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Determination and Comparison of Nutritional Indices in Commercial Silkworm Hybrids during Various Instars

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Abstract: The aim of the present study was generate data on *Bombyx mori* feeding and nutritional indices and characteristics during 1st-5th larval instars and comparison of these parameters among eight commercial hybrids. All insect rearing and experiments were done under special laboratory conditions. The several parameters such as quantity of food consumed, fecal matter excreted and larval growth was determined based on fresh (wet) and dry weight. The experiment was set in a completely randomized design. Also, evaluation index value and sub-ordinate function value were calculated for nutritional indices. From obtained results, gain for total instars (1-5 instars) was maximal in 104×103 (0.67 g DM/larva) and minimum in 151×154 (0.56 g DM/larva). In all the hybrids, ingested food for total larval duration was observed to be above 5.8 g DM/larva. Highest food consumption was recorded in 31×32 (6.31 g DM/larva) followed by 32×31 (6.30 g DM/larva) and 104×103 (6.22 g DM/larva), whereas lowest was recorded in 151×154 (5.80 g DM/larva) followed by 154×151 (5.82 g DM/larva). ECI for total instars (1-5 instars) was maximal in 151×154 (10.35) and minimum in 153×154 (8.37). In all larval duration, approximate digestibility was observed to be above 0.47. Highest AD was recorded in 154×151 (0.507) followed by 153×154 (0.505) and 31×32 (0.504), whereas lowest was recorded in 104×103 (0.475) followed by 103×104 (0.476). After evaluation by both the statistical methods (evaluation index method and sub-ordinate function method), hybrids of 31×32, 104×103 and 32×31 were identified as potential hybrids for further development at distribution between farmers.

Key words: *Bombyx mori*, food, digestibility, feces, dry matter

INTRODUCTION

The mulberry silkworm, *Bombyx mori* L., is a very important economic insect that contributes substantially to the national economy of many countries e.g., China, India, Thailand, Iran etc., (Chen, 2003). Also, the domesticated silkworm, *Bombyx mori* is one of the genetically well-characterized insects next only to the fruitfly, Drosophila and has recently emerged as a lepidopteran molecular model system (Goldsmith, 1995; Nagaraju *et al*., 2001; Nagaraju, 2002).

During the last three decades, silk production increase benefited, to a great extent, from the application of genetic and feeding principles in the silkworm breeding and feeding programmes (Nagaraju, 2002). There is a need to recognition and integrate the physiological and nutritional requirements of the silkworm hybrids under ecological conditions to the silkworm breeding and management programmes to make them need-based (Nagaraju, 2002).

Conventional hybridization is widely used for improving yield potential in silkworm. This strategy generates variability by hybridizing the elite genotype with other improved varieties or local varieties, followed by selection of the desirable recombinants. The breeding objectives were achieved with precise selection starting from the initial choice of parents, meticulous progeny testing and selection and matching ecological and nutritive conditions. To further increase the yield potential of silkworm, heterosis breeding which exploits the increased vigor of the F1 hybrid (improved growth rates, better crop stability by virtue of increased resistance to biotic and abiotic stresses) has been used in silkworm (Nagaraju, 2002).

Recently, many viable hybrids were evolved and authorized at Iran for commercial exploitation which have various production potential (Seidavi *et al*., 2003, 2007a, b). These hybrids although being more productive and robust, but there is not any data and knowledge regarding their nutritional characteristics and indices.

This study reports the nutritional characteristics and performance of eight commercial bivoltine silkworm hybrids, with the aim of enhancing commercial sericulture in Iran and other countries of the region, where this activity is increasingly perceived as a promising alternative source of income generation for rural small-scale farmers. In fact, the present study was designed to generate data on *Bombyx mori* feeding and nutritional indices and characteristics during 1st-5th larval instars. Also, it is compared these parameters among eight commercial hybrids.

MATERIALS AND METHODS

Eight silkworm populations were used for the present research study. After hatching from the eggs, neonates were brushed and reared up separately on fresh leaves of mulberry (*Morus alba*). One-day-old 1st instar larvae of uniform size were selected from all eight hybrids and used for the experiment. All insect rearing and experiments were done under the following laboratory condition at Iran Silkworm Research Center (ISRC) and Islamic Azad University, Rasht branch during 2008-2009.

Commercial pure lines of silkworm were mated together and produced silkworm eggs of eight newly evolved Iranian commercial hybrids namely 31×31, 23×31, 103×104, 104×103, 151×154, 154×151, 153×154 and 154|×153 as composite laying eggs. Composite laying were prepared for each hybrid before rearing and each composite laying consisted of about 500 eggs taken from 10 disease free laying (about 500 eggs per laying). Eggs of the silkworm bivoltine hybrid were obtained from Iran Silkworm Research Center (ISRC) and incubated at breeding laboratory.

