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Nutrigenic Efficiency Change and Cocoon Crop Loss due to Assimilation of Leaf Spot Diseased Mulberry Leaf in Silkworm, *Bombyx Mori L.*

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Abstract

Healthy growth and development of silkworm largely depending on the quality of mulberry leaves. The activity of sericulture is declining due the reduction of mulberry production area in sericulture practicing countries, which lead to adverse effects on silkworm rearing and cocoon production. Screening for nutritional trait change by feeding leaf spot diseased of mulberry leaf to silkworm, *Bombyxmori L.* (Lepidoptera: Bombycidae) is an essential prerequisite for better understanding of reduced food consumption, nutritional efficiency loss and low efficiency conversion. The aim of this study was to identify efficiency and cocoon crop loss due to the consumption of leaf spot diseased mulberry leaves to bivoltine silkworm breeds using the hybrid races as SH6 and NB_4D_2. The 1stday of 5th stage silkworm larvae of bivoltine strains were subjected to standard gravimetric analysis until spinning for two tothree consecutive generations covering two different seasons on 11 nutrigenic traits. Highly significant ($p \leq 0.01$) differences were found among all nutrigenic traits of bivoltine silkworm strains in the treated worms compared to the control worms, where healthy leaves were given. Higher nutritional efficiency conversions were found in the bivoltine silkworm strains on efficiency of conversion of ingesta to cocoon and shell than leaf spot diseased leaf fed worms of the same races. Comparatively smaller consumption index, respiration, metabolic rate with superior relative growth rate, and quantum of food ingesta and digesta requisite per gram of cocoon and shell were found; the highest amount was in healthy leaf fed worms than in the diseased leaf fed worms. The significant weight loss in both the races ranged from the 3.38% to 34.28% in the diseased leaf fed larva as compared to the healthy leaf fed larva Furthermore, based on the overall loss in nutrigenic traits utilized as index or 'biomarkers', the two bivoltine silkworm strains (SH6 and NB_4D_2) were identified as having the high potential for nutrition efficiency conversion, when healthy leaves were provided to the silkworms. The data from the present study advances our knowledge to study the loss of nutritional efficiency conversion due to the leaf spot disease fed mulberry leaves and their effective commercial consequences in the sericulture industry progress and management.

Keywords: Efficiency conversion; Nutrigenic trait; bivoltine breed; leaf spot disease; *Bombyxmori L.*

Introduction

To achieve the goal of production of good quality silkworm cocoon crop, certain factors play important role. The most important factor is the mulberry leaf, contributing about 38.2 % followed by climate (37.0%), rearing techniques (9.3%), silkworm race (4.2%), silkworm egg (3.1%) and other factors (8.2 %) in producing good quality cocoons (1). Hence, quality of mulberry leaf is one of the basic prerequisite of sericulture and plays a pivotal role for successful silkworm cocoon crop. Healthy mulberry leaves influences the growth, development and quality of cocoons formed and thus decide the superiority of silk to a greater extent. Hence production of good quality leaves in terms of nutrition is very important in ensuring quality besides quantity. The mulberry silkworm, *Bombyxmori L.* (Lepidoptera: Bombycidae) is a monophagous insect that feeds exclusively on the mulberry (Morus spp.) foliage for its nutrition and produces the natural proteinaceous silk. 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 the reduction of mulberry production area in sericulture practicing countries on silkworm rearing and silk production. This consensus is more pronounced in countries more advanced in sericulture compared to developing countries in Asia and Pacific regions. Thus, among many factors attributed to reduction in silk production, the major one is the loss of nutrition efficiency conversion in Bivoltine silkworm strains in temperate areas because of feeding the worms with the diseased leaves and mostly the leaf spot diseased leaves. This disease is predominant during the rearing period of the silkworms in the temperate region Therefore, one of the key considerations in developing Bivoltine hybrids for temperate regions could be the need for nutrition feeding the bivoltine strains with healthy mulberry leaves. The recent advances in silkworm breeding and those with nutrition efficiency loss have opened up new avenues to evolve different management strategies to control the leaf spot disease, the deadly disease of mulberry. Sericulture in India is practiced to a limited extent in the temperate environment of Jammu and Kashmir. The existing situation provides scope for creating biovoltine hybrids (crossbreeds) as a commercial venture as hybrids above 90% of total silk production

