MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Insect

The experimental insects were the local multivoltine ' Nistari ' race of the mulberry silk moth Bombix mori. L. Disease free eggs were collected from the Germ Plasm Bank of the Central Sericultural Research & Training Institute, Berhampore, West Bengal where the stock was maintained as pure race for a number of generations.

3.2. Rearing of silkworms

The rearing was undertaken following the schedule (Ullal and Narasimhanna, 1987) recommended for the farmers for incubation, hatching, brushing, feeding, bed cleaning, spacing and other rearing practices in an uncontrolled rearing room maintaining a temperature within 27-32^{o} C and relative humidity within 86-96% and under natural light condition of the rearing room.

The freshly ecdysed fifth instar larvae were taken from stock culture developed from the egg collected initially from the Germ Plasm Bank. Such a pool of larval stock was maintained during the entire period of investigation.

For the following three aspects of investigation larva were taken for both control and each of the treatment sets an

there were 5 replications for each

1. Consumption and utilization of mulberry leaves, larval growth and duration.
2. Efficiencies of conversion of consumed leaves into cocoon and shell.
3. Consumption and utilization of leaf nitrogen for the nitrogen of shell.

Simultaneously, reserve batches of large number of larvae were maintained both for control and each of the treatment sets. The problems of mortality and individual variation were minimised by replacing dead and sick larvae with healthy ones of the same age and size, obtained from reserve batches.

The experiments relating to (4) Larval body water and faecal water with reference to leaf moisture and (8) Reeling character and silk filament character were studied from these reserve batches.

In case of experiment relating to (5) Rearing result the sample size of each was 300 larvae and there were 5 replications. The experiments on (6) Cocoon melting and (7) Reproductive performance were studied from the resultant individuals of experimental batches relating to Rearing result.

Diet

The larvae were fed with leaves of mulberry, Morus alba, S1 variety evolved by the Central Sericultural Research and Training Institute, Berhampore, West Bengal. Counting from the tip of a twig, only the 6th-8th leaves were used from plants 2 months after prunning so that the water content remained almost at the same level. Fresh leaves having a moisture of 76.62 ± 0.5% were given to the control larvae. But the leaves given to the larvae of treatment sets contained 60, 65 and 70% ± 0.5% water Attempt was also made for rearing the larvae with leaves containing 55% water, but the larval survivability was only 12% Hence the leaves having less than 60% water were not considered for further investigation.

3.3. Determination of leaf moisture

This was done with a very little modification of the earlier procedure of Paul et al.(1992). Fresh leaves were weighed, then dried in an oven at 60°C for more than 48 hours till the dry weight became constant. Percentage of moisture control leaves was calculated from the differences in the weights.

In order to make the leaves having desired water level than that of the control leaves, the fresh leaves were spread thinly on a tray and kept under air circulation using a ceiling

fan for variable periods, which again differed on rainy and sunny days. After about 105-110, 75-78 and 40-42 minutes every gram of leaves after loosing water weighed 0.59, 0.67 and 0.78 g which correspounded respectively to the leaves having 60, 65 and 70% moisture. The percentage of water of such treated leaves was determined using the following formula:

$$\text{Percentage of leaf moisture after loss} = \frac{w - (x-y) \times 100}{y}$$

Where, w = initial amount of moisture in the fresh leaves which was determined by : $\frac{\text{Moisture \% of fresh leaves}}{100} \times x$,

x = fresh weight of leaves before drying and y = weight of leaves after loosing the desired quantity of water. The dry matter of leaves of all the categories was determined by substracting the moisture from the leaves.

3.4. Consumption and utilization of mulberry leaves, larval growth and duration.

The measured quantity of mulberry leaves were given 4 times in every 24 hours in such a way that some excess quantity of leaves remained uneaten every time so that the food could not be a limiting factor. Simultaneously, the mulberry leaves were kept as aliquot in a separate rearing tray but without any worms. After every 24 hours the residual food and faeces were carefully separated and dried at 80° C till the weight became constant. The dry weight of food ingested was calculated by substacting the dry weight of residual food from that of the aliquot. The

quantity of food digested was measured by substracting the weigh of the faeces from the quantity of food ingested.

