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Nutrigenetic Traits Analysis for the Identification of Nutritionally Efficient Silkworm Germplasm Breeds

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Abstract: In order to amalgamate the effects of nutrition and its efficiency on genetic traits expression in silkworm germplasm breeds by serial analysis on nutrigenetic traits and utilized as biomarker with an aim to identify nutritionally efficient silkworm germplasm breeds. In the present study, six bivoltine silkworm breeds were subjected for investigation on ingestion, digestion and utilization of dry food matter in single silkworm larva and its sharing on economically important stages 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 analyzed by standard gravimetric method with the aid of INDOSTAT software to understand the nutrigenomic divergence among the silkworm germplasm breeds against different seasons. Results indicated that higher conversion efficiency was observed in most of the new silkworm breeds for Efficiency of Conversion of Ingesta (ECI) to cocoon and shell than control (APS_8) as leaf - cocoon and leaf-shell conversion are the ultimate indices in nutrigenomic analysis. It also demonstrated that relatively smaller amount of Consumption Index (CI), respiration, Metabolic Rate (MR) with superior Relative Growth Rate (RGR) and in relation to quantum of food ingesta and digesta requisite per gram of cocoon and shell found less than the control. This concluded that nutrigenetic traits directly associated to the quantum of food ingesta and digesta g^{-1} of cocoon and shell and such nutrigenetic study pragmatically utilized as biomarkers in order to advocate sturdily the following breeds viz., RBD_1, RBD_4 (peanut type cocoon), RBO_2, RBO_3 (oval type cocoon) were chosen as highly nutritionally efficient bivoltine silkworm germplasm breeds.

Key words: Bivoltine, approximate digestibility, relative growth rate, respiration, ingesta, digesta, excreta, consumption index, metabolic rate

INTRODUCTION

Silkworm, *Bombyx mori* L. is an important economic insect and also a tool to convert leaf protein into silk. The industrial and commercial use of silk, the historical and economic importance of production and its application in all over the world finely contributed to the silkworm promotion as a powerful laboratory model for the basic research in biology (Ramesh-Babu *et al*., 2009). During the last decades, both genome mapping and sequencing methods have advanced significantly to provide a foundation for scientists to understand genome structures and functions in silkworm (Mita *et al*., 2004; Xia *et al*., 2004; Goldsmith *et al*., 2005). The gene expression analysis will be an important tool for unraveling genetic architecture and the connections between genotypic and phenotypic variation, but the results of such studies require careful interpretation. The relationship between the environment and genes has considered bidirectional with food consumption efficiency

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on gene expression varies depending on the genetic background of an organism and expressed physiological or nutritional unit in gene regulation studies (Giacobino *et al*., 2003; Milner, 2004; Kang, 2008; Ogunbanwo and Okanlawon, 2009). Hence, it obviously indicates at higher level, nutrition/physiology play an imperative role of genes expression in genome of an individual and the impact of variations in genome on response to nutrition promises to improve insight into nutrient metabolism and revolutionize biomarkers development to utilize with the genome for its phenotypic expression.

The domesticated silkworm feeds exclusively on the mulberry foliage for its nutrition and produces the natural proteinous silk-the queen of textiles of industrial and commercially significant. The mulberry leaves constituent chiefly proteins beside carbohydrates, vitamins, sterols, phagostimulants and minerals. Silkworm larvae obtain nutrients from mulberry leaves to build up body, sustain life, spin cocoons and egg production. Such nutritional requirement in food consumption have direct impact on the over all genetic traits such as larval and cocoon weight, amount of silk production, pupation and reproductive traits. Silkworm nutrition refers the substances required by silkworm for its growth and metabolic functions and obtained from ingested food of mulberry/artificial diet and remaining other nutritional components are being synthesized itself through various biochemical pathways including proteineous silk fiber of commercial interest (Takano and Arai, 1978; Hamano *et al*., 1986; Zhang *et al*., 2002).

