [37]. 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 nonfeeding 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 certainly it was 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 [9] & [43]. 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 [43]. A slant variation observed in relative growth rate between the breeds was due to less difference in larval duration among bivoltine germplasm breeds. From the present study during spring (2019) the breeds *viz*., B.con 1, BHR 2, CSR50, B.con 4, BHR3, ATR 16, RSJ 14 and NB_4D_2 respectively based on consumption index and efficiency conversion of ingesta to larva, ingesta per gram of the cocoon and shell. It is 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 [44].

5. CONCLUSION

From the data, it was concluded that bivoltine breeds with minimum consumption index and maximum efficiency of conversion of ingesta/cocoon identified/shortlisted as breeds *viz*., B.con 1, B.con 4, BHR 2, ATR 16, BHR 3, CSR 50, RSJ 14 and NB_4D_2 as potential bivoltine breeding resource material for the development of nutritionally efficient breeds/hybrids under North Western India.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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