The nutritional indices were calculated on the basis o procedure designed by Waldbauer (1968) and Reynolds an Nottingham(1985) on dry weight basis.

$$\text{(Absolute) growth rate} = \frac{P}{T}$$

$$\text{(Absolute) consumption rate} = \frac{E}{T}$$

$$\text{Approximate digestibilty \%} = \frac{(E-F) \times 100}{E}$$

$$\text{ECI\%} = \frac{P \times 100}{E}$$

$$\text{ECD\%} = \frac{P \times 100}{(E-F)}$$

Where E = Dry weight (g) of ingested food.

F = Dry weight of faeces (g)

P = Gain in larval weight (g)

T = Duration of larval feeding period (days).

At the beginning of experiment before feeding the initia larval dry weight was recorded based on 5 observations eacl consisting of 20 larvae to get better estimate for initial larva weight. The fullgrown (final) larval weight was taken prior t the onset of spinning when the gut became completely empty. This

avoided the error in calculating the nutritional indices. Because the larval weight along with the food in the gut would give the incorrect results for determining the gain in weight which in turn would show the higher ECI and ECD values. The gain in larval weight was determined by substracting the initial larval weight from the final weight.

3.4.1. Statistical calculations

Correlation coefficients were calculated to determine the relationship between leaf moisture and different nutritional indices and further relationship between the parameters. Linear regression lines were fitted using the equation $Y = a+bx$, wehere Y = quantity consumed, consumption rate, growth rate and final larval weight, x = leaf moisture, and a and b were constant. The regression model employed for quantity digested, approximate digestibilty, ECI and ECD was $Y = a+bx+cx^2$, where Y = parameters, x = leaf moisture, and a, b and c were constant. Finally, the model $Y = ax^b$ was used for fitting the regressior line relating to larval duration, represented by Y. In the figures, the regression line was first drawn on the basis o repsective regression equation. Then mean observed vlaues wit standard error bars were plotted agaisnt the corresponding lea moisture.

The constant values in the rgression model were estimate by standard linear estimation procedure (Yamane, 1970). Fc

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

3.5. Efficiencies of conversion of consumed leaves into cocoon and its shell

Both control and each of the treatment sets of larvae after completion of feeding and gut evacuation were transferred replication wise to spinning trays for the formation of cocoons. The cocoons were harvested on the 6th day of pupation and these were opened carefully and dried in an oven at 60° C till the weight reached a constant for considering the dry weight of cocoons (shell plus pupa) and the shell separately. For each replication 5 male and 5 female cocoons with corresponding shells were considered for calcuclation of conversion efficiency values following the procedure of Waldbauer (1968) and Horie et. al. (1976).

$$\text{ECI\% for cocoon} = \frac{\text{Gain in cocoon wt}}{\text{Wt of dry matter of food ingested}} \times 100$$

$$\text{ECI\% for shell} = \frac{\text{Wt of cocoon shell}}{\text{Wt of dry matter of food ingested}} \times 100$$

$$\text{ECD\% for cocoon} = \frac{\text{Gain in cocoon wt}}{\text{Wt of dry matter of food digested}} \times 100$$

$$\text{ECD\% for shell} = \frac{\text{Dry wt of cocoon shell}}{\text{Wt of dry matter of food digested}} \times 100$$

Quantity of ingested dry matter required for every gram of cocoon shell = $\frac{\text{Wt of ingested dry matter}}{\text{Wt of cocoon shell}}$

The gain in cocoon wt was measured by substracting the initial larval weight from the dry weight of the cocoon.

3.5.1. Statistical calcualtions

The procedures followed for statstoical calculations were similar to those referred under 3.4.1. However, for wt of cocoon and wt of shell the linearity of regression was fitted using the equation $Y = a+bx$. The regression model $Y = a+bx+cx^2$ was employed for ECI for cocoon and shell, ECD for cocoon and shell and quantity of ingested food required for the production of each gram of shell.