Silkworm breeds differ in their nutritional requirements depending on the variety, rearing environment, season and quantum of nutrition upon the physiological and metabolic activities. In silkworm rearing, food is a factor of paramount importance, which regulates its growth, development and silk yield in sericulture. The basic nutritional aspects of silkworm were studied earlier but implications of these aspects in selection of parental breeds for the breeding programs are very scanty and screening of silkworm germplasm stock in respect of nutrigenomic traits for identifying the nutritionally efficient germplasm breeds were found to be meager. Hence, the present study on nutrigenetic traits analysis in 5th instar was undertaken to determine the superiority of newly evaluated promising silkworm germplasm breeds with respect of consumption and conversion efficiency of mulberry leaves. With the objective of analysis for nutrigenetic traits of the new parental silkworm germplasm breeds and utilized them as biomarkers in identification of nutritionally efficient silkworm germplasm breeds.

MATERIALS AND METHODS

Silkworm germplasm breeds: Six bivoltine silkworm germplasm breeds viz., (RBO_2, RBO_3, RBO_4, RBD_1, RBD_2 and RBD_4) drawn after understanding the genomic diversity and germplasm evaluation from gene the bank of Silkworm Breeding and Molecular Genetic Laboratory (SBMGL) in Andhra Pradesh State Sericulture Research and Development Institute (APSSRDI), Hindupur, India.

Silkworm rearing and nutritional parameters: The nutrigenetic experiments were conducted in APSSRDI, Hindupur, AP, during the major seasons in 2006-2007 covering pre-monsoon, monsoon and post-monsoon of the year in a completely randomized block design. Silkworm rearing was conducted for selected breeds in a plastic tray by feeding V_1 variety of mulberry leaves from the well-maintained irrigated mulberry garden by standard rearing procedure as recommended (Datta, 1992). After resumption from fourth moult, 50 healthy silkworm larvae with three replications for each breed were kept separately in a plastic tray. For the experimental batch, accurately weighed fresh mulberry leaves for three feeds day^{-1} was provided and moisture content of leaves was maintained with utmost care by covering wax paper. Simultaneously, an additional batch of larvae for each breed was maintained to determine the dry weight and subsequently daily increment in larval weight separately was determined (Maynard and Loosli, 1962). The healthy larvae counted daily in each replication and missed larvae were replaced from reserved batch. Left over leaves and excreta were collected on subsequent day on daily basis and kept in a paper cover after separating manually. These left over leaves, excreta and reserve batch larvae were dried in hot air oven daily at about 100°C to attain constant weight for three consecutive times at 6 h interval. Further, observations on dry weight of left over leaves, excreta, larvae, cocoon and shell were recorded.

From the data obtained on the nutrigenetic traits viz., 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 were calculated. Further, the ingesta and digesta required for producing one gram of cocoon and shell (I/g and D/g) were worked out as described by standard gravimetric method (Waldbauer, 1968; Scriber and Feeny, 1979). The brief descriptions of the nutrigenetic traits and efficiency parameters applied for the calculation are given below with the respective formulae.

Ingesta: The total intake of the dry weight of mulberry leaves by silkworm larvae during the 5th stage upto spinning or ripening stage:

$$\text{Ingesta} = \text{Dry weight of leaf fed-Dry weight of left over leaf}$$

Digesta: 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:

$$\text{Digesta} = \text{Dry weight of leaf ingested-Dry weight of litter}$$

Excreta: Excreta refer to the non-utilized mulberry leaves in the form of litter from the ingested mulberry leaves of a silkworm:

$$\text{Excreta} = \text{Ingesta-Digesta}$$

Approximate Digestibility (AD): It directly indicated the assimilation efficiency of mulberry leaves and depends on the passage rate of food through gut in silkworm. This is certainly a racial trait in silkworm and higher food intake does not necessarily result in higher digestibility. The approximate digestibility is highest in the first instar but food intake of this instar is less and vice versa in 5th instar:

$$\text{AD} = \frac{\text{Dry weight of digesta}}{\text{Dry weight of food ingested}} \times 100$$

