

**IMPACT OF DIETARY MOISTURE ON THE PERFORMANCE  
OF *Bombyx mori* L. DURING UNFAVOURABLE WET  
PART OF SUMMER IN WEST BENGAL**

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*Dulal Chandra Paul*

**DEPARTMENT OF ZOOLOGY  
CENTRE FOR LIFE SCIENCES  
UNIVERSITY OF NORTH BENGAL**

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*Dr. D. C. Deb*

DEPARTMENT OF ZOOLOGY

UNIVERSITY OF NORTH BENGAL

P. O. NORTH BENGAL UNIVERSITY

RAJA RAMMOHUNPUR, DIST. DARJEELING,

WEST BENGAL, INDIA, SILIGURI—734430.

PHONE : BDR. 498



Ref. No.....

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This is to certify that the thesis entitled "Impact of Dietary Moisture on the Performance of Bombyx mori L. During Unfavourable Wet Part of Summer in West Bengal" embodies the record of original investigation carried out by Mr. Dulal Chandra Paul under my supervision.

Mr. Paul has fulfilled the requirements of the University for submission of thesis. I am pleased to forward his thesis for submission to the University of North Bengal for the award of the degree of Doctor of Philosophy (Ph. D.) in Science (Zoology).

(D. C. Deb.)  
Reader

Department of Zoology  
North Bengal University  
P.O. - 734430

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# INTRODUCTION

## 1. INTRODUCTION

In India sericulture is the second largest cottage industry next to handloom and now ranks second in global production of mulberry raw silk amounting to 11,487 metric tons in 1990 (Statistical Binnual:Silk in India, Central Silk Board, 1992,p. 2). Among the states in India, West-Bengal ranks 4th in mulberry silk production covering an area of 17,165 hectare with an annual production of 829metric tons (Ibid.p.69) and is practised almost throughout the year in most of its districts.

Throughout the world the rearing of silkworm Bombyx mori (Lepidoptera: Bombycidae) is practised exclusively indoor. On account of domestication for a very long period of time the insect has acquired an inherent sensitivity to fluctuating environmental temperature and humidity. Even the temperature and humidity optimum for best metabolic activity, growth and performance of different stages of life cycle such as larval instars, pupa and adult are quite different. In order to get a good economic return the need for an optimum range of temperature and humidity has been emphasized by many authorities ( Matsumura, 1928; Matsumura et. al., 1928; Kogure, 1933; Takada et al., 1961; Ueda and Iizuka, 1962).

In West Bengal the rearing of silkworm by most of the farmers is done usually in impoverished room made up of mud wall and tile or thatch roof. No sophisticated device is adopted for temperature and humidity regulation. With the seasonal fluctation of ambient conditions the rearing room temperature varies from 15 to 37° C and r.h. from 36 to 96%. During wet part of summer in

West Bengal, particularly during July and August the rearing room temperature ranges from 27 to 32° C and r.h. from 86 to 96%. Such a situation has been proved most unfavourable for silkworm rearing (Annual Report of C.S.R.&T.I., Berhampore, 1987-88, pp.114-115).

At higher humidity mulberry leaves wither very slowly, inducing faster consumption, favouring digestibility and conversion efficiency and consequent growth of silkworm larva (Sharada and Bhat,1957). Further, a luxuriant growth of mulberry during July-August provides an additional impetus to the farmers for one large scale rearing of silkworms. However, the advantages face a set back as because the resultant 5th instar larvae become physiologically weak in resisting diseases caused by micropathogens (Yokoyama, 1962), particularly the bacterial disease flacherie causes a serious concern (Chigasaki, 1937). Though the larval duration is shortened at a relatively high temperature coupled with higher humidity (Takada et. al., 1961) the 5th stage larvae become susceptible to mortality (Ueda and Iizuka 1962).

Thus, at high humidity and relatively high temperature during wet part of the summer in West Bengal, like many other tropical silk growing parts of India a considerable amount of crop loss is an imperative due to high rate of mortality of silkworm larvae (Subba Rao et.al.,1991). Further, there is a high incidence of yellow-stained cocoon production as a consequence of excess urination

due to accumulation of excess water in the body during the rainy season (Pathak and Vyas, 1988). Yellow staining impairs considerably the recovery of raw silk fibre from the cocoon shell (Kumararaj, 1972) and thus incurs a heavy crop loss. Added to these is the higher proportion of pupal death followed by melting within the shell (Giridhar et al., 1990).

The differential water and nitrogen contents of many host plants at different seasons have been found to influence the performance of many species of folivore lepidopterans. Acquisition of sufficient energy from nitrogen and other nutrients including water determines the success of these folivores (Scriber and Slansky, 1981). The moisture level of mulberry leaves also varies in different seasons (Pathak and Vyas, 1988) and depending on leaf maturity (Kawase, 1914; Hiratsuka, 1917). The peak level in tropical parts of India reaches during July-August and in the tender leaves.

Silkworm larvae acquire body water from the diet (mulberry leaves), undoubtedly a higher amount of body water accumulates from high-water diets. The avenues of water loss from the body are transpiration and along with the faeces. But a high r.h. restricts a transpiratory loss. With the progress of age the body water of 5th instar larvae is gradually depleted through balanced regulation of urination by diuretic hormone and reduced to the lowest level just prior to spinning (Ueda and Suzuki, 1967).

Thus, an optimum water balance is needed by silkworms for their balanced growth, survivability and silk production. Larval

body water above or below the tolerable range will be lethal and impair silk yield. The dietary water affects this over all performance of B. mori

In order to boost up silk production by exploiting the large scale availability of mulberry leaves and congenial ambient situation favouring quick larval growth during wet part of the summer in tropical situations, one of the possibilities might be the feeding of larvae with mulberry leaves having desired percentage of water. This may restrict water accumulation in the body of larvae and minimize consequent damages. But except an attempt by Narayanprakash et al., (1985) no information is in hand regarding the optimum percentage of mulberry leaf water suitable for better output during the unfavourable period under consideration.

The present investigation is an attempt to know the impact of different levels of foliar water on the local multivoltine Nistari race of B. mori during July-August and to find out an optimum level of dietary water for better performance accompanied with least crop loss.

The multivoltine Nistari race of B. mori has a great commercial importance in West Bengal and is used extensively as mother for the production of hybrid seeds by crossing with bivoltine male races. At present such hybrids are reared commercially only during the favourable seasons. Further apart from round the year rearing Nistari is the only race reared

extensively even during the most unfavourable period. All these stand for choosing this race for investigation.