(2). Crossbreed nutritional efficiency conversion is low when compared to the existing bivoltine (3). Earlier studies have demonstrated fundamental interaction of nutrition/physiology on gene expression (4). Similarly, nutrition or diet/physiology play important roles in insect gene expression (5), some earlier studies addressed the importance of nutritional aspects, but nutrigenics is often neglected in the selection of silkworm breeds with respect to type of nutrition consumption and efficiency conversion for evaluating the loss due to the foliar diseases on nutritionally efficient silkworm hybrids. However, a clear understanding of the genetic basis and variability in the gene expression of productive and qualitative traits during the analysis of loss of nutrigenetic traits are an important step for the production of good quality leaf and to increase the efficiency conversion.

The purpose of this study is to obtain new data about screening the nutritional efficiency loss in bivoltine silkworms due to leaf spot disease, only to augment current knowledge on gene interaction between nutrition efficiency conversion and loss of quantitative traits due to feeding leaf spot diseased leaves to the bivoltine silkworms under varied conditions.

Materials and Methods

Bivoltinesilkworm hybrid races

The two Bivoltine silkworm breeds used for the study were SH6 and NB_4D_2. These strains with varied phenotypic quantitative traits, maintained at Silkworm Breeding and Genetics section of Central Sericulture Research and Training Institute, Gallander, Pampore, India, were utilized for the study.

Silkworm rearing

The disease-free layings (DFLs) from each strain were reared and cocoons were harvested and maintained until eclosion of moths. Healthy female moths emerging on the peak day of eclosion were allowed to mate for 3-4 hours and held until oviposition. The eggs were incubated at 25 ± 1°C temperature and 70- 80% relative humidity (RH) after surface treatment with 2% formalin solution. 20 to 30 eggs were chosen from each brood and pasted onto to egg sheets. Three such egg sheets for each breed were prepared, wrapped in white tissue paper and boxed with black paper to synchronize the embryonic development. On the day of hatching, the eggs were exposed to light in order to obtain uniform hatching, and finely chopped fresh mulberry leaves were fed to the young eclosions. The whole process from silkworm egg incubation to completion of rearing activities was carried out under hygienic conditions in a silkworm-rearing house that had been thoroughly disinfected with bleach followed by formalin solution. Silkworm rearing was conducted for each breed in plastic trays by feeding them with the healthy and Leaf spot diseased Goshoerami, TR-10, Ichinose and Chinese white variety of mulberry leaves from the well maintained irrigated mulberry plots in rearing room of Entamology and Pathology section of the Institute. A standard rearing procedure was adopted as recommended by Krishnaswami et al. (6). The young larvae (1st-3rd instars) were reared at 26-28 °C with 80-90% RH, and late age larvae (4th and 5th instars) were maintained at 24-26°C with 70-80% RH until the resumption of 4th molt. Each batch was divided into six, one of which was maintained as reserved stock under standard rearing conditions and the other five were subjected to standard gravimetric analysis. Reserve batch of 200 worms of each breed (NB_4D_2 and SH6) were fed with the healthy leaves of the selected mulberry varieties in the separate trays in the same rearing room.One batch fed with the healthy leaves and the other three were treated with the 4th grade leaf spot diseased respective leaves.