Reference Ratio (RR): An indirect expression of absorption and assimilation of food and expresses the ingesta required per unit excreta produced. Higher the RR value indicates the rate of digestion and absorption of food is more:

$$\text{RR} = \frac{\text{Dry weight of food ingested}}{\text{Dry weight of excreta}}$$

Consumption Index (CI): It was related on the rate of food intake to the mean weight of the larvae during the feeding period with the high nutritional interest since, it measures the rate at which nutrients enter the digestive system:

$$\text{CI} = \frac{\text{Ingesta}}{\text{5th age mean fresh larval weight (g)} \times \text{5th age larval duration (days)}}$$

Relative Growth Rate (RGR): The physical or phenotypic or biological gain in biomass by growth after utilizing the nutrition fed within the limited duration from the initial stage and it differs among the races. It refers to larval gain biomass and indicates the efficiency of conversion of nutrition into larval biomass:

$$\text{RGR} = \frac{\text{Weight gain of the larva during feeding period}}{\text{5th stage mean fresh larval weight (g)} \times \text{5th stage larval duration (days)}}$$

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:

$$\text{Respiration} = \text{Dry weight of food digested-Maximum dry weight of larvae}$$

Metabolic Rate (MR): It measure of total biochemical reactions involving both catabolic as well as anabolic reactions of an organism and associated with the degradation of macromolecules into smaller unit and vice versa:

$$\text{MR} = \frac{\text{Respiration}}{\text{5th stage mean fresh larval weight (g)} \times \text{5th stage larval duration (days)}}$$

Efficiency Conversion of Ingesta (ECI) to larva: This parameter associated with the efficiency conversion of ingested or digested nutrition into biomass or body matter at different stages and expressed in percentage. ECI to larva is the efficiency of conversion of ingested food into larva:

$$\text{ECI to larva} = \frac{\text{Maximum dry weight of larva}}{\text{Dry weight of ingesta}} \times 100$$

Efficiency Conversion of Digesta (ECD) to larva: The expression of efficiency conversion of digesta into larval biomass and calculated by the following formulae:

$$\text{ECD to larva} = \frac{\text{Maximum dry weight of larva}}{\text{Dry weight of digesta}} \times 100$$

Efficiency Conversion of Ingesta (ECI) to cocoon: The paramount importance in practical sericulture industry and expression of efficiency conversion of ingesta into cocoon. It also referred as leaf-cocoon conversion rate and it was the ultimate indices to evaluate superiority of breed in nutritional efficiency:

$$\text{ECI to cocoon} = \frac{\text{Dry weight of cocoon}}{\text{Dry weight of ingesta}} \times 100$$

Efficiency Conversion of Digesta (ECD) to cocoon: The expression for efficiency conversion of digesta into cocoon and calculated by the following equation:

$$\text{ECD to cocoon} = \frac{\text{Dry weight of cocoon}}{\text{Dry weight of digesta}} \times 100$$

Efficiency Conversion of Ingesta (ECI) to shell: An important economic trait in sericulture industry with the expression efficiency conversion of ingesta into shell. However, it referred as leaf- shell conversion rate and the ultimate indices to evaluate superiority of breed in nutritional efficiency:

$$\text{ECI to shell} = \frac{\text{Dry weight of shell}}{\text{Dry weight of ingesta}} \times 100$$

Efficiency Conversion of Digesta (ECD) to shell: The expression of efficiency conversion of digesta into shell and calculated by the following formula:

$$\text{ECD to shell} = \frac{\text{Dry weight of shell}}{\text{Dry weight of digesta}} \times 100$$

Ingesta per gram (I/g) cocoon: These calculated data having most economical significance to assess the silkworm breed performance in nutrigenomic analysis. This particular trait was the expression of total ingesta required for the production of one gram of cocoon.

$$\text{I/g shell} = \frac{\text{Dry weight of ingesta}}{\text{Dry weight of cocoon}}$$