The silkworm eggs had incubated in the controlled environment chamber. When there were 95% of eggs having little black dots on the surface of eggs, they were shaded with black gobo to prevent the light irradiation for about 48 h for making the larvae emerge form the eggs at one time. After most of them hatched, the silkworm larvae were fed on leaves of mulberry. Brushing was done carefully.

The batches of 50 silkworm larvae were reared from the early-late stages. At the beginning of 1st instar, 200 larvae were counted from each hybrid, divided to four replications (50 larvae per each replication) and retained for studies. Rearing was carried out under hygienic conditions. All hybrids were reared standard rearing techniques (Pallavi and Basavaraja, 2007). The young larvae (1st-3rd instars) were reared at 27-28°C with 85-90% relative humidity and the late age larvae (4th and 5th instars) were maintained at 24-26°C with a relative hnmidity of 70-80%. The larvae were fed *ad libitum* with Ichinose variety mulberry leaves three times a day.

The quantity of food consumed, fecal matter excreted and larval growth was determined based on fresh (wet) and dry weight. Amount of food ingested was calculated by subtracting the weight of residual food from the amount of food given as wet and dry matter. The difference in weight of the food ingested and fecal matter produced was treated as food digested. Samples of leaves, feces and larvae dried in an oven at 80°C to a constant weight to determine the dry weight. Left over food and feces were recorded and removed daily when fresh leaves was given and dried in the same way to get the dry weight. Thus, for each instar, the increase in fresh and dry weights of the larvae, fresh and dry weights of food eaten and digested and dry weight of feces produced were recorded (Rath *et al*., 2003). The fresh leaf mass (L1) of feeding to the silkworms was weighed and recorded each time. Also, consnmed fresh leaf moisture is measured and recorded daily. Weight and moisture amounts of feces and un-used leaves (L2) measured carefully and recorded daily. The actual leaf mass of silkworm consumed was calculated as:

$$\text{Leaf mass} = L1\times \text{moisture (\%)} - L2\times \text{moisture (\%)}$$

where, weight and moisture percentage of larvae measured in first and end of each instar.

The methods of determination of various indices of food consumption and utilization were followed according to Waldbauer (1968) and Rath *et al*. (2003).

$$\text{Approximate Digestibility (AD)} = \frac{\text{Dry weight of food eaten- Dry weight of feces produced}}{\text{Dry weight of food eaten}}\times 100$$

$$\text{ECI} = \frac{\text{Dry weight gain of larva}}{\text{Dry weight of food eaten}}\times 100$$

Larval growth and food utilization were calculated every instar during 1st-5th instars. Differences in average weight of the larvae recorded at the beginning and at the end of the period gave the gain in body weight while the mean larval body weight was calculated using the formula:

$$\text{Weight gain} = \text{Final weight-Initial weight}$$

The experiment was set in a Completely Randomized Design (CRD) with four replications. For the statistical analysis, the data were transformed if necessary and then analyzed using one-way ANOVA and the means were grouped using Duncan's multiple range test by means of SPSS statistical Package version 11.0 for windows using the GLM procedure. Results are expressed as Means±SD (SPSS, 1999).

Also, evaluation index value and sub-ordinate function value were calculated for nutritional indices. Evaluation Index (EI) value for silkworm hybrid performance was calculated by using the following formula (Mano *et al*., 1993; Rao *et al*., 2006):

$$\text{EI} = [(A-B)/C]\times 10+50$$

where, A is mean of the particular trait in a hybrid; B is overall mean of particular trait in all hybrids; C is standard deviation of a trait in all hybrids and 50 is constant.

Sub-ordinate function is calculated by utilizing the following formula based on Gower (1971) and Rao *et al*. (2006):

$$Xu = (X_i - X_{min})/(X_{max} - X_{min})$$

where, Xu is sub-ordinate function; X_i is measurement of trait of tested breed; X_{min} is minimum value of the trait among all the tested breeds; X_{max} is maximum value of the trait among all the tested breeds.

The evaluation index and sub-ordinate function values for the all traits were calculated separately and average index value was obtained. Then studied silkworm hybrids are ranked based on average of evaluation index method and sub-ordinate function method.

RESULTS

Most of the parameters used showed significant differences in all the cultivars ($p<0.01$). From obtained results, it is showed the gain of first instar of the 153|×154 and 154×151 hybrids remained significantly at lower level than other hybrids. An increase in gain of second instar was observed at

31×32 hybrid significantly higher than other hybrids. The gain of third instar in 154×151 hybrid decreased significantly in comparison with other hybrids and followed by a significant increase by 104×103 hybrid (Table 1). Instar to instar consumption and digestion revealed a continuous increase in food consumption and digestion in all hybrids; the highest ingestion and digestion were recorded on fifth instar (Table 1). Food consumption at first instar remained significantly at a lower level in the 153×154 and 154×153 hybrids (0.00005 g DM/larva). This parameter was at the highest level for 103×104, 104×103, 104×103 and 31×32 during second till fifth instars respectively (Table 1).