Estimation of nutritional traits

The nutrigenetic traits estimation study was carried out in July 2012 to August 2012 covering the summer rearing period of the temperate area, 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 hybrid in three replications of 150 larvae each were selected for estimation on nutritional traits analysis. Accurately weighed fresh mulberry leaves were fed 3 times a day to the experimental batches and the control. Simultaneously, an additional batch of larvae for each breed was maintained to determine the dry weight on subsequent daily increments in larval weight were recorded separately as suggested by Maynard and Loosli (7). Silkworm rearing continued using appropriate plastic trays. The healthy larvae were counted daily in each replicate, and any missed larvae were replaced from the reserve batch. Left over leaves and excreta were collected on each subsequent day, separated manually and dried in a hot air oven daily at about 100-115°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 4-5 days later after completion of cocoon spinning. Harvested cocoons were accessed 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 was recorded. The nutrigenetic traits interaction was obtained by utilizing standard gravimetric analysis methods for three consecutive seasons. During the silkworm nutritional study, data were collected on the biomass of larvae and cocoons for the 11 nutrigenetic traits on ingesta, digesta, excreta, reference ratio (RR), relative growth rate (RGR), efficiency conversion of ingesta (ECI) and digesta (ECD) for larva, cocoon, and shell in both the treatments as described by standard gravimetric methods (8-11), the equations with brief description of the nutrigenetic traits evaluated 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).

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).

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) x 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 x 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 x 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 x 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 x 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 x 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 x 100). The data obtained was analyzed using technique of ANOVA as given by Ronald E Walpole to test the effectiveness of leaf spot diseased leaves on the rearing and nutrigenic traits of the treated silkworms.

Results

Negative correlations were found against all the 11 traits and no positive correlations were found among these traits (Figures 1).

Performance on nutrigenic traits

Considerable variations were found for 11 nutrigenetic traits and the average larval weight gain (from fourth to fifth instar) among the bivoltine breeds on nutritional parameters and efficiency loss due to feeding the worms with the leaf spot diseased leaves. Data were obtained for ingesta, digesta, excreta, RR, RGR, ECI, and ECD to larval biomass, ECI and ECD to cocoon and shell, I/g and D/g to cocoon and shell for two diseased leaf fed bivoltine breeds under standard nutritional estimation including control breed. There was evidence of clear declines in consumption efficiency in food conversion to biomass for major nutrigenic traits in experimental bivoltine breeds over the control (healthy leaf fed silkworms) worms. (Tables 1-2).

Ingesta, digesta, excreta and reference ratio

In the experiment, the highest decrease in ingesta 42.6% was found in NB_4D_2 race after feeding the larva with leaf spot infected Chinese white leaves followed by SH6 race when fed with the leaf spot diseased TR-10 leaf. The least decrease was found in SH6 worms fed with diseased Chinese white leaves. The highest decrease of 43%, 38.5% and 37.9% in digesta was found in SH6 worms fed with the infected leaves of Chinese white, KNG and TR-10 varieties respectively. Decrease in digesta for the NB_4D_2 was least compared to SH6. The highest decrease in excreta of more than 50% was found in NB_4D_2 and less than 30% decrease was found in SH6 when treated with the healthy leaf compared to control. Reference ratio value was the highest in SH6 breed worms fed with C. White and TR-10 Leaf spot infected mulberry leaf followed by the NB_4D_2 breed worms.

Nutrigenic traits	GSH			KNG			C. W			TR-10		
	I*	H	%D/(χ2)	I*	H	%D/(χ2)	I*	H	%D/(χ2)	I*	H	%D/(χ2)
Ingesta/L (g)	2.36 {8.62}	3.44 {11.0}	31.5 (0.71)	2.16 {8.45}	3.50{10.7}	37.4 (0.38)	2.21 {8.55}	3.85{11.3}	42.6 (0.42)	2.25 {8.82}	3.70 {10.6}	39.2 (0.51)
Digesta/L (g)	0.84 {4.89}	0.9{5.56}	8.33 (0.98)	0.98 {5.25}	1.07 {5.49}	8.06 (0.99)	0.73 {5.65}	0.94{5.92}	22.8 (0.97)	0.75 {4.97}	0.87 {5.36}	13.7 (0.99)
Excreta/L (g)	1.55 {7.13}	2.74{9.51}	43.7 (0.53)	1.27{6.4}	2.80{9.60}	54.7 (0.34)	1.24 {6.38}	2.75{9.55}	55.1(0.34)	1.54 {7.11}	2.73 {9.50}	43.7 (0.55)
RR (g)	2.30{8.84}	2.9{9.78}	18.3 (0.93)	2.32{8.70}	3.30{10.3}	29.2 (0.66)	1.74 {8.45}	2.52 {9.96}	30.8 (0.78)	2.16 {7.58}	3.0{9.12}	27.8 (0.82)

