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Impact of starvation on productive traits and associated biochemical variations in selected bivoltine silkworm genetic resources

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Abstract
The effect of starvation on selected silkworm genetic resources was studied on economic and biochemical parameters and nutritional indices to identify the genetic hardiness. Approximate digestibility (AD), Consumption index (CI), Reference ratio (RR), Efficiency of conversion of ingesta and of digesta (ECI and ECD) to larval body, cocoon, shell, ingesta and digesta per gram (I/g and D/g) cocoon and shell were used to measure genetic hardiness. A significant improvement on growth and enzyme activity /stability was observed in promising silkworm breed BBE-0164 and BBI-0341 as a result of reduced feeding amount offered the role of feeding restriction on growth and enhanced biochemical activity is discussed. Also these races showed the presence of thermo stable esterase enzyme resisting temperature upto 60̊ C. These parameters may throw a better light on how best these races /breeds could be effectively reared under minimal food availability and utilized for breeding as a better stress tolerant pure breeds.

Keywords: bivoltine silkworm germplasm, biochemical analysis, nutritional indices, stress

Introduction
The genetic variation of base population is essential for genetic improvement in silkworm race. Though the silkworm ingests same amount of mulberry leaves, it shows significant difference in its ability to digest, absorb and convert the leaves to silk. Feed conversion efficiency is an important criterion in the evaluation of silkworm breeds as it contributes to the cost benefit ratio of the silkworm rearing. It is an important physiological phenomenon and the ability of the different silkworm breeds varies in ingestion of mulberry leaf, digest, absorb, assimilate and convert it to silk fiber.. The food ingested influences its survival, growth and development The smaller feed index increased the level of efficiency of conversion of digested food (ECD) and efficiency of conversion of ingested food (ECI) in silkworm, *Bombyx mori L*. Feeding starvation is a stress, which alters the physiology and growth of a particular organism. Starvation prolongs the duration of larval life and results in mortality, decrease larval, pupal and adult weights, affects survivability, cocoon characters and reproductive ability of resulting adults of many insects (Manabu Kamimura and Makoto Kiuchi 2002) [6], including *Bombyx mori* L. (Okada *et al*., 1989) [7].
The esterase is also reported as thermo stable enzyme and used as a marker for identification of thermo tolerant silkworm and also for screening silkworm genetic resources (Wu and Hou, 1993). Thermo tolerance is the ability of cells and organism to with stand severe elevated temperature after brief exposure to mild elevated temperature. The ability of the organism to tolerate and adjust to varying thermal conditions is a key factor affecting both local habited selection and geographic distribution.
The present study is aimed at understanding the role of feeding restriction on the growth physiology, behaviour and biochemical changes. The data on these parameters will be used to select races /breeds which could be effectively reared under minimal food availability and utilized for breeding as a better stress tolerant pure breeds.

Materials and Methods
In the present study, the silkworm races were selected based on high performance on important economical parameters to study the effect of starvation on economic traits and biochemical parameters are given in the Table 1.
The races chosen for the study were reared under controlled agro climatic conditions (Krishnaswami, 1978) [3]. The larvae from each race were starved for 24, 48 and 72 hrs. Various parameters on larval, cocoon characters and nutritional indices were recorded from samples of control and 24 hours starved larvae. Twenty four hours after the period of starvation over, the larval weight and after completion of the required feeding and growth, the parameters viz., cocoon wt, shell wt, and shell ratio % were recorded. Various nutritional indices viz., approximate digestibility (AD), Consumption index (CI), Efficiency of conversion of ingesta and of digesta (ECI and ECD) to larval body, cocoon and shell and ingesta and digesta per gram (I/g and D/g) cocoon and shell was computed using the formula as described by Waldbauer (1968) [10] The starved larval samples were sacrificed for the collection of haemolymph.
Whereas, the activity of various enzymes viz., amylase, protease and alkaline phosphatase from the haemolymph of control and starved samples of 24hrs, 48 hrs and 72 hrs duration was assayed per the protocol adapted by Fitter *et al*., (2001) [2], Zhao *et al*., (2010) [13] and Zahia *et al*., (2009) [12] respectively. Further the stability of the stress related esterase isozyme was also confirmed by thermo stable esterase analysis in a native 7.5 % gel (Laemmli, 1970) [5].