Rates of nutritional conversion showed a gradnal increase, reaching a maximum on the second and fifth instars and decreased before settling for the first, third and fourth molts and during second and fifth instar the maximum rates recorded were on the ECI in all hybrids tested, which decreased thereafter until they showed the signs of spinning.

Table 1: Mean±SD performance of nutritional indices in eight studied silkworm hybrids (g DM/larva)

Hybrid	Gain (1st instar)	Gain (2nd instar)	Gain (3rd instar)	Gain (4th instar)	Gain (5th instar)	Ingested food (1st instar)
31×32	0.00080±0.00000^{a}	0.00275±0.00050^{a}	0.01850±0.00100^{d}	0.08550±0.00300^{bcd}	0.57400±0.02920^{bc}	0.00280±0.00182^{b}
32×31	0.00050±0.00000^{bc}	0.00200±0.00000^{b}	0.02000±0.00081^{c}	0.06925±0.00250^{e}	0.53575±0.00750^{cd}	0.00105±0.00050^{bc}
103×104	0.00052±0.00005^{b}	0.00225±0.00050^{b}	0.01825±0.00050^{d}	0.08150±0.00834^{d}	0.53400±0.01568^{cd}	0.00205±0.00050^{ab}
104×103	0.00045±0.00005^{cd}	0.00250±0.00057^{ab}	0.01725±0.00095^{d}	0.09275±0.00095^{a}	0.58300±0.03101^{a}	0.00105±0.00125^{bc}
151×154	0.00037±0.00005^{e}	0.00200±0.00000^{b}	0.02225±0.00095^{b}	0.09100±0.00509^{ab}	0.44450±0.05604^{e}	0.00080±0.00081^{bc}
154×151	0.00040±0.00000^{de}	0.00200±0.00000^{b}	0.01600±0.00081^{e}	0.08175±0.00457^{d}	0.57450±0.02803^{bc}	0.00055±0.00957^{bc}
153×154	0.00037±0.00005^{e}	0.00200±0.00000^{b}	0.01825±0.00050^{d}	0.08325±0.00221^{cd}	0.55425±0.01992^{bc}	0.00005±0.00125^{c}
154|×153	0.00040±0.00000^{de}	0.00200±0.00000^{b}	0.02525±0.00095^{a}	0.08975±0.00499^{abc}	0.50300±0.00725^{d}	0.00005±0.00095^{c}
Mean	0.00047	0.00219	0.01947	0.08434	0.53787	0.00105
SD	0.00013	0.00039	0.00292	0.00812	0.13499	0.00131
Minimum	0.00037	0.002	0.016	0.06925	0.4445	0.00005
Maximum	0.0008	0.00275	0.02525	0.09275	0.583	0.0028

Hybrid	Ingested food (2nd instar)	Ingested food (3rd instar)	Ingested food (4th instar)	Ingested food (5th instar)	ECI* (1st instar)	ECI (2nd instar)
31×32	0.02325±0.00206^{ab}	0.10900±0.01400^{bc}	0.4565±0.02310^{a}	5.72500±0.13772^{a}	2.28000±1.74500^{a}	8.77500±2.54480^{a}
32×31	0.02275±0.00236^{abc}	0.11325±0.00953^{ab}	0.45700±0.04043^{a}	5.71500±0.12396^{a}	2.10000±1.00000^{a}	11.37500±1.18150^{a}
103×104	0.02400±0.00355^{a}	0.10450±0.01885^{bcd}	0.44625±0.03382^{a}	5.47750±0.16978^{abc}	3.88000±0.55000^{a}	11.07500±3.00930^{a}
104×103	0.02325±0.00150^{ab}	0.12975±0.00427^{a}	0.47425±0.02151^{a}	5.61000±0.10863^{ab}	2.43000±3.20700^{a}	9.70000±2.46170^{a}
151×154	0.02050±0.00264^{abc}	0.10200±0.01055^{bcd}	0.42575±0.01691^{ab}	5.25750±0.18786^{c}	2.38000±2.68900^{a}	10.25000±1.32290^{a}
154×151	0.02025±0.00095^{abc}	0.10450±0.01236^{bcd}	0.4445±0.04645^{a}	5.25500±0.18699^{e}	1.38000±2.39400^{a}	10.12500±0.47870^{ab}
153×154	0.02000±0.00200^{bc}	0.08450±0.01204^{d}	0.42225±0.02196^{ab}	5.37250±0.13574^{bc}	0.10000±3.15300^{a}	10.00000±1.0000^{a}
154|×153	0.01900±0.00282^{c}	0.09200±0.01407^{cd}	0.38825±0.03891^{b}	5.50000±0.16391^{abc}	0.13000±2.39400^{a}	9.50000±1.41420^{a}
Mean	0.02163	0.10492	0.43934	5.4891	1.835	10.1
SD	0.00274	0.01702	0.03792	0.22331	2.373	1.8267
Minimum	0.019	0.0845	0.38825	5.255	0.1	8.775
Maximum	0.024	0.12975	0.47425	5.725	3.88	11.375