Digesta per gram (D/g) cocoon: The total digesta necessary for the production of one gram cocoon and it would be arrived by following formula:

$$\text{D/g cocoon} = \frac{\text{Dry weight of digesta}}{\text{Dry weight of cocoon}}$$

Ingesta per gram (I/g) shell: The total ingesta required for the production of one gram shell and it attained through subsequent formula:

$$\text{I/g shell} = \frac{\text{Dry weight of ingesta}}{\text{Dry weight of shell}}$$

Digesta per gram (D/g) shell: Total digesta required for the production of one gram shell and achieved through given formula:

$$\text{D/g shell} = \frac{\text{Dry weight of digesta}}{\text{Dry weight of shell}}$$

The pooled data obtained was subjected for biometric analysis with the assistance of computer software INDOSTAT to determine the significance among the breeds, seasons and in contrast to the control with regard to nutrigenetic and nutritional efficiency traits (Kogan and Parra, 1981).

RESULTS

Phenotypic diversity among bivoltine germplasm breeds: It was observed that all silkworm breeds were exogenous in origin. APS_8 was a popular commercialized breed and utilized in the study as a control to compare nutrigenetic trait analysis. The egg shell and larval body marking traits observed to be similar in most of the breeds but on body shape was slender in four breeds (RBO_2, RBO_3, RBO_4, RBD_1) and stout in other two breeds (RBD_2, RBD_4) including control breed. All these hibernating bivoltine germplasm breeds spun white color cocoon with fine and medium grains but oval type in RBO_2, RBO_3, RBO_4 and peanut type in RBD_1, RBD_2 and RBD_4 (Table 1).

The highly significant nutrigenetic diversity observed among the bivoltine silkworm germplasm breeds in different seasons on nutrigenomic traits and its conversion efficiency parameters discussed as follows.

Ingesta, digesta, excreta, AD and RR: The ingesta ranged between highest in RBO_4 (3.786 g) and lowest in RBD_2 (3.368 g) with control APS_8 (3.719 g). The maximum digesta unveiled in RBO_4 (1.335 g) with minimum in RBD_4 (1.111 g) and in control APS_8 (1.196 g). Highest excreta revealed in control APS_8 (1.523 g) and among other breeds, it ranged between lowest in RBD_1 (2.251 g) and highest in RBD_4 (2.508 g). Approximate digestibility ranged between RBO_4 (35.22%) to RBD_4 (30.70%) and control with APS_8 (32.13%). The considerable divergence was noticed in respect to reference ratio with maximum value in RBO_4 (1.546) and minimum in RBD_4 (1.444) while in control APS_8 (Table 2).

Table 1: Morphological salient features of bivoltine silkworm germplasm breeds

		Egg		Larval		Cocoon		
Breed	Origin	Chorion color	Shell color	Markings	Body shape	Shape	Color	Grains
RBO_2	Exogenous	Pigmented	White	Plain	Slender	Oval	White	Fine
RBO_3	Exogenous	Pigmented	White	Plain	Slender	Oval	White	Fine
RBO_4	Exogenous	Pigmented	White	Plain	Slender	Oval	White	Medium
RBD_1	Exogenous	Pigmented	White	Plain	Slender	Peanut	White	Fine
RBD_2	Exogenous	Pigmented	White	Plain	Stout	Peanut	White	Fine
RBD_4	Exogenous	Pigmented	White	Plain	Stout	Peanut	White	Fine
APS_8(C)	A.P	Pigmented	White	Plain	Stout	Peanut	White	Medium