Results and Discussion

It is evident from the Table-2 that the larval weight in control ranges from 3.94 g (BBI-0300) to 4.5 g (BBI-0341) and maximum larval weight was recorded in accession number BBI-0341 (4.5g), whereas when the larvae were given with stress (starvation), the larval weight ranges from 3.45g (BBI -0342) to 3.70g (BBI -0341) and the larval weight was reduced to the tune of 0.8g in BBI-0341. In starvation BBI -0341 recorded maximum larval weight of 3.70g, whereas the cocoon weight was less in BBE-0164 (1.66g) and more in BBI-0341 (2.16g). After giving starvation the cocoon weight was reduced by 0.46g. The cocoon weight also ranged from 1.4 to 1.73g.

In case of shell weight, it ranges from 0.35g to 0.39g in control (No starvation) and in treated (after 24 hrs of starvation) it was recorded as 0.25gm to 0.32gm. Especially BBI-0300 (control) was recorded maximum shell weight (0.39g) whereas in treated BBI-0342 was recorded with maximum shell weight of 0.3g. With regard to shell ratio, BBE-0160 was recorded 22.29 % both in control and treated. BBI-0342 was recorded 21.43% shell ratio even after giving 24 hours of starvation. This breed resisted 24 hours starvation as there is only meager difference in shell ratio of between control (21.11) and treated (21.43).

Nutritional indices like ingesta (I/g), digesta (D/g), approximate digestibility (AD) and consumption index of selected bivoltine races has been presented in Table-3. Maximum ingesta were recorded in BBI-0303 (6.31g) whereas minimum was recorded in BBI-0342 (4.9g) in control. Whereas in treated (24 hours starved) maximum ingesta was recorded in the same race BBI-0303 (4.5g) but minimum was in the accession BBE-0160 (2.29g). The digesta was maximum in BBE-0164 (3.28) and minimum BBI-0303 and less consumption index (CI) was noticed in BBI-0341.

Under starvation maximum ingesta (4.50g) and digesta (2.39g) recorded in BBI-0303 and minimum in BBI-0341 (2.67g and 0.31g). The approximate digestibility ranged from 46.02% to 56.08% in control, whereas under starvation it varied from 11.44 to 52.98%. AD was less in accession number. BBI-0303 (46.02%) under normal feeding, and it was maximum (52.98 %) under starvation implies this accession performed well even under stress condition (Table-3).

Nutritional efficiency parameter like efficiency of conversion of ingested food to ody substance (ECI), efficiency of conversion of digested food to body substances (ECD) for larvae, shell with shell ratio was worked out Table-4. ECI of larvae was higher in BBI-0303 (control) (11.53) and under starved condition BBI-0300 recorded higher ECI value (13.60). ECD of larvae ranged from 3.17 to 25.04 in control and it was higher in BBI-0300 (30.22) and less in BBI-0341 (-53.10). Under starved condition ECI, the shell ranged from 5.55 to 7.70 under control and the value was higher in BBE-0160 and minimum in BBI-0300 under starvation**.**

The mean performance of six bivoltine silkworm germplasm accessions for the selected parameters like ingestion cocoon ratio (ICR), ingestion shell ratio (ISR), single cocoon weight and single shell weight for control and treated (24 hours starved) has been presented in the Table-5. In control, BBI-0341 performed well with low Ingestion cocoon ratio (ICR) and Ingestion shell ratio (ISR), whereas under starvation conditions BBE-0160 performed well with low ICR and ISR, but maximum cocoon weight was recorded in BBE-0164 (1.73 g). It was reported that the nutritional requirement and food consumption has a direct impact on larval weight, cocoon weight and quantity of silk production (Kumerasan *et al*., 2001). The relationship between the amount of food intake and cocoon productivity was also reported (Takano *et al*., 1978) [8]. Studies on quantitative analysis of amylase, alkaline phosphatase and protease in the digestive juices of six accessions were taken up to know the effect of starvation. The results on amylase, alkaline phophatase and protease are given in Table-6 and as a graphical representation in Fig.1-3. The amylase activity was found maximum (576 units/g/hr) in BBE-0160 and minimum (76 Units/g/hr) in BBI-0342. This study indicates that the BBE-0160 could withstand a maximum starvation effect upto 48 hrs indicating its ability to cope up stress conditions. Work carried out in lobster under starvation showed that they were not gaining sufficient growth due to low activity of amylase (Danielle J. Johnston *et al*., 2001) [1].

