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IMPACT OF DIETARY MOISTURE ON NUTRITIONAL INDICES AND GROWTH OF *BOMBYX MORI* AND CONCOMMITANT LARVAL DURATION

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Abstract—Fifth-instar larvae of *Bombyx mori* were reared on mulberry leaves having artificially depleted moistures of 60, 65 and 70%, which were regarded as treatments and a naturally occurring moisture of 76.62% regarded as control, during the wet part of summer (July–August) at a temperature of 27–32°C and humidity of 86–90%.

Absolute consumption and growth rates/day/larva (measured as dry weight) increased with increasing percentage of leaf moisture. The quantity of dry matter consumed and digested, the values of efficiency of conversion of ingested food (ECI%) and efficiency of conversion of digested food (ECD%) and final larval weight increased significantly with increasing leaf water showing a high positive correlation. Approximate digestibility increased progressively up to 70% leaf moisture but was reduced at the control dietary water level. Larval duration was prolonged under low water content but without a corresponding increase in the quantity of leaf consumed, ECI% and ECD%. Conversely, a short larval duration with a high leaf moisture was associated with an increased quantity of food consumed, ECI% and ECD%, indicating a physiological adjustment of fifth-instar larvae of *B. mori* for quick growth and transformation to the next developmental stage during the wet part of the summer.

Key Word Index: *Bombyx mori*; dietary moisture; nutritional efficiency; growth

INTRODUCTION

Consumption and utilization of mulberry leaves by the bivoltine race of *Bombyx mori* L. have been studied by many investigators (Matsumura and Takeuchi, 1950; Matsumura *et al.*, 1955; Takeuchi *et al.*, 1964; Udea, 1965; Udea and Suzuki, 1967; Horie and Watanabe, 1983). The percentage of water in mulberry leaves varies according to the age (Kawase, 1914; Hiratsuka, 1917) and season (Pathak and Vyas, 1988). Dietary water affects and nutritional efficiency of insects (Soo Hoo and Fraenkel, 1966; Matson and Scriber, 1987). A high water content affects both edibility and assimilability of leaves in the silkworm (Parpiev, 1968) while a low water content affects energy expenditure nutritional efficiency and the growth of herbivorous insects (Scriber, 1977; Reese and Beck, 1978; Martin and Van't Hof, 1988; Schmidt and Reese, 1988; Van't Hof and Martin, 1989).

The multivoltine "Nistari" race of *B. mori* is of great commercial importance in West Bengal. During the wet part of the summer (July–August) when the rearing room temperature is 27–32°C and the humidity is 86–96%, mulberry leaves grow profusely and contain more than 76% water. This level of water is favourable for quick larval growth but results in high mortality of both fifth-instar larvae and pupae, which results in a substantial economic loss. Moreover fifth-instar larvae alone consume about 87% of the total amount of food required for the completion of the life cycle.

The present investigation includes measurement of the consumption and utilization of food, larval growth and duration of the "Nistari" race of *B. mori* reared on mulberry leaves containing artificially various depleted percentages of moisture.

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MATERIALS AND METHODS

Larvae

Freshly ecdysed, fifth-instar larvae were obtained from the stock culture reared on fresh mulberry leaves at 27–32°C temperature and 86–96% relative humidity. For each set of experiments there were five replications, and in each replication there were 20 larvae.

Diet

The larvae were fed with the leaves of *Morus alba* var S_1 of C.S.R. & T.I., Berhampore, West Bengal. The leaves given to the experimental larvae contained 60, 65 and 70% $\pm$ 0.5% water. Trials were made with leaves containing 55% water, but larval survivability was only 12%. Hence, the leaves with water lower than 60% were not used for futher investigation. Counting from the tip of a twig, the 6th–8th leaves were given from plants 2 months after pruning so that the water content remained almost at the required level. Excess food was provided four times in every 24 h. For determining the amount of food consumed the unused residual food was collected at each feeding period.