The 5th instar larvae alone consumes about 87% of the total consumption by all the larval instars (Matsumura and Takeuchi, 1950). The mortality is also highest at the 5th stage in general in all seasons, more particularly during wet part of the summer. This is why the investigation has been undertaken from this stage onwards.

With the above objectives the following experiments have been designed to know the impact of mulberry leaf moisture on:

1. Consumption and utilization of mulberry leaves, larval growth and duration
2. Efficiencies of conversion of consumed leaves into cocoon (Pupa with shell) and shell alone
3. Consumption and utilization of leaf nitrogen for the nitrogen of shell
4. Larval body water and faecal water with reference to leaf moisture
5. Rearing result
6. Cocoon melting
7. Reproductive performance and
8. Reeling character of cocoon and silk filament character.

The experiments at serial numbers 1-3 are basically concerned with the nutritional efficiency. But these are treated here in compartmentalized way so that the essential commercial aspects can well be clarified.

# REVIEW OF LITERATURE

## 2. REVIEW OF LITERATURE

### 2.1. CONSUMPTION AND UTILIZATION OF MULBERRY LEAVES, LARVAL GROWTH AND DURATION

The consumption and utilization efficiencies of food are essential traits of insects that qualify for their growth, development and reproduction. The amount, rate and quality of food consumed particularly by lepidopteran larvae like those of B. mori having non-feeding, short-lived and only reproductive adult stage, influence larval growth rate, weight, developmental time and concomitant reproductive performance (Slansky and Scriber, 1985). In general, folivore caterpillars have a higher efficiency of conversion of leaf dry matter to growth as because the foods are highly nutritious and the larvae require a fast growth with accompanying food reserve (Soo Hoo and Fraenkel, 1966). As for example, the ECD of B. mori on mulberry leaves is 63% (Hiratsuka, 1920).

Phytophagous insects have very poor ability for water conservation. Hence, they suffer from deleterious effects if dietary water is not adequate (Waldbauer, 1962, 1964, 1968). Dietary moisture level in such insects, particularly in the caterpillars, is an important factor in the nutritional

efficiency ( Scriber, 1977; Reese and Beck, 1978). This acts as a primary limiting factor for the folivores (Schroeder, 1986). Low foliar water has been considered nutritionally poor ( Soo Hoo and Fraenkel, 1966; Mattson and Scriber, 1987), hence reduces the growth rate of caterpillars (Scriber, 1977; Reese and Beck, 1978; Slansky and Scriber, 1985; Martin and Van't Hof, 1988; Timmins et al., 1988; Van't Hof and Martin, 1989). A low - water imposed impaired growth has also been implicated by Scriber (1979) in 16 species of forb-, shrub- and tree - feeding caterpillars.

Consumption and utilization of mulberry leaves by the bivoltine races of B. mori have been studied by several workers in Japan ( Matsumura and Takeuchi, 1950; Matsumura et al., 1955; Takeuchi et al. , 1964; Ueda, 1965; Ueda and Suzuki, 1967; Horie and Watanabe, 1983 a). In Bulgaria a sex- limited differential nutritional efficiency has been observed by Tzenov(1993) and Tzenov and Petkov(1993). The percentage of water in mulberry leaves varies according to the age(Kawase, 1914; Hiratsuka, 1917) and season (Pathak and Vyas, 1988). Parpiev(1968) emphasized that high moisture content of mulberry leaves favours the palatability and also assimilability of leaf nutrients by silkworm. The over all performance of insects must depend on weight gain from larval feeding(Kestner and Smith, 1984) which is again directly related to the amount of food consumed and efficiency of utilization therefrom (Slansky, 1980). The final weight of silkworm larvae is intimately associated with

the quantity and quality of food consumed (Sunioka et al., 1982). This is evident from the results of many experiments involving restriction of feeding duration (Muthukrishnan et al., 1978; Mathavan et al., 1987), food rationing (Radhakrishna and Delvi, 1987; Pillai, 1989 ) and number of feeds per day (Haniffa et al., 1988).

While maximum emphasis has been laid on the negative aspects of nutritional efficiency associated with low - water diets, literature on the optimum water requirement for best efficiency, or the impact of dietary water in excess of optimum requirement is scanty. The observations obtained by using diets diluted with excessive water (Timmins et al., 1988; Slansky and Wheeler, 1989) do not implicate strictly the impact of excess water on nutritional efficiency of the caterpillars. However, Narayanaprakash et al. (1985) attempted to study the effect of food utilization efficiency in a bivoltine hybrid and a multi x bi cross breed of B. mori. The authors worked under tropical situation using mulberry leaves with natural high water and those made artificially to contain low levels of water. A breed specific thriving ability and utilization efficiency of food by the larvae on variable water levels were reported.

The present attempt is to measure the consumption and utilization of food, larval growth and duration of the Niatari race of B. mori reared on mulberry leaves containing different levels of water.

## 2.2. EFFICIENCIES OF CONVERSION OF CONSUMED LEAVES INTO COCOON AND SHELL

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Dietary water level is important to the efficiencies of conversion of nutrients into biomass of phytophagous insects (Soo Hoo and Fraenkel, 1966; Feeny, 1975; Slansky and Scriber, 1985; Mattson and Scriber, 1987). Depending on the age older mulberry leaves contain progressively poor percentage of water and protein ( Nakajima, 1931). Scriber and Slansky (1981) also agree that leaf water and nitrogen content of leaves of plants generally decline with age.

Several reports are known on the consumption and utilization efficiency of mulberry leaves into cocoon and shell( Kawase, 1914; Hiratsuka, 1917; Matsumura et. al., 1955; Ueda, 1965; Ueda and Suzuki, 1967; Horie et al., 1976; Horie and Watanabe, 1983a) Both the weight of the cocoon and shell tend to decrease when the larvae are fed with leaves in descending order from the apex of the plants. One of the causes for such decline lies with the fact that water and nitrogen contents are inversely proportional to the ages of mulberry leaves (Kawase, 1914; Hiratsuka, 1917; Nakajima, 1931; Hassanein and El Shaarawy, 1962a). Further, the varietal differences of mulberry leaves due to differential amount of water and other nutrients result in the differential efficiency of cocoon and shell production

(Machi and Katagiri, 1990). Apart from other factors, water in the leaves has been considered to limit the nitrogen utilization efficiency and thereby resulting in the differential growth and final biomass production (Feeny, 1970; Scriber, 1977; Slansky and Scriber, 1985).