Table 1: List of silkworm germplasm, their origin and morphological characters.

Accession No.	Name of the Race	Donor	Origin	Larval Pattern	Cocoon color and shape
BBE-0160	(N112.C110)(N124.C124)	CSRTI, Pampore	Exotic	Plain	White, EC
BBE-0164	Shongetsu Hosho	CSRTI, Pampore	Exotic	Marked	White, EC
BBI-0300	YS-5	RSRS, Sahaspur	India	Marked	White, Oval
BBI-0303	KSO-1	KSSRDI, Bangalore	India	Plain	White, Oval
BBI-0341	NK-1	KSSRDI, Bangalore	India	Plain	White, ENC
BBI-0342	NK-2	KSSRDI, Bangalore	India	Plain	White, Oval

EC-Elongated and constricted, ENC- Elongated and not constricted

Table 2: Effect of starvation in larval and cocoon characters of selected BV silkworm germplasm

	Larval weight		Cocoon weight		Shell weight		Pupal weight		Cocoon Shell ratio	
ACC. No.	**Control**	**24 hrs**	**Control**	**24 hrs**	**Control**	**24 hrs**	**Control**	**24 hrs**	**Control**	**24 hrs**
BBE-0160	4.12	3.52	1.66	1.55	0.37	0.25	1.15	1.27	22.29	16.13
BBE-0164	4.36	3.70	1.84	1.73	0.37	0.28	1.33	1.38	20.11	16.18
BBI-0300	3.94	3.60	1.89	1.5	0.39	0.28	1.52	1.27	20.63	18.67
BBI-0303	4.31	3.69	1.95	1.55	0.35	0.29	1.38	1.26	17.95	18.70
BBI-0341	4.5	3.70	2.16	1.7	0.38	0.28	1.65	1.40	17.59	16.47
BBI-0342	4.3	3.45	1.8	1.4	0.38	0.32	1.46	1.21	21.11	21.43

(All the values are expressed in grams)

Table 3: Nutritional indices of selected BV silkworm germplasm

ACC. No.	I/g		D/g		AD%		CI	
	Control	24 hours	Control	24 hours	Control	24 hours	Control	24 hours
BBE-0160	6.01	2.29	3.19	0.53	53.10	23.01	0.73	0.65
BBE-0164	6.12	2.74	3.28	0.49	53.59	17.91	0.70	0.74
BBI-0300	5.69	3.38	3.19	1.52	56.08	45.01	0.72	0.94
BBI-0303	6.31	4.50	2.90	2.39	46.02	52.98	0.73	1.22
BBI-0341	5.83	2.67	2.69	0.31	46.11	11.44	0.65	0.72
BBI-0342	4.93	3.57	2.35	1.71	47.54	47.83	0.57	1.04

I-Ingesta per gram, D-Digesta per gram, AD-Approximate digestibility, CI-Consumption Index

Table 4: Nutritional efficiency parameters of selected BV silkworm germplasm

	ECI larvae		ECD larvae		ECI shell		ECD shell	
ACC. No.	Ctrl	24 hrs	Ctrl	24 hrs	Ctrl	24 hrs	Ctrl	24 hrs
BBE-0160	8.91	-1.66	16.79	-7.22	6.15	10.94	11.59	47.53
BBE-0164	3.24	0.99	6.04	5.50	6.05	10.22	11.29	57.03
BBI-0300	7.69	13.60	13.72	30.22	6.85	8.30	12.21	18.43
BBI-0303	11.53	10.06	25.04	18.99	5.55	9.77	12.06	18.45
BBI-0341	1.46	-6.07	3.17	-53.10	6.52	10.50	14.14	91.77
BBI-0342	4.84	4.06	10.19	8.49	7.70	8.40	16.20	17.56

Efficiency of conversion ingesta and digesta (ECI and ECD)

Table 5: Mean performances of feeding indices for selected BV silkworm germplasm

ACC. No.	Ingesta		cocoon wt (gm)		shell wt(gm)		ICR		ISR	
	control	24 hrs	control	24 hrs	control	24 hrs	control	24 hrs	control	24 hrs
BBE-0160	6.01	2.29	1.66	1.55	0.37	0.25	3.62	1.47	16.25	9.14
BBE-0164	6.12	2.74	1.84	1.73	0.37	0.28	3.32	1.58	16.53	9.79
BBI-0300	5.69	3.38	1.89	1.50	0.39	0.28	3.01	2.25	14.60	12.05
BBI-0303	6.31	4.50	1.95	1.55	0.35	0.29	3.23	2.90	18.02	15.52
BBI-0341	5.83	2.67	2.16	1.70	0.38	0.28	2.70	1.57	15.34	9.53
BBI-0342	4.93	3.57	1.80	1.40	0.38	0.30	2.74	2.55	12.98	11.90