Hybrid	ECI (3rd instar)	ECI (4th instar)	ECI (5th instar)	AD* (1st instar)	AD (2nd instar)	AD (3rd instar)
31×32	5.87500±0.74110^{b}	5.27500±0.35000^{bc}	9.95000±0.36970^{bc}	_0.19700±0.83020^{a}	0.54580±0.04381^{a}	0.39700±0.65036^{a}
32×31	5.62500±0.37750^{bc}	6.52800±0.60760^{a}	10.62500±0.28720^{ab}	_0.97500±0.66400^{a}	0.55650±0.04374^{a}	0.40425±0.04126^{a}
103×104	5.72500±1.03720^{bc}	5.55000±1.00830^{b}	11.02500±1.12950^{a}	0.10900±0.14850^{a}	0.50230±0.07384^{a}	0.27725±0.13109^{a}
104×103	7.52500±0.25000^{a}	5.07500±0.25000^{bcd}	9.6000±0.47610^{e}	2.30000±6.18140^{a}	0.53680±0.02573^{a}	0.42800±0.03363^{a}
151×154	4.55000±0.047960^{d}	4.67500±0.30960^{cd}	9.58500±0.29770^{c}	2.00000±5.69370^{a}	0.50580±0.06097^{a}	0.32875±0.07016^{ab}
154×151	6.47500±0.85000^{b}	5.4000±0.48990^{bc}	9.12500^{e}±0.29860	4.71200±6.45450^{a}	0.55530±0.03689^{a}	0.37950±0.11825^{a}
153×154	4.67500±0.78050^{cd}	5.05000±0.19150^{bcd}	9.67500±0.51230^{a}	5.88800±5.41920^{a}	0.55780±0.06272^{a}	0.17800±0.18933^{b}
154|×153	3.62500±0.66520^{d}	4.30000±0.47610^{d}	10.90000±0.21600^{e}	2.60000±5.54060^{a}	0.55850±0.06520^{a}	0.28350±0.16377^{ab}
Mean	5.509	5.231	10.06063	2.055	0.5398	0.33453
SD	1.3071	0.776	1.7186	4.6874	0.05232	0.12921
Minimum	3.625	4.3	9.125	-0.975	0.5023	0.178
Maximum	7.525	6.528	11.025	5.888	0.5585	0.428

Hybrid	AD (4th instar)	AD (5th instar)	Gain (total instars)	Ingested food (total instars)	ECI (total instars)	AD (total instars)
31×32	0.31275±0.05185^{a}	0.51925±0.01393^{bc}	0.66040±0.03150^{bc}	6.31250±0.12447^{a}	9.56500±0.38354^{a}	0.50400±0.01751^{a}
32×31	0.29975±0.02163^{ab}	0.49675±0.00899^{cd}	0.62215±0.00801^{cd}	6.30250±0.15521^{a}	10.08250±0.29871^{a}	0.48275±0.00485^{a}
103×104	0.20650±0.04631^{c}	0.50200±0.01803^{bcd}	0.62440±0.01884^{cd}	6.05000±0.17321^{bc}	9.70000±0.45461^{a}	0.47525±0.02005^{a}
104×103	0.30500±0.02264^{ab}	0.49150±0.01369^{d}	0.67842±0.03136^{a}	6.22500±0.09574^{ab}	9.17250±0.41532^{ab}	0.47500±0.01677^{a}
151×154	0.28475±0.03971^{abc}	0.51400±0.01883^{bcd}	0.56012±0.05416^{e}	5.80000±0.17833^{d}	10.35492±0.29462^{a}	0.49425±0.01822^{a}
154×151	0.31225±0.09313^{a}	0.52525±0.01117^{b}	0.67665±0.03018^{a}	5.82000±0.13772^{cd}	8.60000±0.16833^{b}	0.50725±0.00834^{a}
153×154	0.21775±0.04261^{bc}	0.52775±0.02137^{b}	0.65190±0.02060^{bc}	5.89500±0.13772^{cd}	8.37500±1.54561^{b}	0.50500±0.01428^{a}
154|×153	0.21775±0.05185^{bc}	0.55325±0.02098^{a}	0.60340±0.00850^{d}	5.97250±0.13841^{cd}	9.89500±0.12662^{a}	0.49375±0.07516^{a}
Mean	0.26956	0.51622	0.63468	6.0472	9.46811	0.49216
SD	0.0653	0.02387	0.0085	0.24145	1.46761	0.02931
Minimum	0.2065	0.4915	0.56012	5.8	8.375	0.475
Maximum	0.31275	0.55325	0.67842	6.3125	10.35492	0.50725

Mean values in each column followed by the same superscript letters are not significantly different at α = 0.01 ECI: Efficiency of conversion of ingested food; AD: Approximate digestibility

The efficiency of conversion of ingested food (ECI) in first instar among hybrids ranged between 0.1 (153×154) and 3.88 (103×104) and average ECI was found to be 1.83. Lowest ECI for second instar was recoded in 8.77 (31×32) and highest was 11.37 (32×31) with an average of 10.10. The ECI in third instar among hybrids ranged between 3.62 (154×153) and 7.52 (104×103) and average ECI was found to be 5.50. Lowest ECI for 4th instar was recoded in 4.30 (154×153) and highest was 6.52 (32×31) with an average of 5.23. Also, the ECI in 5th instar among hybrids ranged between 9.12 (154×151) and 11.025 (103×104) and average ECI was found to be 10.06.