Table 2: Nutrigenetic traits of bivoltine silkworm germplasm breeds

Breeds	Ingesta/larva (g)	Digesta/larva (g)	Excreta/larva (g)	AD (%)	RR	CI	RGR	Respi-ration (g)	MR
RBO_2	3.601	1.228	2.373	34.07	1.518	0.243	0.043	0.445	0.029
RBO_3	3.689	1.253	2.436	33.93	1.514	0.266	0.045	0.484	0.034
RBO_4	3.786	1.335	2.452	35.22	1.546	0.252	0.044	0.589	0.039
RBD_1	3.396	1.145	2.251	33.73	1.509	0.242	0.047	0.354	0.025
RBD_2	3.368	1.232	2.447	33.49	1.505	0.245	0.038	0.553	0.036
RBD_4	3.618	1.111	2.508	30.70	1.444	0.261	0.046	0.341	0.025
APS_8 (C)	3.719	1.196	2.523	32.13	1.474	0.244	0.037	0.527	0.034
SE±	0.0160	0.0140	0.0115	0.2928	0.0065	0.0017	0.0001	0.0134	0.0009
CD at 5%	0.0456	0.0400	0.0328	0.8368	0.0187	0.0049	0.0004	0.0383	0.0025

Table 3: Nutritional efficiency conversion parameters in bivoltine silkworm germplasm breeds

	Larva (%)		Cocoon (%)		Shell (%)		Cocoon (g)		Shell (g)	
Breeds	ECI	ECD	ECI	ECD	ECI	ECD	I/g	D/g	I/g	D/g
RBO_2	21.69	63.68	18.01	52.92	9.17	26.94	5.57	1.90	10.93	3.72
RBO_3	20.79	61.31	16.66	49.15	8.77	25.87	6.03	2.04	11.42	3.87
RBO_4	19.68	56.31	13.24	46.47	8.48	24.19	6.20	2.19	11.81	4.16
RBD_1	23.24	68.99	19.86	58.97	9.58	28.40	5.82	1.72	10.45	3.52
RBD_2	18.44	55.44	15.20	45.66	8.70	26.07	6.62	2.22	11.53	3.87
RBD_4	21.25	69.87	17.43	57.26	8.99	29.46	5.77	1.78	11.16	3.44
APS_8 (C)	17.92	55.77	15.01	46.75	6.99	21.77	6.68	2.14	14.32	4.60
SE±	0.1352	0.7026	0.0809	0.6195	0.0911	0.4078	0.0299	0.0235	0.1180	0.0575
CD at 5%	0.3864	2.0083	0.2313	1.7708	0.2605	1.1656	0.0855	0.0672	0.3373	0.1643

CI, RGR, Respiration and MR: The Consumption Index (CI) found to be highest in RBO_3 (0.266) which followed by RBO_4 (0.261) and lowest in RBD_1 (0.242) with compared to control APS_8 (0.244). There was significant difference observed in Relative Growth Rate (RGR) with maximum in RBD_1 (0.047) followed by RBD_4 (0.046) and minimum in RBD_2 (0.038). A momentous difference reveal with respect to respiration and found relatively more in RBO_4 (0.589 g) followed by RBD_2 (0.553 g), while in control, APS_8 (0.527 g) and less in RBD_4 (0.341 g). The distinguishable divergence for Metabolic Rates (MR) among bivoltine breeds which ranged between top in RBO_4 (0.039) and bottom shared in RBD_1 and RBD_4 (0.025) compared to control (Table 2).

ECI and ECD to larval biomass: The efficiency of mulberry leaves ingested converted into silkworm larval biomass varied significantly among the bivoltine breeds (Table 3). The highest efficiency conversion of ingesta (ECI) to larva recorded in RBD_1 (23.24%) followed by RBO_2 (21.69%) and least in control APS_8 (17.92%). With regard to Efficiency Conversion of Digesta (ECD) to larva, significant variation was observed and more efficient conversion of digested food into larval biomass in RBD_4 (69.87%) followed by RBD_1 (68.99%) and less efficient in RBD_2 (55.44%) while in control APS_8 (55.77%).

ECI and ECD to cocoon and shell: Efficiency Conversion of Ingesta (ECI) to cocoon revealed higher in RBD_1 (19.86%) followed by RBO_2 (18.01%) and lower in RBO_4 (13.24%) and in control APS_8 (15.01%). Efficiency Conversion of Digesta (ECD) to cocoon varied significantly among the breeds which ranged between 58.97% (RBD_1) to 45.66% (RBD_2) and with control, APS_8 (46.75%). ECI to shell observed to be maximum in RBD_1 (9.58%) and minimum in control APS_8 (6.99%). With regard to ECD for shell high conversion in RBD_4 (29.46%) followed by RBD_1 (28.40%) and low in control, APS_8 with 21.77% (Table 3).