In the bivoltine races of B. mori, generally the ECI and ECD values for female pupa and male cocoon-shell are higher than those for male pupa and female cocoon-shell (Horie and Watanabe, 1983a). Correspondingly the efficiency of storage of absorbed energy to pupa and cocoon-shell show sex-limited differences (Horie and Watanabe, 1985).

Narayanaprakash et al. (1985) have recorded an increased shell weight and fibroin content of the cocoons with increasing diet moisture of mulberry leaves in case of both bivoltine and multi x bi cross breeds of B. mori under tropical situation.

However, no information is available for the multivoltine 'Nistari' race of B. mori regarding the conversion efficiency of leaf into cocoon and shell under differential moisture content of mulberry leaves particularly during the wet part of summer.

### 2.3. CONSUMPTION AND UTILIZATION OF LEAF NITROGEN FOR THE NITROGEN OF SHELL

The classical review of Hiratsuka (1920) and a relatively recent work of Horie et al. (1978) reveals the efficiency of leaf nitrogen utilization by the larvae of B. mori. Over 60 % of the ingested nitrogen is digested and absorbed and about 65% of digested nitrogen is utilized by the 5th instar larvae for silk protein synthesis. This general quantification is for bivoltine races.

The effects of graded levels of protein in the diet on larval growth and silk production have been demonstrated by several workers (Ito and Tanaka, 1962; Ito and Mukaiyama, 1964, 1970; Kamioka et al., 1971; Horie et al., 1971; Horie and Watanabe, 1983 b). The observations of these authors suggest that an optimum level of dietary nitrogen accelerates larval growth and silk production, the best performance is found with highly nutritive proteins. A comprehensive information on nitrogen utilization by B. mori has been obtained from the works of several workers (Hiratsuka 1917; Fukuda 1951; Nakano and Monsi 1968).

A sex-specific compartmentalization of nitrogen utilization for cocoon shell and egg production by the 5th stage larvae of B. mori reared on mulberry leaves has been

demonstrated by Horie and Watanabe (1986). The amount of ingested and digested nitrogen converted to shell was 46 and 70% for the male and 43 and 63% for the female. This clarifies that under optimum life conditions the efficiency of conversion of nitrogen in the shell is very high in the silkworm.

The study of Subba Rao et al. (1989) provides an excellent evidence of nitrogen utilization by the multivoltine Nistari race of B. mori during both favourable and unfavourable seasons. When the larvae were reared on mulberry leaves fortified with variable concentrations of protein meal (1-10%), 1-3% protein enrichment yielded good result. Further, fortification with a 3% protein meal showed a very low nitrogen utilization during the unfavourable season (July-September), but the utilization was several times higher during the favourable season (November-December). The authors implicated higher ambient temperature during unfavourable season to the low nitrogen utilization. A low digestibility of protein and efficiency of conversion of digested protein into cocoon shell have been obtained by Shen (1986) at 30°C rearing temperature than those recorded at 20°C.

Utilization of nitrogen by other lepidopteran larvae has been related to the water content of food. A limiting effect of low leaf water on the nitrogen utilization has been clarified by Scriber (1977) in Hyalophora cecropia. Larvae which were fed

leaves low in water, grew more slowly and were less efficient in plant nitrogen utilization than those larvae fed with leaves supplemented with water. This was equally true for both nitrogen utilization efficiency and relative accumulation rate of nitrogen. Scriber and Slansky (1981) reviewed the relationship of leaf water and nitrogen with the nutrition of folivore larvae belonging to 25 species of Lepidoptera. They concluded that the two nutrients under laboratory conditions predict upper limits for larval performance. Nutritionally unbalanced protein may also reduce larval growth rate by imposing a metabolic load as advocated by Slansky and Scriber (1985) and Schroeder (1986). These authors emphasize that excess protein with accompanying low water in the diet reduces nitrogen utilization. Schroeder (1986) further explained that in such situation excess protein instead of contributing to conversion of larval biomass, added up to metabolic costs for increased catabolism and nitrogen excretion.

Martin and Van't Hof (1988) further emphasized that the reduced efficiency of conversion of digested nitrogen into larval biomass under low dietary water was due to limitation in the amount of water available for the synthesis of new hydrated tissue, and reduced efficiency was not due to the imposition of higher food processing cost. Timmins et al. (1988) opined for Manduca sexta larvae that both higher and lower water than normal lead to increased costs in food processing and lower the efficiencies of conversion into larval biomass.

With this background of knowledge and considering the commercial aspect, consumption and utilization of leaf nitrogen for the nitrogen of shell by the Nistari race of B. mori during wet part of summer in West Bengal at different leaf-moisture have been attempted for investigation.

#### 2.4. LARVAL BODY WATER AND FAECAL WATER WITH REFERENCE TO LEAF MOISTURE

A chronological change in the percentage of body water has been recorded from the first to the fifth instar larvae of B. mori. The lowest amount exists immediately after hatching, increases considerably with the resumption of feeding, almost a steady state is maintained from second to fourth instar. The highest percentage is attained in the newly moulted fifth stage, but declines considerably towards the end of this stage (Hiratsuka, 1917; Ueda and Suzuki, 1967). Thus the first instar appears to be the period of water accumulation and the fifth stage is the period of dehydration.

Legay (1957) observed that the larvae of B. mori fed with mulberry leaves containing 70% water retained much of the ingested water. About 30% was passed out with faeces, 10% was lost as vapour and 60% was retained.

Evans (1939) stated that lepidopteran larvae supported on high-water leaves (>60-90%) absorbed relatively little from the ingested food water (20-30%), while on low-water leaves (<60-50%) absorbed much of the ingested food water (60-80%).

Delvi et al. (1988) obtained a corroborative result in B. mori and Philosamia ricini through their food ration experiment. The larvae retained higher amount of dietary water by increasing water retention efficiency from 22% at 100% ration to 61% at 25% ration. In none of the insects, irrespective of ration level, absorption of water was influenced. But in Danaus chrysippus increasing ration level boosts up dietary water intake, absorption and retention (Pandian et al., 1978). Thus, lepidopteran larvae have evolved either to the strategies for adapting against water constraints by absorbing higher amount of dietary water or by increasing the efficiency of retention.