The amount of Approximate Digestibility (AD) in first instar ranged from -0.97 to 5.88 with the highest of AD recorded for 153×154 and the lowest of AD recorded for 32×31. The AD in second instar ranged from 0.50 to 0.55 with highest of AD recorded for 154×153 and the lowest of AD recorded for 103×104. In all the hybrids, AD for 3rd instar was recorded more than 0.27 except in 153×154 (0.17) and the average AD was 0.33. Meanwhile, AD in 4th instar was found to be highest in 31×32 (0.31) and lowest in 103×104 (0.20) with an average of 0.26. Finally, maximum AD in 5th instar was recorded in 154×153 (0.55), lowest in 104×103 (0.49).

Gain for total instars (1-5 instars) was maximal in 104×103 (0.67 g DM/larva) and minimum in 151×154 (0.56 g DM/larva). In all the hybrids, ingested food for total larval duration was observed to be above 5.8 g DM/larva. Highest food consumption was recorded in 31×32 (6.31 g DM/larva) followed by 32×31 (6.30 g DM/larva) and 104×103 (6.22 g DM/larva), whereas lowest was recorded in 151×154 (5.80 g DM/larva) followed by 154×151 (5.82 g DM/larva) (Table 1).

ECI for total instars (1-5 instars) was maximal in 151×154 (10.35) and minimum in 153×154 (8.37). In all larval duration, approximate digestibility was observed to be above 0.47. Highest AD was recorded in 154×151 (0.507) followed by 153×154 (0.505) and 31×32 (0.0504), whereas lowest was recorded in 103×104 (0.475) followed by 103×104 (0.476) (Table 1).

Also, based on nutritional indices of hybrids were assessed on different parameters like gain, food consumption, efficiency of conversion of ingested food and approximate digestibility using the evaluation index (Table 2) and sub-ordinate function (Table 3). Among commercial hybrids, as per the evaluation method, the hybrids 31×32 (1329.21), 104×103 (1311.461), 32×31 (1228.219) and 154×151 (1222.71) showed higher evaluation index values and 103×104 (1192.131), 153×154 (1135.865), 154×153 (1115.31) and 151×154 (1065.093) showed lower index (Table 2, 4).

The other important method used in hybrid evaluation was sub-ordinate function method (Gower, 1971). As per this method, among all hybrids, 31×32 (16.66799), 104×103 (14.77702), 32×31 (14.19177) and 103×104 (11.82723), have shown higher index values, whereas 154×151 (11.40779), 154×153 (9.372836), 151×154 (8.616624) and 153×154 (8.415133) showed lower index values (Table 3, 4).

Table 2: Evaluation index values for nutritional indices in 8 studied silkworm hybrids

Hybrid	Gain (1st instar)	Gain (2nd instar)	Gain (3rd instar)	Gain (4th instar)	Gain (5th instar)	Ingested food (1st instar)	Ingested food (2nd instar)	Ingested food (3rd instar)	Ingested food (4th instar)	Ingested food (5th instar)	ECI* (1st instar)	ECI (2nd instar)
31×32	74.90385	64.42308	46.68236	51.42395	52.67612	63.35878	55.93066	52.3869	54.52433	60.56547	51.87526	42.74648
32×31	51.82692	45.19231	51.81935	31.41164	49.84258	50.00000	54.10584	54.88396	54.65618	60.11766	51.11673	56.97980
103×104	53.36538	51.60256	45.8262	46.49784	49.71294	57.63359	58.66788	49.74295	51.82127	49.48222	58.61778	55.33749
104×103	47.98077	58.01282	42.40154	60.35252	53.34284	50.00000	55.93066	64.57844	59.20523	55.41568	52.50737	47.81026
151×154	41.82692	45.19231	59.52483	58.19735	43.08282	48.0916	45.89416	48.27409	46.41515	39.63045	52.29667	50.82115
154×151	44.13462	45.19231	38.12072	46.80573	52.71316	46.18321	44.98175	49.74295	51.35977	39.51849	48.0826	50.13686
153×154	41.82692	45.19231	45.8262	48.65302	51.21305	42.36641	44.06934	37.99207	45.49215	44.78024	42.68858	49.45256
154|×153	44.13462	45.19231	69.7988	56.65794	47.41648	42.36641	40.41971	42.39865	36.52591	50.48979	42.815	46.71539