I/g and D/g to cocoon and shell: The highest ingesta per gram cocoon (I/g) observed in control APS_8 (6.88 g) and among new breeds it ranged between maximum in RBD_2 (6.62 g) and minimum in RBD_4 (5.77 g). The amount of digesta per gram cocoon (D/g) observed was high in RBD_2 (2.22 g) and low in RBD_1 (1.72 g) control (APS_8) with 2.14 g. With regard to ingesta per gram (I/g) shell noticed to be high in control, APS_8 (14.32 g) and among other breeds it ranged between RBO_4 (11.81 g) and RBD_1 (10.45 g) followed by RBO_2 (10.93 g). The digesta required to one gram (D/g) shell revealed more in control, APS_8 (4.60 g) and among new breeds it ranged between maximum in RBO_4 (4.16 g) and minimum in RBD_4 (3.44 g) (Table 3).

Seasonal variations on nutrigenetic traits: The bivoltine germplasm breeds established to be significant at p#0.001 and p#0.05 on seasonal variation among different seasons against nutrigenetic traits and its conversion efficiency parameters. It was noticed that among pre-monsoon season, highest values were obtained in following nutritional traits such as consumption index (0.268), respiration (0.532 g), metabolic rate (0.040), ingesta and digesta required per gram cocoon (6.48 and 2.16 g) and shell (12.06 and 4.01 g), respectively. Highest nutritional value obtained in monsoon season for the following traits viz., digesta (1.225 g), approximate

Table 4: Analysis of variance on nutrigenetic traits with seasons among bivoltine silkworm germplasm breeds

Source of variations	Ingesta/Larva	Digesta/Larva	Excreta/Larva	AD	RR	CI	RGR	Respi- ration	MR
Breed	0.1396***	0.0486***	0.0761***	19.505***	0.0097***	0.0008***	0.00014***	0.0828***	0.00026***
Season	0.2677***	0.0348***	0.1875***	18.370***	0.0096***	0.0092***	0.0102***	0.1628***	0.00212***
Breed x Season	0.0177***	0.0187***	0.0006***	6.393***	0.0033***	0.0001***	0.0000*	0.0180***	0.00008***
SE±	0.0276	0.0243	0.0199	0.5071	0.0113	0.0030	0.0002	0.0232	0.0015
CD at 5%	0.0759	0.0693	0.5685	1.4493	0.0323	0.0085	0.0007	0.0663	0.0044

*Significant at 5 %; ***Significant at 0.1%.

Table 5: Analysis of variance on nutritional efficiency conversion parameters with seasons among bivoltine breeds

	Larva (%)		Cocoon (%)		Shell (%)		Cocoon (g)		Shell (g)	
Source of variations	ECI	ECD	ECI	ECD	ECI	ECD	I/g	D/g	I/g	D/g
Breed	31.66***	341.07***	25.835***	265.36***	6.053***	59.567***	2.938***	0.3734***	14.13***	1.4202***
Season	97.53***	1219.9***	42.857***	589.25***	1.418***	20.528***	5.122***	0.8197***	2.615***	0.3974***
Breed x Season	0.5061**	44.57***	0.8196***	33.79***	0.1277	9.141***	0.0614***	0.0514***	0.2234	0.1857***
SE±	0.2342	1.2170	0.1402	1.0731	0.1579	0.7063	0.0518	0.0407	0.2044	0.0995
CD at 5%	0.6693	3.4785	0.4006	3.0671	0.4512	2.0188	0.1480	0.1164	0.5842	0.2845