Determination of leaf moisture

Fresh leaves were weighed then dried to constant weight in an oven at 60°C for 48 h. Percentage of leaf water of control leaves was determined from the difference in the two weights.

In order to obtain leaves with desired low levels of water the leaves were spread thinly on a tray and were subjected to air circulation under a ceiling fan for variable periods. The timing varied for rainy and sunny days. After approx. 105–110, 73–76 and 40–42 min each gram of leaves weighed 0.59, 0.67 and 0.78g which corresponded respectively to the leaves with 60, 65 and 70% water which were determined by the following formula:

$$\% \text{ leaf moisture after loss} = \frac{W-(x-y)}{y},$$

where, W = % moisture of control leaves x = fresh weight of leaves before drying and y = weight of leaves after loosing the required quantity of water.

Determination of nutritional efficiency

Nutritional indices were calculated on the basis of procedures designed by Waldbauer (1968) and Reynold and Nottingham (1985) on dry weight basis

$$\text{(absolute) growth rate} = P/T,$$

$$\text{(absolute) consumption rate} = E/T,$$

$$\text{approximate digestibility} = 100\ (E-F)/E,$$

$$\text{ECI\%} = 100 \cdot P/E,$$

$$\text{ECD\%} = 100 \cdot P/(E-F),$$

where E = dry wt (g) of ingested food, F = dry wt (g) of faeces left, P = gain in larval dry wt (g), and T = duration of larval feeding period (days).

As a continuous feeder, the gut of silkworms always contains food. Only prior to the onset of spinning does the gut become completely empty and remains so for a short period in the newly ecdysed next instar. In order to avoid errors in calculating ECI%, ECD%, growth rate and final larval weight, newly ecdysed fifth-instar larvae prior to the onset of feeding and mature larvae just before the beginning of spinning were taken for weighing.

Problems of mortality and individual variations were minimized by replacing the sick larvae with healthy ones of the same age and size, obtained from the reserve batch.

Statistical calculations

Correlation coefficients were calculated to determine the relationship between leaf moisture and other nutritional indices and further relationship between the parameters. Linear regression lines were fitted using the equation

$$Y = a + bx,$$

where Y was the quantity consumed, consumption rate, growth rate and final larval weight, x was the leaf moisture and a and b were constant. For handling the quantity digested, approximate digestibility, ECI% and ECD% the regression model employed was $Y = a + bx + cx^2$, where Y represented the parameters, x was leaf moisture and a, b and c were constant. Finally, a model $Y = ax^b$ was used to calculate larval duration, represented by Y. In Figs 1–9 the regression line was first drawn on the basis of the regression equation. Then mean observed values were plotted against the corresponding foliage water.

The constant values in the regression model were estimated by a standard linear estimation procedure (Yamane, 1970). For measuring the goodness of

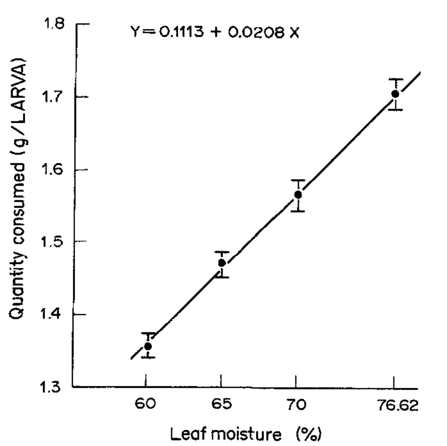

Fig. 1. The effects of leaf moisture on dry weight of food consumed ($r^2 = 0.986$, df = 18).