3.6. Consumption and utilization of leaf nitrogen for the nitrogen of shell

For estimation of nitrogen of mulberry leaves, the material was dried in an oven at 60° C till the weight became constant. The dry leaves were finely ground into powder by morter and pestle. 3 samples of leaf powder each of 0.5 g were taken for estimation of nitrogen by Kjeldal method. The silkworm faeces of each replication of both control and treatments were similarl

prepared for nitrogen estimation. Taking into consideration the estimated nitrogen contents of mulberry leaves and faecal samples, and based upon the amount of dry matter of leaves ingested and digested, the total amount of nitrogen ingested and digested by each larvae were calcualted. The estimation of nitrogen (g) in cocoon shell was also done by the same Kjeldal method.

The nutritional indices of nitrogen consumption and utilization for cocoon shell were calculated according to Waldbauer (1968) and Horie and Watanabe (1986) on dry wt basis.

Approximate digestibility of nitrogen %

$$= \frac{\text{Nitrogen ingested - nitrogen in faeces}}{\text{Nitrogen ingested}} \times 100$$

Efficiency of conversion of ingested nitrogen to nitrogen of cocoon shell (ECI% for nitrogen of shell)

$$= \frac{\text{Quantity of nitrogen in cocoon shell}}{\text{Nitrogen ingested}} \times 100$$

Efficiency of conversion of digested nitrogen to nitrogen of cocoon shell (ECD% for nitrogen of shell)

$$= \frac{\text{Quantity of nitrogen in cocoon shell}}{\text{Nitrogen digested}} \times 100$$

3.6.1. Statistical calculations

Here too, the procedure followed for statistical calculations were similar to those referred under 3.4.1. The regression model $Y = a+bx$ was used for only the amount of nitrogen ingested. But for the amount of nitrogen digested, apaproximate digestibility of nitrogen, ECI for nitrogen of shell and ECD for nitrogen of shell the regression model employed was $Y = a+bx+cx^2$

3.7. Larval body water and faecal water with reference to leaf moisture

The study on larval body water and faecal water was done on the larvae of reserve batches maintained in connection with the experiment on nutritional efficiencies (Chapt. 3.2).

3.7.1. Determination of larval body water

Spanning the entire duration of 5th instar larvae, a daily record was taken on the fresh weight of the sample larvae at a fixed time, then these were dried at 60°C till a constant weight was attained. The percentage of body moisture was determined from the difference of the two weights. There were 5 observations/day/ experimental set.

3.7.2. Determination of faecal water

The moisture percentage of the faeces was also determined daily at the same time as in case of larval body water determination. In order to minimise the loss of moisture from the faeces if exposed for longer duration considerable number of larvae at the peak feeding stage were taken in a glass petridish and kept under glass cover for 5 minutes. The faeces ejected from the body within 5 minutes were collected for recording the fresh weight and dried in an oven at 60° C till the attainment of constant weight. The moisture percentage of the faeces was determinded from the difference of the two weights. There were 5 observations/day/ experimental set.

The data collected on moisture percentage of larval body and faeces were subjected to statstical analysis.

3.8. Rearing result

Twenty five disease free layings (eggs laid by a single female = dfl, each consisting of about 400 eggs) were reared in a mass from hatching upto the end of 4th instar. After the 4th moult randomly 1500 larvae were taken from the mass culture for each treatment and control. These 1500 larvae were divided into 5 replications each of 300 larvae, and reared with leaves having respective moisture level. When the larvae became ready for

spinning these were transferred to spinning tray for formation of cocoons. During the period of investigation the spinning was completed within 2.5 days. The cocoons were harvested on 6th day from the onset of spinning as this duration is sufficient for transfromation of the larvae into pupae.

In accordance with the practical need in silkworm rearing the following parameters were considered :

1. Larval duration (days).
2. Wt of 10 mature larvae (g).
3. Effective rate of rearing by number (ERR No.) which was calculated as

$$\text{ERR No.} = \frac{\text{No of cocoons harvested}}{\text{No of larvae reared}} \times 10{,}000$$

4. Effective rate of rearing by weight (ERR Wt) which was calculated as

$$\text{ERR Wt} = \frac{\text{Wt of cocoons harvested (Kg)}}{\text{No of larvae reared}} \times 10{,}000$$

5. Single cocoon Wt (g)
6. Single shell wt (g)
7. Cocoon shell ratio (SR%) which was determined as

$$\text{SR\%} = \frac{\text{Wt of single shell}}{\text{Wt of single cocoon}} \times 100$$

8. Absolute silk content (g) which was determined by ERR No x wt of single shell.

All the parameters were recorded on fresh weight basis. 20 samples (10 males and 10 females) were considered for cocoon assessment against each replication. All values on rearing results were subjected to ANOVA test.