Ingestion cocoon ratio (ICR), Ingestion shell ratio (ISR),

Table 6: Studies on quantitative analysis of three enzymes in digestive juices

Enzyme	Amylase				Alkaline phosphatase				Protease			
Acc. no	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
BBE-0160	180	348	364	576	200	120.4	160	100.4	68.8	76	84	104
BBE-0164	100	364	124	204	220	140.8	147.2	100	184.8	84	68.8	96
BBI-0300	160	312	184	264	220.8	121.2	147.2	120	74.4	88	71.2	88
BBI-0303	160	168	184	152	241.2	140	160	100.4	128	98.4	80	76
BBI-0341	152	180	212	152	300	121.6	180	100.4	141.6	100.8	59.2	59.2
BBI-0342	264	212	228	76	141.6	121.6	147.2	100.4	141.6	102.4	86.4	121.6

T1-control, T2-24 hours starved, T3-24 starved & fed, T4-48 hours starved.
(All the values are expressed in units/g/hour of enzyme activity)

The alkaline phosphatase activity was found maximum (120 units/g/hr) in accession BBE-0300 and minimum (100 Units/g/hr) in BBE-0164. This study indicates that BBE-0300 could withstand the maximum starvation effect up to 48 hrs among these six races. All the accessions showed a decline in alkaline phosphatase activity indicating this enzyme was highly sensitive to the starvation stress. These results were similar to the studies done on rats by Thompson (1989) where, Starvation caused a significant fall in alkaline phosphatase activity.

The results on resistance to the temperature viz., 40ºC, 60ºC, and 80ºC were identified from the enzyme sensitive to the temperature given in Fig. 4 to 6. The results on heat treatments from 40º C to 60 º C did not affect the activity of the enzyme in all the races, whereas at higher temperature treatment of 80ºC, the enzyme activity was completely inhibited suggesting a possible denaturation at this particular temperature. This temperature was found highly inhibitive to the enzyme in the haemolymph samples of all the races. Most probably all these accessions possess more sensitivity to higher temperature.