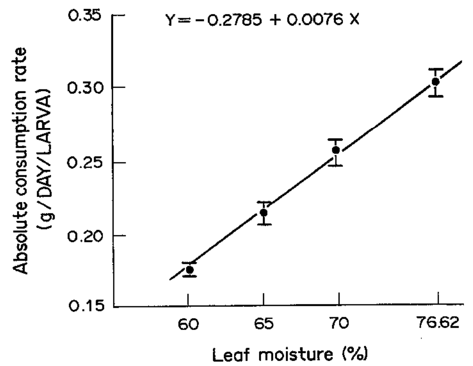

Fig. 2. The effects of leaf moisture on absolute consumption rate ($r^2 = 0.984$, df = 18).

fit and testing the linearity, the coefficient of determination (r^2) was used.

RESULTS

Effect of leaf moisture on nutrition

All the nutritional indices except approximate digestibility, increased progressively with an increase in moisture level (Table 1). For approximate digestibility too, such an increase occurred in the larvae which were fed with leaves containing up to 70% water but this decreased slightly at 76.62% dietary water. This decrease, however, did not differ significantly from the value obtained at 70% water. The values for the indices quantity consumed, quantity digested, consumption rate, ECI% and ECD% were highly significant for all the treatments i.e. diet with 60, 65 and 70% water.

The correlation coefficient study showed a significant positive correlation between the leaf water and each of the parameters and between the pairs of parameters. The level of significance in all the cases was at least $P < 0.01$ (Table 2).

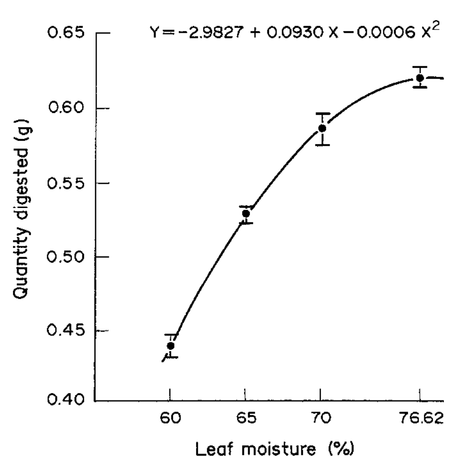

Fig. 3. The impact of foliage water levels on quantity of leaf dry matter digested ($r^2 = 0.994$, df = 18).

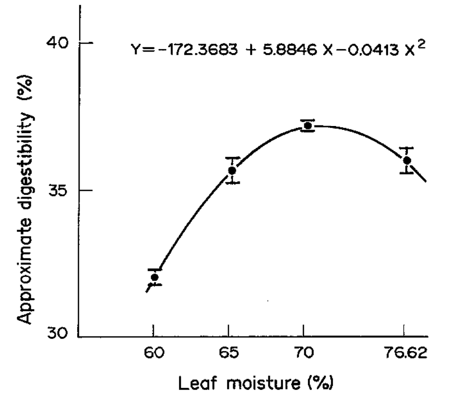

Fig. 4. The effects of leaf water content on larval approximate digestibility (%) ($r^2 = 0.982$, df = 18).

In the regression characteristics of different nutritional indices on leaf water, linearity was observed for quantity of consumption and consumption rate (Figs 1 and 2) with highly significantly values for coefficient of determination (r^2). But for the values quantity digested, approximate digestibility, ECI% and ECD% the relationship was also significant but curvilinear (Figs. 3–6). The r^2 values were also highly significant (Table 3).

With a view to testing the effectiveness of the regression equations of all the indices on leaf water levels the predicted values derived from the regression equation were compared with the observed values (Table 2). The marginal deviation of predicted values from the observed values were indicative of improved goodness of fit of the regression equations as the r^2 values calculated from different parameters were highly significant (Table 3).

From the regression lines (Figs 3–6) it appeared that the pattern of increase in the values of different indices was not uniform with respect to different

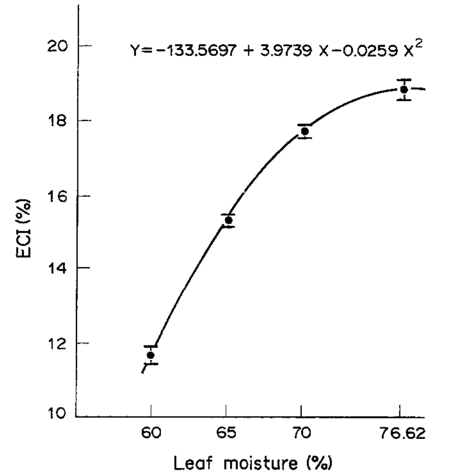

Fig. 5. The effects of foliage water levels on efficiency of conversion of ingested food (ECI%) ($r^2 = 0.997$, df = 18).