Hybrid	ECI (3rd instar)	ECI (4th instar)	ECI (5th instar)	AD* (1st instar)	AD (2nd instar)	AD (3rd instar)	AD (4th instar)	AD (5th instar)	Gain (total instars)	Ingested food (total instars)	ECI (total instars)	AD (total instars)
31×32	52.79722	50.55896	49.35631	45.19643	51.13723	54.83467	56.61371	51.26990	80.25882	60.9883	50.66015	54.04086
32×31	50.88459	66.70586	53.28392	43.53666	53.18234	55.39577	54.62289	41.84384	35.25882	60.57414	54.1863	46.79077
103×104	51.64964	54.10277	55.61140	45.84924	42.82301	45.56681	40.34265	44.04326	37.90588	50.11648	51.58002	44.23192
104×103	65.42059	47.98164	47.31977	50.52348	49.41705	57.23386	55.42688	39.64443	101.45880	57.36436	47.98574	44.14662
151×154	42.66028	42.82700	47.23249	49.88346	43.49197	49.55257	52.32580	49.07049	-37.71760	39.76237	56.04251	50.71435
154×151	57.38754	52.16978	44.55589	55.66919	52.95298	53.48028	56.53714	53.78351	99.37647	40.5907	44.08484	55.14969
153×154	43.61659	47.65947	47.75617	58.17804	53.43081	37.88552	42.06547	54.83085	70.25882	43.69694	42.55173	54.38204
154|×153	35.58354	37.99452	54.88406	51.16349	53.56460	46.05052	42.06547	65.51372	13.20000	46.90671	52.90871	50.54376

Table 3: Sub-ordinate function values for nutritional indices in 8 studied silkworm hybrids

Hybrid	Gain (1st instar)	Gain (2nd instar)	Gain (3rd instar)	Gain (4th instar)	Gain (5th instar)	Ingested food (1st instar)	Ingested food (2nd instar)	Ingested food (3rd instar)	Ingested food (4th instar)	Ingested food (5th instar)	ECI* (1st instar)	ECI (2nd instar)
31×32	1.00	1.00	0.27027	0.691489	0.935018	1.00	0.85	0.541436	0.793605	1.00	0.57672	0.00
32×31	0.302326	0.00	0.432432	0.00	0.658845	0.363636	0.75	0.635359	0.799419	0.978723	0.529101	1.00
103×104	0.348837	0.333333	0.243243	0.521277	0.646209	0.727273	1.00	0.441989	0.674419	0.473404	1.00	0.884615
104×103	0.186047	0.666667	0.135135	1.00	1.00	0.363636	0.85	1.00	1.00	0.755319	0.616402	0.355769
151×154	0.00	0.00	0.675676	0.925532	0.00	0.272727	0.30	0.38674	0.436047	0.005319	0.603175	0.567308
154×151	0.069767	0.00	0.00	0.531915	0.938628	0.181818	0.25	0.441989	0.65407	0.00	0.338624	0.519231
153×154	0.00	0.00	0.243243	0.595745	0.792419	0.00	0.20	0.00	0.395349	0.25	0.00	0.471154
154|×153	0.069767	0.00	1.00	0.87234	0.422383	0.00	0.00	0.165746	0.00	0.521277	0.007937	0.278846

Hybrid	ECI (3rd instar)	ECI (4th instar)	ECI (5th instar)	AD* (1st instar)	AD (2nd instar)	AD (3rd instar)	AD (4th instar)	AD (5th instar)	Gain (Total instars)	Ingested food (total instars)	ECI (total instars)	AD (total instars)
31×32	0.576923	0.437612	0.434211	0.113362	0.774021	0.876	1.00	0.449393	0.847675	1.00	0.601034	0.899225
32×31	0.512821	1.00	0.789474	0.00	0.964413	0.905	0.877647	0.08502	0.524345	0.980488	0.862409	0.24031
103×104	0.538462	0.561041	1.00	0.157948	0.00	0.397	0.00	0.17004	0.543364	0.487805	0.669219	0.007752
104×103	1.00	0.347846	0.25	0.477197	0.613879	1.00	0.927059	0.00	1.00	0.829268	0.402794	0.00
151×154	0.237179	0.168312	0.242105	0.433484	0.062278	0.603	0.736471	0.364372	0.00	0.00	1.00	0.596899
154×151	0.730769	0.493716	0.00	0.828646	0.94306	0.806	0.995294	0.546559	0.985038	0.039024	0.113641	1.00
153×154	0.269231	0.336625	0.289474	1.00	0.987544	0.00	0.105882	0.587045	0.775824	0.185366	0.00	0.930233
154|×153	0.00	0.00	0.934211	0.520909	1.00	0.422	0.105882	1.00	0.36585	0.336585	0.767708	0.581395

Table 4: Ranking of 8 studied silkworm hybrids based on average of evaluation index method and sub-ordinate function method

Hybrid	Evaluation index method: Value	Evaluation index method: Rank	Sub-ordinate function method: Value	Sub-ordinate function method: Rank
31×32	1329.210	1	16.66799	1
32×31	1228.219	3	14.19177	3
103×104	1192.131	5	11.82723	4
104×103	1311.461	2	14.77702	2
151×154	1065.093	8	8.616624	7
154×151	1222.710	4	11.407790	5
153×154	1135.865	6	8.415133	8
154| ×153	1115.310	7	9.372836	6

DISCUSSION

The results on nutritional performance of the different silkworm hybrids tested in the present study indicate breed significant effects on nutritional characteristics. Previously Vyjayanthi and Subramanyam (2002) stated multivoltine silkworms had significantly higher rates of feeding, assimilation and conversion with increased efficiency of conversion of ingested and digested food to body substance when compared with bivoltine silkworms.