RGR	0.43 {4.11}	0.47{4.65}	9.86(1.0)	0.52 {3.86}	0.66{5.52}	21.7 (0.99)	0.46 {3.73}	0.90{3.93}	50.9 (0.81)	0.74 {4.93}	0.97 {5.64}	23.4 (0.97)
ECIL (%)	53.6 {42.4}	56.3{43.9}	4.86(0.9)	59.5 {40.1}	61.0 {41.9}	2.30 (0.82)	45.7 {38.9}	55.4 {43.0}	17.5 (0.08)	49.5 {41.6}	53.4 {45.8}	7.20(0.77)
ECDL (%)	18.6 {21.4}	20.2{22.4}	7.82 (0.72)	17.7 {21.6}	23.0 {23.1}	23.8 (0.15)	19.6 {20.7}	25.4 {23.1}	22.8 (0.05)	22.9 {19.6}	25.5 {21.4}	10.0(0)
ECIC (%)	45.5 {47.0}	48.3 {48.6}	5.64(0.8)	41.6 {50.5}	45.0 {51.3}	7.15 (0.82)	39.5 {44.7}	46.6 {46.9}	15.2 (0.23)	44.3 {42.5}	51.5 {40.0}	14.0 (0.19)
ECDC (%)	13.3 {25.5}	14.6 {6.7}	8.33 (0.94)	13.6 {24.8}	15.0 {28.8}	12.4 (0.62)	12.5 {26.2}	15.5 {30.1}	19.1 (0.52)	11.4 {28.6}	13.4 {30.3}	15.3 (0.73)
ECIS (%)	16.4 {23.8}	17.7 {24.8}	7.66 (0.74)	20.3 {26.7}	21.0{27.6}	5.61 (1.0)	16.5 {23.9}	18.2 {25.2}	9.35(0.9)	20.4 {26.8}	21.5 {27.6}	5.37 (0.90
ECDS (%)	7.66 {14.6}	8.46 {15.9}	9.53 (0.69)	7.54 {15.9}	8.30 {16.7}	9.62 (0.8)	6.44 {16.0}	7.56 {16.8}	14.8 (0.76)	8.54 {16.9}	9.46 {17.9}	9.76 (0.85)
SEm±/F-test	0.43	0.68	**	0.52	0.58	**	0.49	0.70	**	0.35	0.44	**
CD at 5%	1.26	2.0		1.52	1.71		1.46	2.05		1.04	1.29	
CV (%)	3.94	5.83		4.66	4.80		4.66	5.91		3.22	3.64	
SD±	17.94	18.72		18.67	19.22		16.19	18.01		16.40	19.23	

Table 1: Effect of leaf spot diseased mulberry leaf on various nutrigenic parameters of NB_4D_2 race of silkworm *Bombyxmori L.* in the fifth instar. H= healthy,I= Infected, %D= Percent Decrease, Values in smallBrackets () are the χ2-test values, values in long Brackets{} are arc sinevaluesD/L, digesta per larva; I/L, ingestaperLarva; ECD, efficiency conversion of digesta; ECI, efficiency of conversion of ingesta; RR, reference ratio; RGR, relative growth rate;