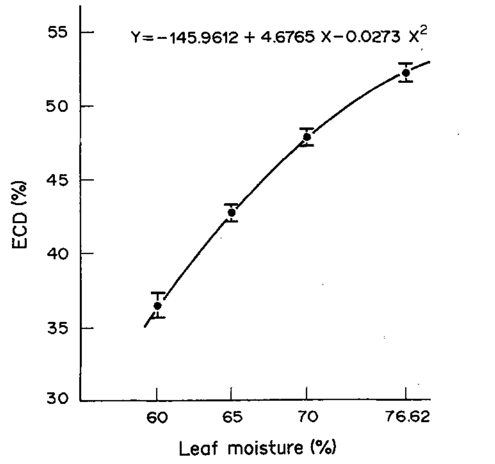

Fig. 6. The impact of foliage water levels on efficiency of conversion of digested food (ECD%) ($r^2 = 0.992$, df = 18).

moisture levels. A pronounced increase in quantity consumed, approximate digestibility, ECI% and ECD% occurred in the diet with 60% water. At the subsequent higher water levels the increase, though progressive, was less pronounced. But for quantity consumed and consumption rate (Figs 1 and 2) the rates were lower at 65% moisture, then increased at the subsequent higher moisture levels.

Effect of leaf moisture on growth

Water content of the leaves influenced larval growth. Both growth rate and final larval weight increased progressively with the increase in water. The mean final larval weights were 0.204, 0.268, 0.325 and 0.367 g and the mean growth rates were 0.020, 0.032, 0.045 and 0.057 g/day/larva, respectively, at 60, 65, 70 and 76.62% dietary moisture (Table 1). Regression characteristics of these two indices on the leaf water showed a distinct linearity (Figs 7 and 8). The r^2 values were also significant (Table 3). The

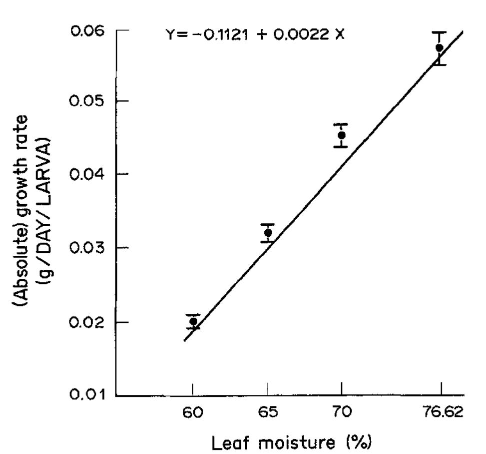

Fig. 7. The effects of foliage water levels on absolute growth rate ($r^2 = 0.984$, df = 18).

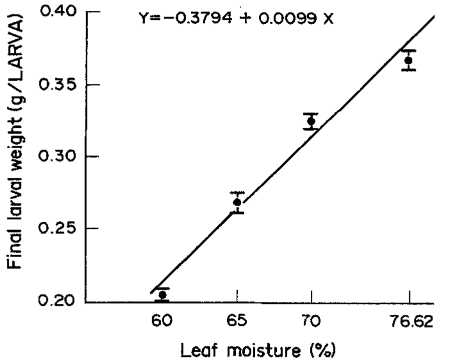

Fig. 8. The influence of foliage water levels on final larval weight ($r^2 = 0.972$, df = 18).

pattern of increase in the growth rates and final larval weights in relation to the increasing water content was almost uniform.