It is expected low intake of food results to reduced larval period. This finding clearly indicates that the varieties with high conversion efficiencies may reduce the larval span and consequently less quantity of the food is needed to support optimal growth which confirms earlier findings of Sarkar and Fujita (1994). Also, it has been stated that the efficiently converted mulberry varieties may be consumed less to support the optimal growth. Therefore, varieties possessing high nutritional indices will have better convertibility and so intake of such varieties by the silkworm is low. This appears to be very significant point that silkworms consume fewer mulberries from the varieties having high nutritional indices and produce higher cocoon yield. Sarkar and Fujita (1994) concluded that the high nutritional indices may be treated as the final indicator for the evaluation of value of hybrids.

The silkworm larvae were almost fed with mulberry leaves exclusively, because the steroid could not be synthesized by the larvae themselves and must be obtained from the exoteric environment such as the mulberry leaves (Yua *et al*., 2008). Also, food is a critical factor of paramount importance which regulates the growth of silkworm, development and silk yield in sericulture. Therefore, nutritional indices are very important in sericulture industry. To date, in spite of assessment of silkworm as human food source (Mishra *et al*., 2003), several important nutritional traits like digestion coefficients, have not been considered and handled very successfully in traditional breeding schemes so far. One reason for this may be the low heritability and lack of application of appropriate statistical tool for analysis of phenotypic data. In fact, the most important traits of sericulture, as in agriculture are not controlled by a single gene but the concerted action of several genes (polygenic or quantitative traits) and non-hereditary factors. Dissecting such traits require substantially enhanced efforts on the part of silkworm geneticists and nutritionists (Nagaraju, 2002). On the other hand, nutritional efficiency of the food ingested by silkworms is usnally evaluated in terms of various characteristics as proportion of cocoon shell weight to the amount of food ingested or production efficiency of cocoon shell etc.

Results of present experiment can introduce and select those hybrids have high nutritional parameters. In *Bombyx mori*, it has been demonstrated that the levels of DNA synthesis in prothoracic gland cells undergo characteristic changes with a dramatic increase preceding the increase in ecdysteroidogenesis during the third, fourth and last larval instars (Gu and Chow, 2005; Chen and Gu, 2006). Moreover, Chen and Gu (2006) showed that activation of DNA synthesis in gland cells during the middle stages of the last larval instar is nutrition-dependent, with starvation on day 3 inhibiting DNA synthesis. Therefore, there is direct correlation between nutritional indices and silk production. Hence, selections of those hybrids have high nutritional parameters is important economically.

This insect is a polyphagous herbivore and depends mainly on the qnality of mulberry leaves and enviroumental conditions for its development. Nutritious leaves play an important role in the silkworm growth and overall silk cocoon production (Adolkar *et al*., 2007). Any effort to improve the yield requires considerations of cumulative effect of the major traits which influences the silk yield. To judge the superiority of the silkworm hybrid impartially a common index method was found very much essential (Bhargava *et al*., 1994; Rayar, 2008). Hence, attempt has been made to identify the potential hybrids based on the evaluation index value and similar approaches. It must notice to in this experiment nutritional traits were evaluated using two reliable statistical methods, i.e., evaluation index method and sub-ordinate function method to assess the performance of the studied hybrids. Earlier many breeders (Gower, 1971; Mano *et al*., 1993; Rao *et al*., 2006; Rayar, 2008) analyzed their breeds by adopting the above methods either individually or together based on production traits. The evaluation index is one such method that increases the accuracy of selection of hybrids by a common index giving full weight age to all the nutritional contributing traits (Rao *et al*., 2006).

The regulation of feeding in insects is clearly very complex and includes positive and negative sensory feedback, distension of the alimentary canal, effects of nutrients and the release of peptides and hormones from the gut or brain (Audsley and Weaver, 2008). The interaction of neuropeptides, such as myoactive peptides that regulate gut motility, plays an important role in determining the amount of food ingested and the duration of and time interval between meals. It is unclear why insects possess such an array of different peptides, some with multiple copies or homologues that stimulate (e.g., proctolin, allatotropin, sulfakinins and tachykinins) or inhibit (e.g., myosuppressins, myoinhibitory peptides and allatostatins) gut motility. It is also unclear how the differential release of these peptides, sometimes from the same neurone, is regulated. Some of these peptides will also have roles other than on the visceral muscles (Audsley and Weaver, 2008).