Moisture loss is controlled in insects to maintain the balance at an appropriate level depending on the ambient situation and conserved or eliminated according to the state of hydration in the body (Chapman, 1972). In India investigation on water balance in other phytophagous lepidopterans has also been undertaken. Goel and Singh (1987) observed in Lymantria marginata that the moisture in the body and egesta decreased with the increase in larval biomass in successive instars. In Trabala vishnu the relationship between food water and larval body water

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was positively significant (Krishna Rao and Goel, 1987). Similar result was obtained in one multivoltine race of B. mori during rainy season (Pathak and Vyas, 1988). The authors further relate high body water to excessive urination by the spinning larvae during this season. The rate of urination is enhanced by climatic conditions. High rate of urination during rainy season by multivoltine silkworm was earlier recorded by Kumararaj (1969)

Larvae of B. mori have only diuretic hormone secreted from brain neurosecretory cells. The secretion of diuretic hormone is more during rainy season. Therefore, the diuretic hormone controls osmoregulation of spinning larvae of B. mori (Pathak, 1991). Nicolson (1980) and Nicolson and Miller (1983) have made significant study on the hormonal control of diuresis in butterflies. Vyas (1991) recently examined the effects of some drugs on diuresis of a multivoltine hybrid silkworm.

Thus, during rainy season there is high ambient r.h. and more accumulation of larval body water from high-water leaves causing to pass excess moisture with faeces. Excess moisture of the faeces further adds to rearing bed moisture. The condition favours the growth of micropathogen population, particularly that of bacteria, which causes considerable crop loss. In order to mitigate all these problems and resultant loss the present investigation has been designed using variable leaf moisture.

## 2.5. REARING RESULT

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In the tropical conditions of India, apart from some genetical relevance of silkworm races, most of the works on rearing results have so far been concentrated on the impact of different mulberry cultivars on this or that bivoltine or multivoltine breeds or races of B. mori. A comprehensive information regarding varietal contribution of mulberry leaves to larval growth and economic characters of silkworm is available from Yokoyama (1963), but the information is on bivoltine breeds or races. The differential nutritive value of the same mulberry cultivar has also been recorded on different seasons, and even depending on the agro-practices.

Apart from other nutritional qualities of leaves, water content surely has a bearing on the rearing results as because the water percentage of mulberry leaves, irrespective of cultivars, differs in different seasons (Pathak and Vyas, 1988; Sinha et al., 1993) and at different ages (Kawase, 1914; Hiratsuka, 1917). Some of the important works on the impact of mulberry varieties can be referred to Krishnaswami et al. (1970a), Tayade and Jawale (1984), Sharma et al. (1986), Tayade et al. (1988), Das and Vijayaraghavan (1990).

Giridhar and Sivarami Reddy (1991a) reported differential larval weights and cocoon and shell characters of bivoltine

breeds on different seasons, the best performance was on rainy season irrespective of mulberry varieties. Further, a highly significant interaction between mulberry varieties and seasons was obtained in respect of both ERR No and ERR Wt (Giridhar and Sivarami Reddy 1991b).

With regard to leaf age and consequent rearing results Krishnaswami et al. (1970b) undertook investigation. Top tender leaves, including buds were found to be superior to lower leaves. Even, if the mature middle or bottom leaves were sprinkled with water prior to feeding, ERR No and ERR Wt were depressed. But the tender leaves are not available in bulk and are not suitable for late-aged larvae for increased larval and pupal mortality particularly during the rainy season.

Because of differences in nutritive value of mulberry cultivars, both cocoon yield and mean cocoon weight vary (Penkov et al. 1988), and also the cocoon weight and shell weight (Periasamy<sup>w</sup> and Radhakrishnan, 1985).

A study was undertaken by Narayanaprakash et al. (1985) using artificially depleted water in mulberry leaves. The experimental 5th stage larvae were a bivoltine hybrid and a multi x bi cross breed. The larvae were fed with both tender and mature leaves having differential water percentages. Depending on the age and water content of leaves there was considerable variation

in the cocoon-shell weight. In general, bivoltine hybrid thrived well on tender leaves and spun larger cocoons while the cross breed thrived on mature leaves and produced heavier cocoons.

Besides the variation in water content, the nitrogen contents, particularly some amino acids such as methionine, histidine and threonine of mulberry leaves of different cultivars vary at different seasons. Thus, Machii and Katagiri (1990) found a seasonal cultivar dependant shell production efficiency in the same race of B. mori positively correlated with the amino acid contents.

Nistari race reared on mulberry leaves enriched with 1% protein meals showed a significant increase in ERR no during August-September (unfavourable season). Whereas, during November-December (favourable season) an enrichment with 1-3% protein meal yielded variably an improved rearing performance in respect of larval wt, ERR no, ERR wt, Cocoon wt, shell wt, SR% and absolute silk content (Subba Rao et al. 1989).

Schroeder (1986) reported that when the fifth stage larvae of leaf-eating lepidopterans were fed with mature leaves having low water but fortified with protien, showed lower ingestion rate and growth efficiency but higher apparent digestion efficiency and apparent assimilation rate. Treated larvae pupated at a lower weight. The low leaf water acted as primary limiting nutrient so that surplus protein imposed a

metabolic load on larvae perhaps by increased catabolism and excretion of excess amino acids causing osmotic imbalance. Slansky and Scriber (1985) are also of the same opinion.

Fifth stage larvae alone consume about 87% of the total leaf consumption during larval life. The ingestion of high-water leaves raises larval body water at directly proportional level, leading to proportionately increased urination, consequent disease induction, hence, larval and pupal mortality. However, there is no information on effective remedy for crop loss during rainy season. This justifies the present investigation aiming at finding out the rearing performance at different dietary water levels.

## 2.6. COCOON MELTING

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Cocoon melting is the death of pupa and followed by decomposition within the shell enclosure. This is affected by factors such as climatic conditions during larval spinning (Krishnaswami, 1986; Benchamin and Nagaraj, 1987) and during the fifth larval stage of rearing. Seasonal climatic conditions also affects cocoon melting. High humidity and relatively high temperature during rainy season result in highest percentage of cocoon melting (as experienced by farmers and at the Silkworm Seed Production Centres.). However, the literature on cocoon melting is very scanty. Compared to bivoltine breeds less melting

occurs in the multivoltine races (Benchamin and Krishnaswami, 1981a, b). The authors further observed among the bivoltines a breed - specific variation in melting percentage.

Giridhar et al. (1990) undertook an investigation on melting using different bivoltine breeds of silkworms, reared on different mulberry cultivars and in different seasons. Their results revealed maximum melting during rainy season among Japanese races than the Chinese races and on a particular mulberry variety (S4 ). Rao et al. (1990) also observed a differential melting percentage (2.8 to 5.1%) among four bivoltine breeds reared on the same mulberry cultivar.