**Significant at 1 %; ***Significant at 0.1%

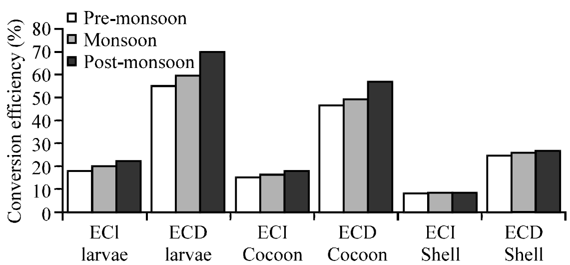

Fig. 1: Seasonal variance on nutritional efficiency conversion in bivoltine silkworm germplasm breeds

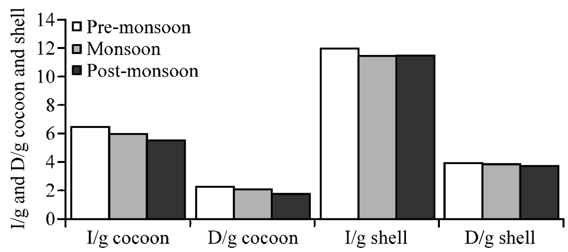

Fig. 2: Seasonal variance on nutrition required per gram cocoon and shell in bivoltine germplasm breeds

digestibility (34.27%), reference ratio (1.523), relative growth rate (0.044) and Efficiency Conversion of Ingesta (ECI) to shell (8.87%). However, maximum values attained in post -monsoon season for nutritional traits namely ingesta (3.745 g), excreta (2.531 g), RGR (0.044), ECI to larva (22.58%), Efficiency Conversion of Digesta (ECD) to larva (69.93%), ECI to cocoon (18.33%) and ECD to cocoon (56.95%) among the experimental trial in different seasons (Fig. 1, 2).

The mean squares values obtained on biometrical analysis and its significance for analysis of variance among the breed, season and breed x season interaction in understanding of nutrigenetic traits and conversion efficiency parameters in bivoltine germplasm breeds. Highly significant (p#0.001) difference observed between the breeds and season as source of variance for all the nutrigenomic traits. Highly significant (p#0.001) variations were observed for major traits and non-significant in Efficiency Conversion of Ingesta (ECI) to shell and ingesta required per gram of shell traits. Significant effect (p#0.01) for ECI to larva and significant (p#0.05) in Relative Growth Rate (RGR) noticed against breed versus season interaction as source of variance (Table 4, 5).

DISCUSSION

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 (Iftikhar and Hussain, 2002; Phillips *et al*., 2008). 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 (Mano *et al*., 1991; Zhang *et al*., 2002). Further, it was established that silkworm derives over 70% of the protein from the mulberry leaves and in 5th instar upto 96% of ingested protein is used for silk protein synthesis and variation in the quantity or quality of nutrition have profound effect on insect development

(Fukuda *et al*., 1963). 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% of the total food intake during the last two instars and the feed utilization study confined to 5th instar larvae as 80-85% of the total leaves consumed in this instar as silkworm very active metabolically at this stage (Rahmathulla *et al*., 2005). Hence, the present study was chosen to conduct experiment to confine to 5th inster or stage of the silkworm rearing only. The feed efficiency or nutrigenetic traits, its genetic expression and its inheritance prototype in silkworm comprehensively discovered (Ding *et al*., 1992; Tzenov *et al*., 1995, 1997). 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 (Hamano *et al*., 1986).

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 (Magadum *et al*., 1996; Gokulamma and Reddy, 2005). 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 (Hassanein *et al*., 1972). 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 control than those of new silkworm breeds indicate that new 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 of the Anantharaman *et al.* (1995) and Rahmathulla *et al.* (2003). 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 (Rahmathulla *et al*., 2003). A slant variation observed in relative growth rate between the breeds was due to less difference in larval duration among bivoltine germplasm breeds. Further, highly significant variance was revealed for the respiration, metabolic rate and other nutrigenetic traits among silkworm germplasm breeds with interaction of different seasons. It was also noticed that the less ingesta with high CI and RGR might be due to shorter larval duration in pre-monsoon and in other two seasons fluctuate with varying at possible extent indicative of the seasonal effect on nutrigenomic traits (Gokulamma and Reddy, 2005). More, Critical Difference (CD) value (1.4493) was found in Approximate Digestibility (AD) and less value (0.0007) in Relative Growth Rate (RGR) among nutrigenetic traits. Among nutritional conversion efficiency parameters, Efficiency Conversion of Digesta (ECD) to larva exhibit more Critical Difference (CD) values (3.4785) followed by ECD to cocoon (3.0671) and less in digesta gG^{1} cocoon (0.1164). Hence, highly significant effect was noticed against almost all nutrigenetic traits and its conversion efficiency parameters on seasons.