Nutrigenic traits	GSH			KNG			C W			TR-10		
	I*	H	%D/(χ2)	I*	H	%D/(χ2)	I*	H	%D/(χ2)	I*	H	%D/(χ2)
Ingesta/L(g)	2.29 {8.70}	3.52 {10.8}	34.8 (0.64)	2.46 {9.01}	3.46 {10.7}	29.1 (0.76)	2.24 {11.9}	4.31 {8.59}	48.1 (0.07)	3.44{11.1}	3.74{10.6}	8.83 (0.96)
Digesta/L(g)	0.76 {4.99}	0.86 {5.32}	11.7(1.0)	0.73 {4.88}	1.28 {6.41}	43.0(0.77)	0.53 {5.75}	1.01 {4.17}	47.5 (0.05)	0.63{5.32}	0.86 {4.53}	37.5 (0.95)
Excreta/L(g)	1.59 {7.25}	2.68 {9.41}	40.5 (0.62)	1.76 {7.62}	2.55 {9.18}	31.0(0.80)	1.67 {10.9}	3.58 {7.42}	53.4 (0.05)	2.79{9.58}	2.78 {10.9}	0.43(1.0)
RR (g)	2.31 {8.73}	2.84 {9.69}	18.7 (0.94)	2.43 {8.96}	3.20{10.2}	23.9 (0.85)	1.98 {11.3}	3.90{8.09}	49.1 (0.08)	2.32{0.72}	3.46 {11.3}	49.3(0.51)
RGR	13.9 {3.95}	15.1 {4.30}	8.01(1.0)	14.6 {3.89}	16.3 {4.26}	10.7(1.0)	12.5 {4.37}	15.4 {3.84}	18.9 (0.98)	13.0{4.30}	14.3 {4.37}	9.85(1.0)
ECIL (%)	0.48 {21.8}	0.56 {22.8}	15.4 (0.94)	0.46 {22.4}	0.56 {23.8}	16.8(0.86)	0.45 {23.3}	0.58 {20.7}	22.9 (0.45)	0.45 {22.2}	0.57 {23.1}	25.9 (0.86)
ECDL (%)	16.7 {40.6}	18.5 {43.0}	9.91 (0.68)	18.4 {40.5}	20.5 {41.8}	10.4 (0.86)	20.4 {43.8}	23.4 {40.1}	12.8 (0.26)	18.5 {44.6}	21.8 {43.8}	17.8 (0.04)
ECIC (%)	42.5 {24.0}	46.5 {25.4}	8.76 (0.87)	42.3 {25.3}	44.5 {26.9}	5.03 (0.70)	41.6 {28.9}	47.9 {26.8}	13.2 (0.63)	40.4 {27.8}	49.3 {28.9}	22.2 (0.39)
ECDC (%)	52.3 {46.2}	54.6 {47.6}	4.27 (0.88)	53.2 {46.8}	58.3 {49.7}	8.73 (0.61)	48.5 {46.9}	53.3 {44.1}	9.05 (0.59)	50.3 {48.0}	55.3 {46.9}	9.90(0.44)
ECIS (%)	16.6 {16.2}	17.3 {16.8}	3.77 (0.94)	20.2 {15.6}	22.6 {16.5}	10.7 (0.93)	17.8 {15.5}	20.1 {17.0}	11.4 (0.71)	15.2 {18.0}	21.4 {15.5}	41.1 (0.24)

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ECDS (%)	7.87 {24.0}	8.39 {24.5}	6.24 (0.98)	7.28 {26.6}	8.13 {28.3}	10.5 (0.48)	7.24 {24.9}	8.57 {26.6}	15.5 (0.78)	6.99 {27.5}	9.56 {24.9}	36.7 (0.01)
SEm±/f-test	0.27	0.28	**	0.34	0.33	**	0.29	0.20	**	0.30	0.33	**
CD at 5%	0.79	0.80		1.00	0.96		0.84	0.57		0.88	0.95	
CV (%)	2.91	2.81		3.61	3.24		2.83	2.12		2.94	3.52	
SD±	17.03	17.95		17.29	18.52		17.56	16.76		18.59	16.23	

Table 2: Effect of leaf spot diseased mulberry leaf on various nutrigenic parameters of SH6 race of silkworm Bombyxmori L. in the fifth instar. %D= Percent Decrease, H= healthy, I= Infected, Values in short Brackets () are the χ2-test values, values in long Brackets{} are arc sine values D/L, digesta per larva; I/L, ingesta per Larva; ECD, efficiency conversion of digesta; ECI, efficiency of conversion of ingesta; RR, reference ratio; RGR, relative growth rate.