No scientific information is available regarding the precise causes of cocoon melting, particularly from the physiological and or consequent pathological stand point. Neither there is any scientific study on this aspect on the multivoltine Nistari. But large scale rearing is required both for silk production and seed production. The density of larvae in a large rearing population in case of practical sericulture adds up moisture in the rearing bed particularly during the rainy season. The overall situation may favour the incidence of carried over diseases in the pupae causing melting.

The present attempt is intended to find out a solution for reducing the pupal melting using leaves containing suitable amount of water.

## 2.7 REPRODUCTIVE PERFORMANCE

Reproductive function of insects is the result of interaction of nutritional, hormonal and environmental factors (Engelmann, 1970; Calow, 1973; De Wilde and De Loof, 1973 a,b). The fertility and fecundity are very sensitive to quantitative and qualitative changes in the food of silkworms and thus nutrition affects both egg number and egg vigour (Legay, 1958). It is also evident from the experiments on fifth instar larvae of B. mori by way of food rationing (Radhakrishna and Delvi, 1987; or by restricting the feeding regimes including feeding frequency/day (Haniffa et al., 1988), deprivation of food on different days (Radhakrishnan et al., 1985) or even on different hours of a day (Mathavan et al., 1987) that the nutritional regulations affect considerably the reproductive performance of B. mori. All these works perhaps aimed at economizing rearing labour and mulberry leaf quantity.

Observation on different bivoltine and multivoltine breeds of B. mori by Pillai and Krishnaswami (1989) under tropical conditions revealed that variation in fecundity was influenced by both intrinsic factors of the breeds and ambient conditions.

A mulberry variety dependent fecundity has been advocated from the observation on variable number of egg production in

B. mori (Nacheva et al., 1988). Similarly food quality dependent egg production has also been claimed in other phytophagous insects (Muthukrishnan and Pandian, 1987).

B. mori like many other lepidopterans having non-feeding adult stage, stores abundant food resource largely during the last larval instar. For the purpose, the last stage attains 'critical weight' through its early part of essential or obligatory feeding period. This feeding pertains largely to reproductive resource allocation (Inagaki and Yamashita, 1983, 1986).

In relating fecundity to pupal weight of B. mori Tanaka (1988) advocates that an optimum range of cocoon weight results in maximum egg production, higher cocoon weight causes greater mortality and production of increased number of unfertilized egg-laying moths. Watanabe (1961) and Yokoyama (1973) are of the opinion that the heterosis in egg production among the breeds is not influenced by mere body weight. Watanabe (1961) further proposed the term 'Luxuriance' on the relation of higher pupal weight to fecundity. Apparently, a high significant positive correlation exists between the moths resulting from heavy pupae and their fecundity not only in multivoltine breeds of B. mori (Rahaman et al., 1978; Jayaswal et al., 1991) but also in other sericigenous moths such as Antheraea mylitta (Siddique et al., 1985), Philosamia ricini (Singh and Prasad, 1987) and Samia cynthia ricini (Nagalakshamma et al., 1988; Kotikal et al., 1989).

A contradictory result has been claimed by Narasimhanna (1988) that the egg laying efficiency is reduced in those moths obtained from heavy pupae.

However, male pupal weight has no correlation with the fecundity (Gowda et al., 1988). Jayaswal et al. (1991) studied the fecundity of a newly evolved A-25 multivoltine breed at three different seasons in the plains of West Bengal. The fecundity was directly proportional to the female pupal weight, though the average fecundity per range of pupal weight was not constant in all the seasons. A seasonal variation in the fecundity has also been reported by Tazima (1958).

A study on the Nistari race of B. mori by Subba Rao et al. (1989) reared on mulberry leaves fortified with protein meal reveals that the larval growth and consequent fecundity results are season dependant. In general the fecundity increases upto an optimum level of protien addition, but the increase is several times higher during favourable seasons (November-December) than that during unfavourable season (July-September).

The foregoing survey of literature reveals a diversity of opinion regarding pupal weight and fecundity of resultant female moths. The luxuriant vegetation, high moisture and endurable temperature during the wet part of summer provide a congenial condition for gaining high larval and pupal weight rapidly. This apparent body weight and good fcundity turn into overall less recovery of eggs due to high rate of larval and pupal mortality

and failure of the female moths to mate and oviposit optimally. However, no information is available regarding abatement of such losses if the larvae are fed with mulberry leaves having depleted water during wet part of summer in tropical regions. This justifies the undertaking of present investigation.

#### 2.8. REELING CHARACTER OF COCOON AND SILK FILAMENT CHARACTER

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In tropical humid situation silkworm larvae acquire high dietary water and accumulate the same in body because of low transpiratory loss. Thus, during rainy season a higher amount of urination occurs, which is directly proportional to the leaf water and body water (Pathak and Vyas, 1988). Excess urination causes higher proportion of yellow-stained cocoon production. Such cocoons incur considerable economic loss by way of poor reelability of fibres due to frequent breaks, more droppings and increased waste percentage of fibres leading to increased renditta value (requirement of green cocoons in Kg. to produce one Kg. of raw silk) (Kumararaj, 1969; Kumararaj, 1972).

The filament characters such as filament length and denier (fibre thickness) obtained from a particular silkworm race have been implicated by several workers to different mulberry cultivars (Hassanein and El Shaarawy, 1962b; Periaswami and Radhakrishnan 1985; Penkov et al., 1988). The impact of filament characters may be due to differences in nutrition obtained from different cultivars.

Absorbed urine being alkaline, dissolves the sericin coating of a silk filament, thus exposes core fibroin fibre (Tanaka, 1964; Kumararaj, 1972 ).

An elaborate study has been made by Tsukuda et al. (1989) on the impact of absorbed moisture on the fibroin component of a silk filament. They examined the fibroin fibre by thermomechanical analysis, X-ray diffractometry and on the basis of refractive index and strength elongation measurement. Increased moisture absorption causes a decreased birefringence of silk fibre and elongation strength and thus induces filament break during reeling.

With a view to combat excessive urination by the spinning larvae attempts have been made to exploit endocrine mechanism underlying diuresis. Pathak (1991) observed in a multivoltine hybrid silkworm that the brain neurosecretory cells producing diuretic hormone are influenced by high ambient humidity. In the same hybrid silkworm Vyas (1991) undertook both in vitro and in vivo studies using diuretic hormones. The work was aimed at reducing water accumulation in larval body through the enhancement of early urination in pre-spinning larvae for reducing cocoon staining. The author found 5- hydroxytryptamine a promising hormone.

The present investigation is an attempt to understand whether the differential nutritional efficiency under different dietary water levels affect the silk filament characters and damage due to excessive urination could be ameliorated.