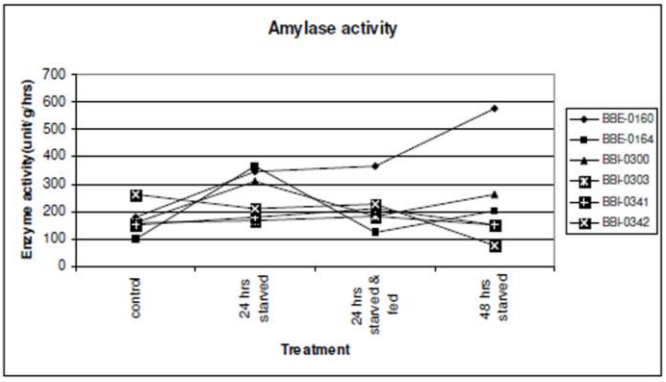

Fig 1: Amylase enzyme activity on starvation

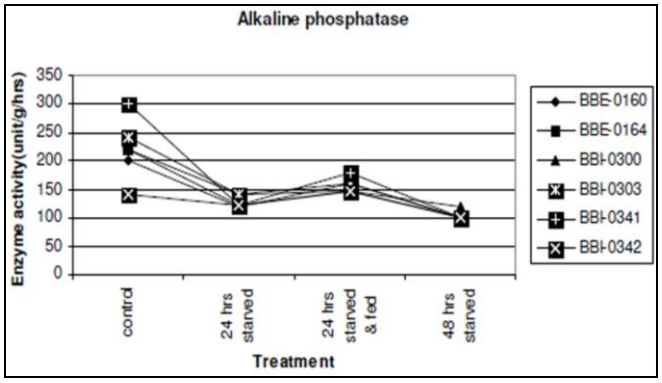

Fig 2: Alkaline phosphatase enzyme activity on starvation

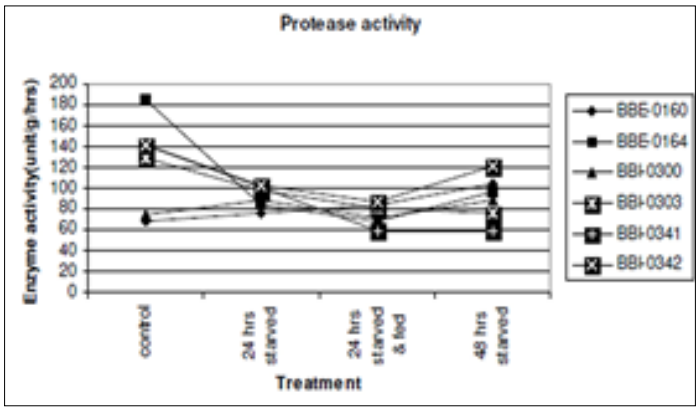

Fig 3: Protease enzyme activity on starvation

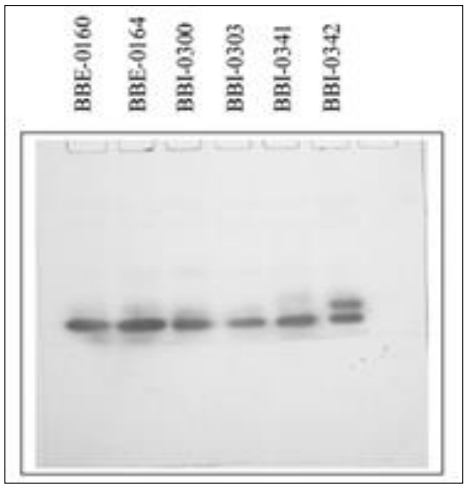

Fig 4: Thermostability of silkworm races at 40ċ

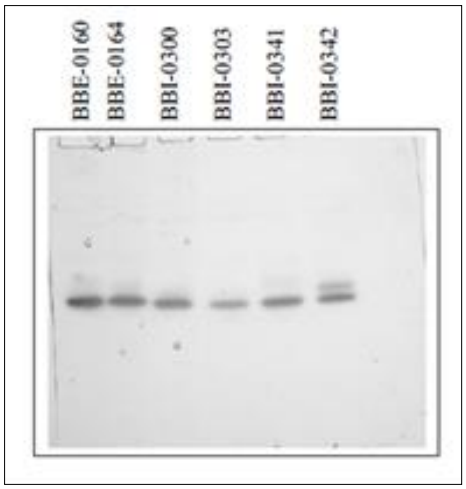

Fig 5: Thermostability of silkworm races at 60ċ

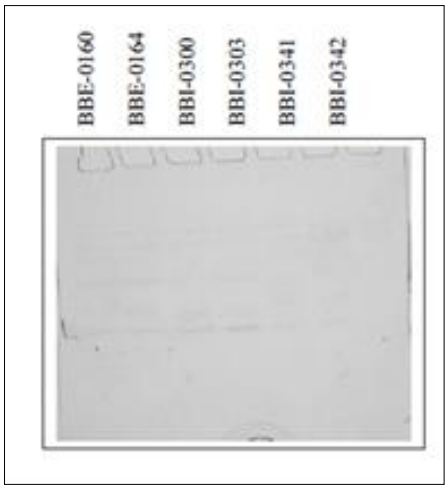

Fig 6: Thermostability of silkworm races at 80ċ

Conclusion

Under starvation condition the accessions BBE-0164 and BBI-0341 performed better for larval weight (3.7gm), cocoon (1.73gm and 1.7gm) and shell weight 1.28gm. Whereas the pupal weight was more in these two accessions, it reflects on reduced cocoon shell ratio (16.18 and 16.47%). The accession BBI-0303 showed a maximum ingesta and digesta in starved conditions. Consumption index was less in BBE-0160 and BBI-0341, BBI-0303 and BBI-0300 showed maximum ECI for larval weight under starvation conditions. Under stress condition, BBE-0160, BBE-0164 and BBI-0341 performed better as shown by high shell weight. All races showed a thermo tolerance up to 60^0C for the esterase enzyme activity when treated in native gel electrophoresis. Maximum amylase activity was observed in BBE-0160 under longer starvation period (48 hrs). All the races showed a reduction in alkaline phosphatase activity indicating an imbalance in acid-base system. BBI-0342 showed higher protease activity under starvation condition.

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