3.9. Cocoon melting

The cocoons obtained from the experiment on rearing result were simultaneosly used for observation of melting percentage. On the 7th day from the onset of spinning the cocoons were opened with the help of a sharp knife for counting the melted dead pupa inside the cocoon and melting percentage was calculated against each replication. The data on cocoon melting were analysed statstically for " test of significance ".

3.10. Reproductive performance

Pupal growth and reproductive performance was studied on the viable cocoons obtained after the observation of melting. The study was based on :

a) Pupal growth, fecundity, fertility and egg vigour.

b) Percentage of mating and oviposition success.

For evaluating the pupal growth initially the weight of 25 male and 25 female pupae was recorded. These pupae were labelled serially. Final pupal weight was considered on only 15

individuals of each sex based on subsequent better reproductive performance. Therefore, the fecundity, fertility and egg vigour were recorded on the resultant moths obtained from those 15 pupae of each sex. The fecundity in particular was recorded on 15 healthy mated females that survived upto 5 days after emergence. All these procedures were followed with a veiw to minimising reasonably the impact of disease carried over from the larvae.

The sex separation was done at pupal stage on the 7th day on the onset of spinning. The male and female pupae were kept separately. After emergence in the morning (which is the usual time of emergence of B. mori.) mating was allowed for 3 hours between male and female of the same treatment and of the control. After depairing the females were allowed to lay eggs on cloth which was previously soaked with starch solution and then dried up. This ensured easy separation and collection of eggs from the cloth. Each mated female was covered by a plastic cellule to avoid the mixing of eggs laid by different females. The eggs o individual female moth were collected separately and allowed for hatching for fertility test. The determination of egg vigour was based on the weight of 100 eggs each obtained from 5 individuals out of 15 females at random for each set.

For the assessment of mating and oviposition success the male and female moths were collected at random after emergence. For each experimental set 100 individuals (50 male and 50 females

per replication were considered. At random one female was allowe to mate with a single male moth for 3 hours, but every 3 minutes observation was recorded for their mating capacity.

After mating the males were carefully removed and femal were kept separately on egg card covered by plastic cellule fo egg laying for a period of 24 hours which was the activ oviposition period. After 24 hours of oviposition all the mate females were dissected for observing the retention of matur (chorionated) eggs in the ovarioles if any, thus ascertainin the cmoplete or partial oviposition. Data on the mating succes and the oviposition success (most of the mature eggs laid) b mated females were recorded. These data were analyse statistically for testing the level of significance.

3.11. Reeling character of cocoon and silk filament character

The cocoons produced by the larvae of reserve batche maintained in connection with the experiment on consumption an utilization of mulberry leaves, larval growth and duration were used as material for examining the filament and reelin characters of cocoons. For each set of treatments and control 2 cocoons were collected at random and their floss (outer loos silk filaments) was removed. In order to kill the pupae and t avoid emergence of moth the cocoons were dried at 100^{0} C for abou 2 hrs. Further, in order to soften the sericin coating of th

fibre for easy separation from the compact shell the cocoons were treated with hot water successively in 3 basins maintaining different temperatures and for different durations. In the first basin the cocoons were kept at 90° C for 1 minute, in the second at 65° C for 30 seconds and finally, at 90° C for 1-2 minutes (Krishnaswami et al., 1972). After boiling the cocoons were transferred to a pot containing hot water of 50° C and silk filament of each cocoon was reeled out along with record of filament length with the help of a single cocoon reeling machine called approvate. The number of breaks of the filament during reeling was also recorded. The entire silk filament thus collected was dried at 60° C in an oven. The dry weight of the silk fibre and its length were considered for determination of denier (thickness of the fibre) by gravimetric method using the formaula :

$$\text{Denier} = \frac{\text{Wt of the silk fibre}}{\text{Total lenght of fibre}} \times 9000$$

The data on filament length, denier and number of breaks were analysed statistically.