Effect of leaf moisture on larval duration

The duration of larval life became progressively shorter with the rise in water. At 60% it was 7.700 days while at the control level of moisture it was 5.650 days on average (Table 1). The relation between short larval duration and the rise in water levels was negative but highly significant. The regression characteristic was curvilinear. The shortening was maximum at 65%, then with the rise in water content it was relatively lower and almost uniform (Fig. 9).

DISCUSSION

The larvae of *B. mori* have a rapid growth rate which is due to a high rate of food consumption and great efficiency of conversion of food to larval biomass. Though rate of food consumption increases with a rise in temperature (Legay, 1958), dietary water seems to have an overall influence on the nutritional performances of *B. mori* and consequently

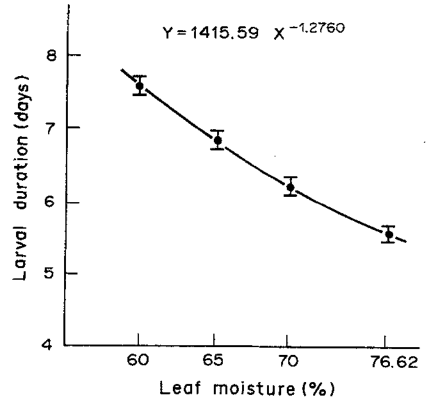

Fig. 9. The impact of foliage water levels on larval duration ($r^2 = 0.963$, df = 18).

Table 1. Mean observed and predicted (in parentheses) values of different nutritional indices at different levels of leaf moisture

Parameters	Moisture in the leaves (%) 60 ± 0.5	65 ± 0.5	70 ± 0.5	76.62 ± 0.5 (Control)
Final larval weight (g)	0.204 (0.215)	0.268 (0.264)	0.325 (0.313)	0.367 (0.379)
Quantity consumed (g)	1.356 (1.359)	1.470 (1.463)	1.567 (1.567)	1.705 (1.704)
Quantity digested (g)	0.438 (0.437)	0.522 (0.527)	0.588 (0.587)	0.618 (0.620)
Approximate digestibility (%)	32.262 (32.028)	35.540 (35.638)	37.548 (37.184)	36.232 (36.052)
ECI%	11.764 (11.624)	15.224 (15.306)	17.914 (17.693)	18.956 (18.861)
ECD%	36.476 (36.349)	42.836 (42.669)	47.750 (47.625)	52.308 (52.084)
Growth rate (g)	0.020 (0.019)	0.032 (0.030)	0.045 (0.041)	0.057 (0.056)
Consumption rate (g)	0.176 (0.177)	0.214 (0.215)	0.255 (0.253)	0.302 (0.303)
Larval duration (days)	7.700 (7.621)	6.850 (6.881)	6.150 (6.260)	5.650 (5.578)

on growth and larval duration. Within the experimental range of dietary water levels, the performance of the fifth-instar larvae in respect of all the parameters except approximate digestibility is always better at high-water levels than with low-water content. Approximate digestibility, however, increases up to the 70% dietary water level but decreases non-significantly at the control level.

The quantity of food consumed by the control larvae decreases to 20.47, 13.78 and 8.09% by comparison with the larvae reared on diet with 60, 65 and 70% water respectively. ECI% and ECD% also decrease with the decreasing water. The influence of dietary water on the conversion efficiency of food to larval biomass has also been emphasized in the herbivorous insects by Soo Hoo and Fraenkel (1966) and Feeny (1975). The present findings strongly corroborate the observations of Scriber (1977) in *Hyalophora cecropia* and Martin and Van't Hof (1988) in *Manduca sexta*. In fifth-instar larvae of *B. mori* a positive correlation exists between the dietary water and all of the nutritional indices as seen from the *r* values (Table 2).