Silkwom Race	Parameters	Variety											
		GSH			KNG			C W			TR-10		
		H	I*	% Loss	H	I*	% Loss	H	I*	% Loss	H	I*	% Loss
NBD	Average wt. in4th Instar	5.05	4.22	3.38 (0.83)	5.94	5.34	10.2 (0.97)	6.93	4.55	34.28 (0.35)	7.72	6.31	18.3(0.73)
	Average larval wt. in 5th instar	11.6	10.4	9.28 (0.91)	14.06	12	14.7 (0.75)	12.6	10.74	14.65 (0.78)	13.3	11.1	17.1(0.61)
SH6	Average wt. in4th Instar	5.59	4.78	3.97 (0.90)	5.36	4.73	11.8 (0.95)	6.83	4.86	28.88(0.5)	5.36	4.52	15.6(0.3)
	Average larval wt. in 5th instar	12.9	11.2	13.5 (0.81)	13.02	11.2	14.1 (0.77)	113	93.52	17.48 (0.03)	134	108	6.17(0)
CV (%)/f-test		1.60	4.34	**	2.86	3.61	**	2.95	8.90	**	1.72	8.09	**
Cd at 5%		1.19	2.96		2.37	2.65		2.65	6.57		0.57	6.70	

Table 3: Larval weight of NB_4D_2 and SH6 silkworm breed in 4th and 5th instars. Values in Brackets are the χ2-test value.

Relative growth rate

The highest relative growth rate of more than 50% was found in NB_4D_2 race when fed with the diseased Chinese white leaf, followed by NB_4D_2 when fed with 4th grade leaf spot diseased Goshoerami leaf.

Efficiency of conversion of ingesta and digesta to larval biomass

The efficiency of mulberry leaf ingested and digested in conversion to silkworm larval biomass or body varied prominently among the bivoltine breeds (Table 1 and 2) when fed with leaf spot infected mulberry leaf. The highest decrease of 17.5% efficiency conversion of ingesta was found in NB_4D_2 when fed with leaf spot diseased C. White leaf and 23.8% decrease in efficiency conversion of digesta for larva was recorded in NB_4D_2 fed with Leaf spot diseased KNG leaf.

Efficiency of conversion of ingesta and digesta to cocoon and shell

The significant decrease of 19.1% in efficiency conversion to cocoon was shown in NB_4D_2 race when fed with C. white Leaf spot diseased leaf followed by more than 15% decrease in TR-10 fed diseased leaf in NB_4D_2 and SH6. The 14.6% significant decrease in efficiency conversion of digesta to cocoon was seen in SH6 when C. white leaf was fed and 3.77% least significant in NB_4D_2, when fed with diseased Goshoerami leaf. The significant decrease in efficiency conversion of ingesta to shell ranged from 14.8% in NB_4D_2 to 3.36% in SH6 when both fed with the leaf spot diseased Goshoerami leaf. In the fourth instar the significant larval weight loss of 41.3% due to leaf spot disease was found in NB_4D_2 when fed with diseased mulberry and 56.7% weight loss in fifth instar. The least decrease was found in fifth instar when the worms become the voracious feeders followed by 36.4% and 33.1% in SH6 race when fed with leaf spot infected Chinese white and Goshoerami leaves The least decrease of 3.70% in larval weight loss was found in NB_4D_2 when fed with Goshoerami Leaf Spot diseased leaves.