## 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<sup>o</sup> 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 pruning so that the water content remained almost at the same level. Fresh leaves having a moisture of  $76.62 \pm 0.5\%$  were given to the control larvae. But the leaves given to the larvae of treatment sets contained 60, 65 and 70%  $\pm 0.5\%$  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  $60^{\circ}\text{C}$  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

far for variable periods, which again differed on rainy and sunny days. After about 105-110, 70-78 and 40-42 minutes every gram of leaves after loosing water weighed 0.59, 0.67 and 0.78 g which corresponded respectively to the leaves having 60, 55 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 subtracting 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 60° C till the weight became constant. The dry weight of food ingested was calculated by subtracting the dry weight of residual food from that of the aliquot. The

quantity of food digested was measured by subtracting the weight of the faeces from the quantity of food ingested.

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

$$\begin{aligned} \text{(Absolute) growth rate} &= \frac{P}{T} \\ \text{(Absolute) consumption rate} &= \frac{E}{T} \\ \text{Approximate digestibility \%} &= \frac{(E-F) \times 100}{E} \end{aligned}$$

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

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

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 initial larval dry weight was recorded based on 5 observations each consisting of 20 larvae to get better estimate for initial larval weight. The fullgrown (final) larval weight was taken prior to 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 subtracting 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  $Y = a + bx$ , where  $Y =$  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 digestibility, 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 regression line relating to larval duration, represented by  $Y$ . In the figures, the regression line was first drawn on the basis of respective regression equation. Then mean observed values with standard error bars were plotted against the corresponding leaf moisture.

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

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 calculation 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

$$\text{shell} = \frac{\text{Wt of ingested dry matter}}{\text{Wt of cocoon shell}}$$

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

### 3.5.1. Statistical calculations

The procedures followed for statistical 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 mortar 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 similar

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 calculated. 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 %

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

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, approximate 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 determined 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 statistical 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 transformation 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 simultaneously 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 statistically 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 view 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 of 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 allowed to mate with a single male moth for 3 hours, but every 30 minutes observation was recorded for their mating capacity.

After mating the males were carefully removed and females were kept separately on egg card covered by plastic cellulose for egg laying for a period of 24 hours which was the active oviposition period. After 24 hours of oviposition all the mated females were dissected for observing the retention of mature (chorionated) eggs in the ovarioles if any, thus ascertaining the complete or partial oviposition. Data on the mating success and the oviposition success (most of the mature eggs laid) by mated females were recorded. These data were analysed statistically for testing the level of significance.

### 3.11. Reeling character of cocoon and silk filament character

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

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 approve. 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 formula :

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

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

## RESULTS

## 4. RESULTS

### 4.1. CONSUMPTION AND UTILIZATION OF MULBERRY LEAVES, LARVAL GROWTH AND DURATION

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#### 4.1.1. Effect of leaf moisture on nutrition

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The quantity of leaves consumed, quantity digested, absolute consumption rate, ECI% and ECD% increased progressively with the increase of leaf moisture. The values of all these nutritional indices were highly significant for all the water levels i.e. leaves with control and different depleted percentage of moisture (Table 1). But the approximate digestibility was a little exception. The value of this index also increased in the larvae which were fed with leaves containing upto 70% water but then decreased significantly at 76.62% leaf moisture. Even this decrease differed significantly from the value obtained at 70% dietary water.

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

In the regression characteristics of different nutritional indices of leaf moisture, linearity was observed for quantity of leaf consumption and absolute consumption rate (Figs 1 and 2) with highly significant values for coefficient of determination

Table 1. Observed and predicted (in paranthesis) mean values of different nutritional parameters at different levels of leaf moisture. C.D. was calculated only for the observed values.

Parameters	Moisture (%) in the leaves				C.D. at 5% level of significance
	60±0.5	65±0.5	70±0.5	control	
Quantity consumed (g)	1.356 (1.359)	1.470 (1.463)	1.567 (1.567)	1.705 (1.704)	0.023
(Absolute) Consumption rate (g)	0.176 (0.177)	0.214 (0.215)	0.255 (0.253)	0.302 (0.303)	0.009
Quantity digested (g)	0.438 (0.437)	0.522 (0.527)	0.588 (0.587)	0.618 (0.620)	0.008
Approximate digestibility (%)	32.262 (32.028)	35.540 (35.638)	37.548 (37.184)	36.232 (36.052)	0.388
ECI%	11.764 (11.624)	15.224 (15.306)	17.914 (17.693)	18.956 (18.861)	0.223
ECD%	36.476 (36.349)	42.836 (42.669)	47.750 (47.625)	52.308 (52.084)	0.784
(Absolute) Growth rate (g)	0.020 (0.019)	0.032 (0.030)	0.045 (0.041)	0.057 (0.056)	0.002
Final larval weight (g)	0.204 (0.215)	0.268 (0.264)	0.325 (0.313)	0.367 (0.379)	0.004
Larval duration (days)	7.700 (7.621)	6.850 (6.881)	6.150 (6.260)	5.650 (5.578)	0.176

Table 2. Correlation coefficients between leaf moisture and pairs of nutritional index parameters

	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	-0.961	-0.839	-0.987	-0.993	-0.991	-0.984

All are significant at p<0.01 level, df = 18

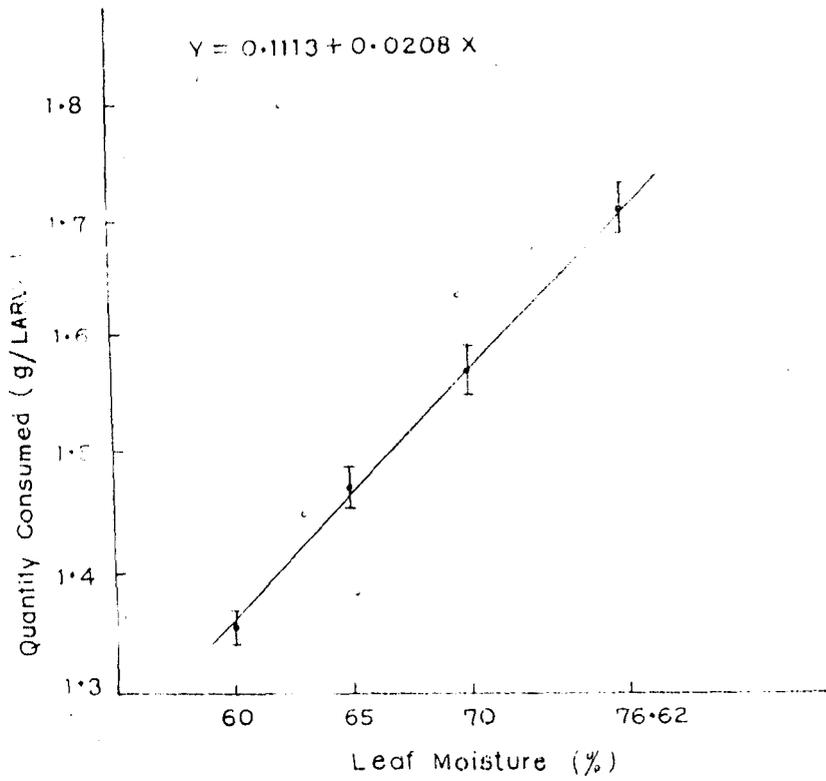


Fig.1. Effects of leaf moisture on the quantity of food consumption ( $r^2 = 0.986$ ,  $df = 18$ ).