Food utilization parameters have been studied in many insects (Rath *et al*., 2003). The nutritive value of mulberry leaves depends on various agro-climatic factors and any deficiency of nutrients in leaves affects silk synthesis by the silkworm. Nutritional management directly influences the quality and quantity of silk production (Hiware, 2006). Therefore, in recent years, attempts have been made to fortify the leaves with nutrients, including spraying with antibiotics, juvenile hormones, juvenile-hormone mimicking extracts of plants, etc., to improve the quality and quantity of silk (Hiware, 2006). Earlier research work was carried out in various agro-climatic zones of Asia and Africa and several varieties were screened for high qnality and production of silk for sericulture practices (Fotedar and

Dandin, 1997; Adolkar *et al*., 2007). Successful sericulture depends on increased production of mulberry leaves with high nutritive values (Krishnaswami, 1978; Adolkar *et al*., 2007). Furthermore, mulberry leaf N, K, S, Zn, Ca, Mg and Cu are significantly influenced by the status of soil nutrients (Rupa *et al*., 1993; Adolkar *et al*., 2007). Meanwhile, the pH levels affect the availability of micro and macro nutrients in the filed soil. In sericulture, macro nutrients like N, P and K are required in large quantities, while micro nutrients only in small quantities (Adolkar *et al*., 2007).

Other factors affect on nutritional indices. Deranged metabolism resulting in decreased growth may be due to nutritional stress caused by the parasitization leading to increased utilization of nutrients by the parasite under rapid multiplication and/or suppression of de novo synthesis (Rath *et al*., 2003). Food utilization efficiency has been affected following parasitization by other parasites including parasitoids (Rath *et al*., 2000, 2003). Metabolic profile of the hemolymph and fat body declined in *Bombyx mori* and other insects because of host-parasite interactions, as a result of which growth is inhibited. Food ingestion, digestion, gain in body weight, and efficiencies of utilization significantly decline following microsporidian infection (Rath *et al*., 2003). Therefore, it must notice parasitization situation in nutritional studies.

On the other hand, patterns of insect feeding have been extensively documented to characterize factors controlling the timing of feeding initiation (meal-start) and termination. Continuous observations of various insects indicate that many insect species have patterned feeding cycles and different nutritional indices (Nagata and Nagasawa, 2006). The physiology of silkworm has been studied extensively due to the economic importance of silk production over the centuries. Structural identification of dietary components essential for feeding in *Bombyx mori* larvae was followed years later by detailed investigations on the relationships between dietary components and growth. Further studies on food preferences and optimal nutrient levels for maximum larval growth and silk production eventually led to the development of an artificial diet (Nagata and Nagasawa, 2006). They believed feeding occurred at regular intervals in *Bombyx mori* larvae throughout larval development. They reported long-term diet-deprived *Bombyx mori* larvae did not begin wandering or foraging until food was replaced indicates that starvation-associated locomotion activity might be controlled by chemical attractants in food. Their quiescence in the absence of food also suggests that long term diet-deprivation might induce a reduced metabolic state in the larva that would minimize energy loss or nutrient depletion. It is unclear, however, whether locomotion activity and the initiation of feeding behavior are controlled by the same factors (Nagata and Nagasawa, 2006). Colasurdo *et al*. (2007) also reported range of protein and carbohydrate concentrations tested did not influence the caterpillars probability to initiate feeding upon contact with the food or the duration of the first feeding event, suggesting no, or only minor, differences in phagostimulatory responses of native insects to the foods. In insects like caterpillars, durations of both interfeed intervals and meals appear to be regulated to a large extent by volumetric feedback from gut stretch receptors and by nutritional feedback from haemolymph metabolite content (Colasurdo *et al*., 2007). Also, researchers believed haemolymph provides internal information on the nutritional status of the insect because its composition fluctuates dramatically as nutrients are absorbed from the gut and processed.

Vyjayanthi and Subramanyam (2002) stated in the silkworm, *Bombyx mori*, feeding behavior depends on the niche, amount of food offered, qnality of food, age and health of the larva. As most phytophagus lepidoptera are voracious feeders any imbalance in the inputs from various factors affect food intake and result in poor larval development (Waldbauer, 1968; Vyjayanthi and Subramanyam, 2002). Therefore, obtained results in this experiment are a case study ouly for studied hybrids based on the enviroument and management conditions.

Finally, to date there is not report regarding investigation and assessment of nutritional characteristics of silkworm hybrids using evaluation index method and sub-ordinate function method. Hence, it can claim this report is the first report regarding application of these methods for comparison of nutritional traits in silkworm.

As conclusion, after evaluation by both the statistical methods (evaluation index method and sub-ordinate function method), hybrids of 31×32, 104×103 and 32×31 were identified as potential hybrids for further development at distribution between farmers. Also, in the present study, variations in nutritional characteristics were observed in eight studied silkworm hybrids and hybrids of 31×32, 104×103 and 32×31 had the best performance. Thus development of mentioned hybrids in necessary based on regional conditions.

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