Efficiency of the nutrition almost nullified by the increase in consumption result in increased production of cocoon, shell and understood that dietary factors and related metabolic interactions has direct and indirect influence on specific gene expression. Feed conversion efficiency contributes directly and indirectly to the major chunk of the cost benefit ratio of silkworm rearing and considered as an important physiological criterion for evaluating the superiority of silkworm breeds. The Efficiency Conversion of Ingesta (ECI) to cocoon and shell, which otherwise be referred as leaf-cocoon and leaf-shell conversion rate and ultimate indices to evaluate nutritional efficient silkworm breed in terms of the production of cocoon/shell (Ding *et al*., 1992; Junliang and Xiaofeng, 1992; Maribashetty *et al*., 1991; Prabhakar *et al*., 2000). Among the total cost of cocoon production, more than 60% of contribution is associated with mulberry cultivation itself in sericulture industry. Therefore, silkworm breeds with lower ingesta and higher conversion efficiency of nutrition into cocoon and shell attracts the attention of the silkworm physiologist and breeder in evolving nutritionally efficient breeds. In analogous to the above, the present study major aims to identify nutritionally efficient silkworm breeds with lower food ingesta with higher conversion rate on to larval, cocoon and shell biomass. A significant difference between the new silkworm germplasm breed and influence of season on conversion efficiency traits found to exhibit its gene expression. But, in comparison with the control most of new germplasm breeds shown higher conversion efficiency. These observations made in the present study on nutrigenetic traits analysis and its phenotypic expression of gene product was utilized as a biomarker in identification of nutritionally efficient silkworm breed as suggested by Ommen (2004), Rajesh and Haemanand (2005).

Further, in relation to the quantum ingesta requisite g^{-1} of cocoon and shell found to demonstrate in all the breeds less than the control among bivoltine germplasm breeds. For quantum of digesta essential per gram of cocoon and shell found to express that less than in bivoltine germplasm breeds. It was also noticed that high feed intake leads to comparatively high digesta to certain extent and quantum of food ingesta and digesta per gram of cocoon and shell quite understandingly related. The ingesta and digesta inversely proportional to the efficiency of conversion and directly proportional to the ingesta and digesta of the bivoltine silkworm breed respectively. However, highly significant variance for the respiration, metabolic rate and other nutrigenetic traits in silkworm breeds, between the breeds, season and its interaction were observed (Junliang and Xiaofeng, 1992; Maribashetty *et al*., 1991; Gokulamma and Reddy, 2005). In addition to above, it also unveiled that in pre-monsoon season maximum values were observed in CI, respiration and metabolic rate was due to less larval duration among bivoltine breeds and these observations suggest that bivoltine breeds prone to seasonal fluctuations than that of polyvoltine for important nutritional traits as revealed by Anantharaman *et al.* (1995). 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 (Periaswamy *et al*., 1984).

In view of above 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 present study sturdily suggested the four bivoltine silkworm germplasm breeds viz., RBD_1, RBD_4 (peanut type cocoon), RBO_2, RBO_3 (oval type cocoon) as nutritionally efficient based on nutrigenetic traits analysis for future synthesis of nutritionally efficient hybrids in hybridization program (Fig. 3a-d).

Fig. 3: Nutritionally efficient bivoltine silkworm germplasm breeds, (a) RBD_1, (b) RBD_2, (c) RBO_2 and (d) RBO_3

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