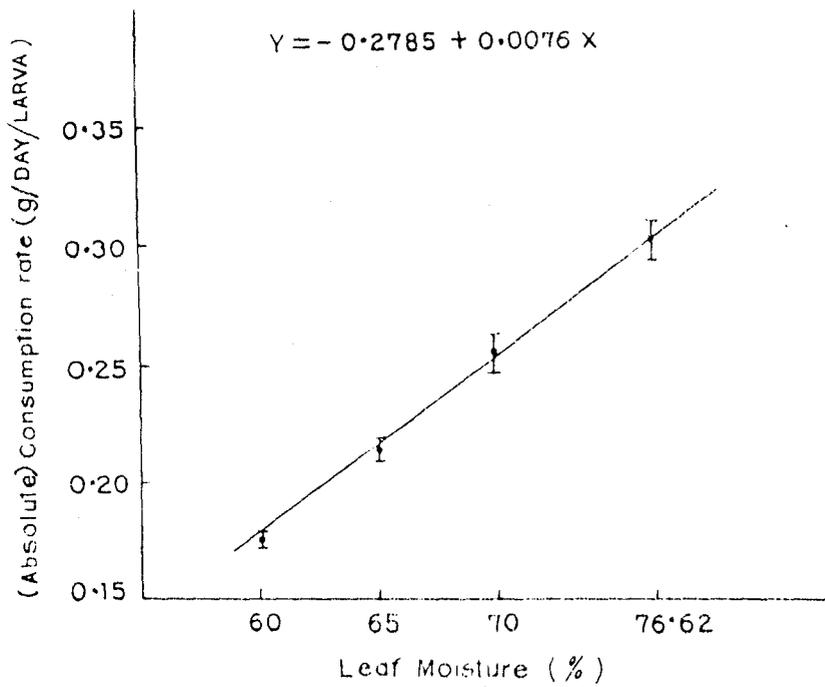


Fig.2. Effects of Leaf moisture on absolute consumption rate ( $r^2 = 0.986$ ,  $df = 18$ ).

$r^2$ ). But for the values for 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 test the effectiveness of the regression equations of all the parameters on different levels of leaf moisture the predicted values derived from regression equation were compared with the observed values (Table 1). The marginal deviations of predicted values from the observed values were indicative of better goodness of fit of the regression equations as the  $r^2$  values calculated for different parameters were highly significant (Table 3).

From the regression figures it appeared that the degree of increase in the value of different parameters from one moisture level to the next level was not uniform. With the elevation of moisture from 60 to 65% there was higher rate of increase in the quantity digested, approximate digestibility, ECI% and ECD%. At the subsequent rise of water levels the increase, though progressive, was comparatively lower (Figs 3 - 6). But for the quantity consumed and absolute consumption rate initially the values increased at a relatively low rate compared with the values obtained at higher moisture levels (Figs 1 and 2).

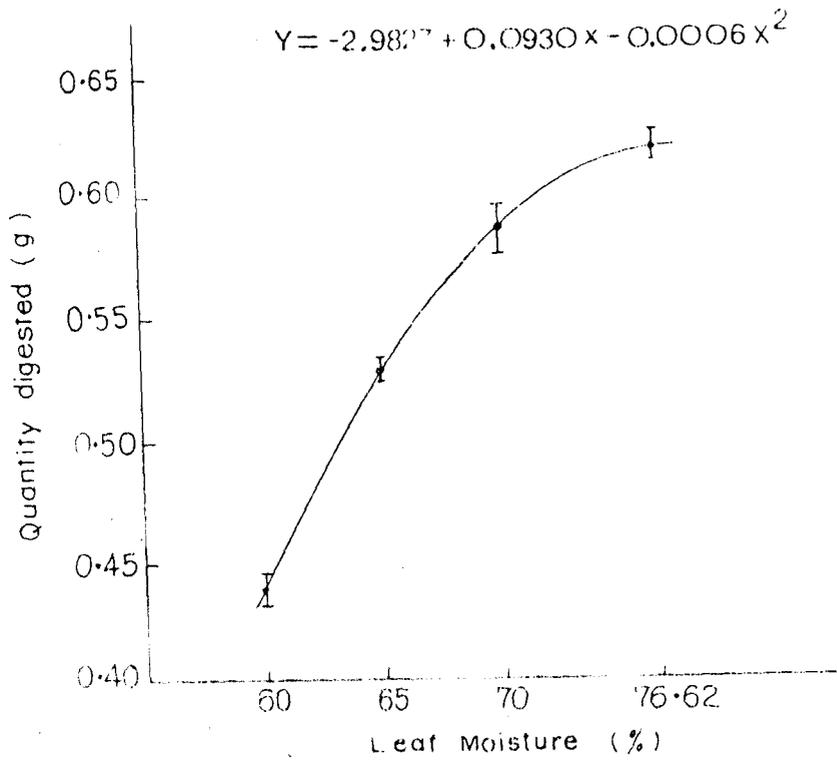


Fig.3. Effects of leaf moisture on the quantity of food digested ( $r^2 = 0.994, df=18$ ).