Based on all morphological, nutrigenic traits and lower consumption of mulberry leaves and maximum efficiency of conversion of nutrients, with highly significant ($p \leq 0.001$) differences in bivoltine breeds for 11 nutrigenetic traits, two bivoltine silkworm breeds, NB_4D_2 and SH6 were identified as potential nutritionally efficient breeding resources for comparative studies in effect of diseased and healthy leaves on larval, cocoon and shell parameters. Silkworm breeding can be defined as the science of improving the genetic entity of silkworms in relation to their economic utility. Silk producing countries in Asia and Pacific regions experience serious problems in the field of silkworm rearing on healthy leaf. This investigation intends to serve as a guideline to organize or revive healthy silkworm feeding programs, as well as a quick reference to sericulture loss. It also offers a brief background on the change in silkworm nutrition and physiology. It also outlines the necessary facilities and tools required to establish modern silkworm breeding programs for the sustenance of sericulture in the temperate regions. The study of the interactions between nutrition and quantitative traits, the major physiological and biochemical traits of silkworm showed a greater decline in consumption with decrease of food efficiency conversion into biomass in experimental bivoltine breeds compared to the healthy leaf fed silkworms of the same breed. A similar result was reported for polyvoltine and commercial hybrid silkworms by Maribashetty et al. (12) and Meneguim et al. (13) respectively. Such dietary factors and related metabolic interactions on specific gene expression were also reported by Walker and Blackburn. Nutrition affects nearly all biological processes including the rates of biochemical and physiological reactions (14,15), and eventually can affect the larval quality or quantity of cocoon crops in the silkworm. Several reports (15-18) demonstrated that silkworms were more responsive to nutrition supplement during the 4th and 5th stages, which are recommended for the recognition and selection of nutritional efficient changes due to leaf spot disease. Hence, the nutrition utilization study was confined to the 5th instar larvae, since 80-85% of total leaf consumption was observed in this stage of silkworm development (16,18) and the silk gland stimulation starts at this stage. For instance, bivoltine breeds reared in temperate countries are known to be less nutritionally efficient, which is also true with cross breeds that have evolved for a tropical climate (3,19-20). It is essential to analyze nutrigenetic traits reduction to understand the racial difference among bivoltinegermplasm breeds for commercial purposes. Recently, the effects of nutrigenetic traits for bivoltine germplasm breeds also have been shown by Ramesha et al. (3). The success of the sericulture industry depends upon several factors, including production of quality mulberry leaves. This factor is of vital importance, since it accounts for 60% of total expenditures (21). It is well understood that the majority of the economically important genetic traits of silkworm are qualitative in nature, and phenotypic expression is greatly influenced by the environmental and leaf factors such as temperature, relative humidity, light, diseased leaf and nutrition (3,6,22-23). Therefore, it is essential to gauge the degree of phenotypic difference of the economical traits to understand the genetic steadiness under the controlled nutrition conditions. The problem of balancing and fixing the desirable traits for a given environment is a challenging task for the silkworm breeder. Hence, understanding the range of a reaction of the selected breeds to variable nutritional conditions especially to feeding with diseased leaf, is important for the breeder to utilize them appropriately in hybrid programs. In order to achieve greater success in this regard, it is important to understand the level of nutrition efficiency in bivoltine silkworm breeds and to analyze the least significant loss to cocoon crop because of leaf spot disease. The main objective of this study was to identify the conversion index and efficiency of conversion of ingesta, when larva were fed with the leaf spot diseased mulberry leaves, to biomass through standard gravimetric method for three successive generations on different races and varieties of mulberry, is supported by earlier observations (24-27). Our emphasis was on the phenotypic manifestation of 11 nutrigenetic traits. The results revealed highly significant ($p \leq 0.001$) variability among the two bivoltine breeds with respect to 11 nutrigenic traits over, when the larvae were fed ith the leaf spot diseased mulberry leaf. Although earlier studies showed that somebivoltine breeds are moderately nutritionally efficient (12,28-29), We concluded that bivoltine breeds with minimum consumption index and maximum efficiency of conversion of ingesta/cocoon identified strains NB_4D_2 and SH6 as potential bivoltine breeding resource material for the development of nutritionally efficient breeds/hybrids in Asia especially Indian subcontinent, pacific regions and other temperate regions(30-36).

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