In the present investigation all factors influencing growth and nutrition of *B. mori* remain constant for both experimental and control larvae apart from leaf moisture. Hence, whatever effects have been observed should be due to dietary water. There is experimental evidence that even with additional proteins in the diet growth could not be induced under water-stress condition (Schroeder, 1986). High temperature and realtive humidity during the period of investigation

Table 2. Correlation coefficients between pairs of nutritional indices

	Leaf moisture (%)	Final larval weight (g)	Quantity consumed (g)	Quantity digested (g)	Approximate digestibility (%)	ECI %	ECD%	Growth rate (g)	Consumption rate (g)
Final larval weight (g)	+0.986								
Quantity consumed (g)	+0.993	+0.984							
Quantity digested (g)	+0.960	+0.991	+0.964						
Approximate digestibility (%)	+0.748	+0.840	+0.746	+0.896					
ECI%	+0.956	+0.990	+0.950	+0.993	+0.903				
ECD%	+0.986	+0.996	+0.979	+0.980	+0.882	+0.987			
Growth rate (g)	+0.992	+0.995	+0.989	+0.977	+0.794	+0.975	+0.994		
Consumption rate (g)	+0.992	+0.987	+0.992	+0.961	+0.750	+0.957	+0.986	+0.997	
Larval duration (days)	−0.974	−0.992	−0.969	−0.981	−0.839	−0.987	−0.993	−0.991	−0.984

All are significant at $P < 0.01$ level, with 18 degree of freedom.

Table 3. Regression co-efficients of leaf moisture on different nutritional indices

Parameters	Regression equations (Y)	Co-efficient of determination (r^2)*
	(A) Linear Regression Equations	
Final larval weight (g)	$-0.3794+0.0099x$	0.972
Quantity consumed (g)	$0.1113+0.0208x$	0.986
Growth rate (g)	$-0.1121+0.0022x$	0.984
Consumption rate (g)	$-0.2785+0.0076x$	0.984
	(B) Curvilinear Regression Equations	
Quantity digested (g)	$-2.9827+0.0930 \times -0.0006\,x^2$	0.994
Approximate digestibility (%)	$-172.3683+5.8846 \times -0.0413\,x^2$	0.982
ECI%	$-133.5697+3.9739 \times -0.0259\,x^2$	0.997
ECD%	$-145.9612+4.6765 \times -0.0273\,x^2$	0.992
Larval duration (days)	$1415.59x^{-1.2760}$	0.963

*All values are significant at $P < 0.01$ level, with 18 degrees of freedom.

were both important for speedy growth of larvae. Yet, the larvae reared on a diet with low water show retarded growth as seen from a lower final larval weight even after prolongation of larval duration. This further strengthens the need of optimal amounts of dietary water for optimal nutritional performances in *B. mori*. Prolongation of larval life under depleted dietary water has also been observed by Van't Hof and Martin (1989) in *M. sexta*.

Theoretically a voraciously feeding larva of *B. mori* should consume a higher quantity of food during an extended larval life. Moreover a long life span means a high maintenance cost. Both should force a higher quantity of food to be consumed. But a reverse situation has been observed. In spite of prolongation of the feeding period, the quantity of food consumed using 60% of water has been lowered by 20.47% relative to larvae reared on the control diet. This implies that water imposes a limiting factor for food utilization and its efficient conversion to larval biomass. A low final larval weight even after prolongation of larval duration may further be due to a high rate of respiratory metabolism which compensates for water deficiency by supplementing metabolic water.

During the wet part of the summer, although the factors for silkworm growth are good, yet there is a high mortality of fifth-instar larvae and pupae. Hence, this season is considered unfavourable for the silkworm. This is possibly due to a high water content of mulberry leaves and a low transpiratory loss from the larval body. On the other hand, a low water content of 60% also accounts for larval mortality to a considerable extent. But the mortality on diet with 65 and 70% water is very low. In order to obtain a better yield during the wet summer the silkworms should be reared on a diet with optimal range of water so that their nutritional and growth performances are likely to be better and mortality to be lowest. Such an investigation is under progress.

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