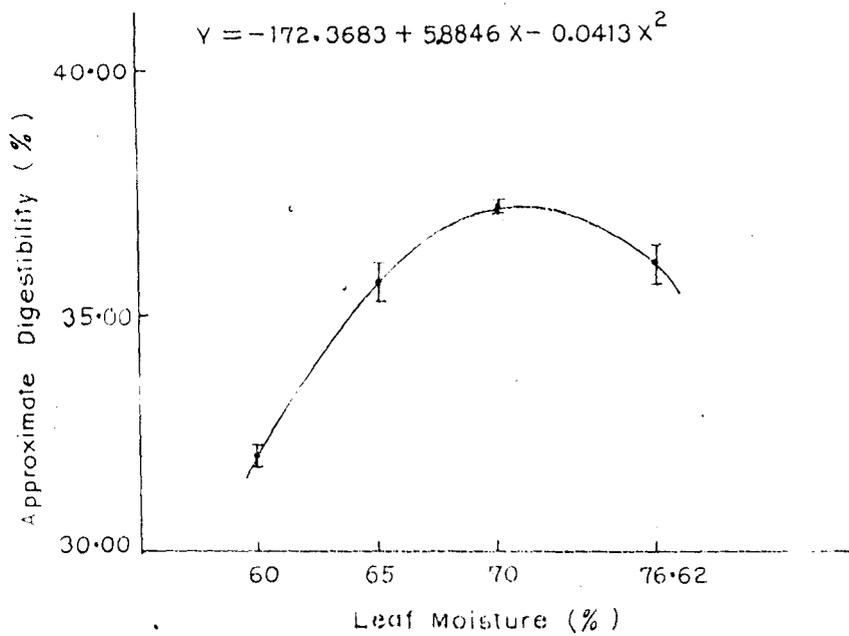


Fig.4. Effects of leaf moisture on approximate digestibility (%) ( $r^2 = 0.982, df=18$ ).

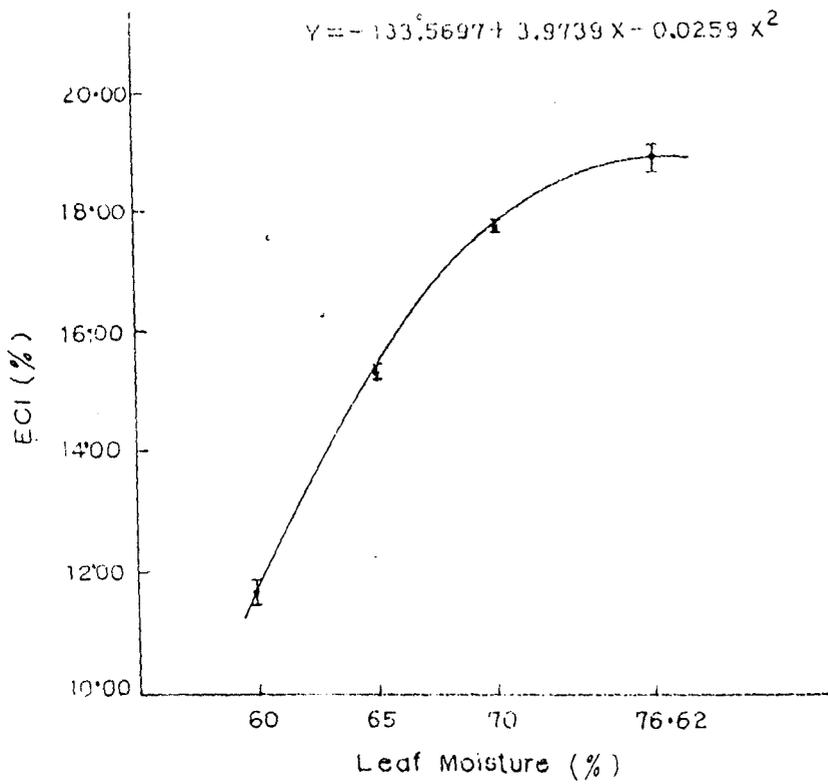


Fig.5. Effects of leaf moisture on efficiency of conversion on ingested food (ECI%) ( $r^2 = 0.997$ ,  $df=18$ ).

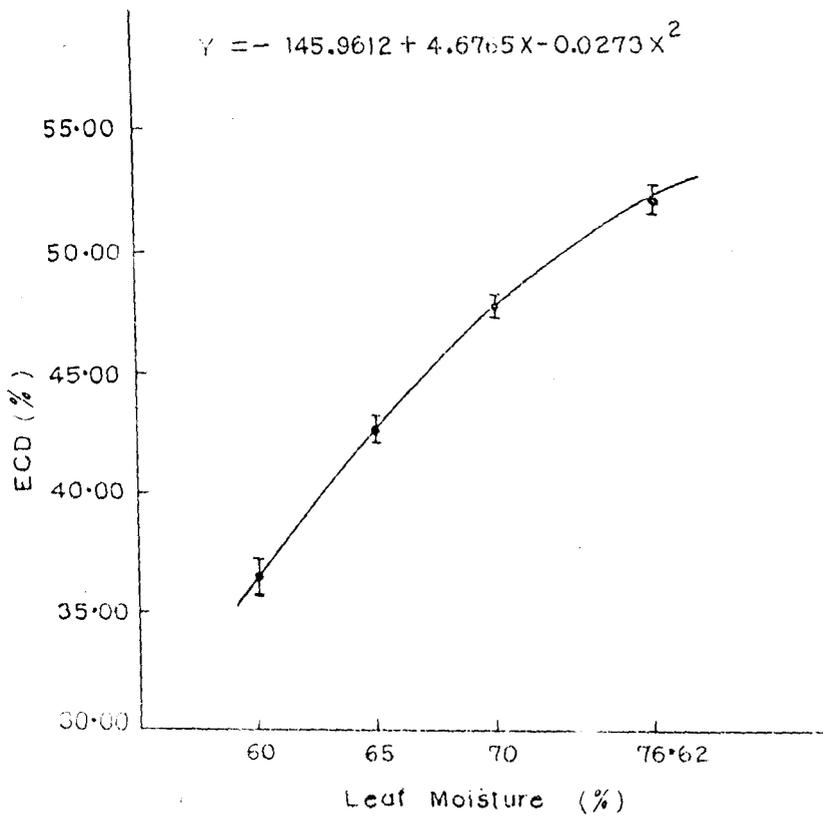


Fig.6. Effects of leaf moisture on efficiency of conversion of digested food (ECD%) ( $r^2 = 0.992$ ,  $df=18$ )

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

Parameters	Regression equations(y)	Co-efficient of determination (r <sup>2</sup> ) *
A. Linear Regression Equations		
Quantity consumed(g)	0.1113+0.0208x	0.986
consumption rate (g)	-0.2785+0.0076x	0.984
Growth rate (g)	-0.1121+0.0022x	0.984
Final larval weight (g)	-0.3794+0.0099x	0.972
B. Curvilinear Regression Equations		
Quantity digested(g)	-2.9827+0.0930x-0.0006x <sup>3</sup>	0.994
Approximate digestibility (%)	-172.3683+5.8846x-0.0413x <sup>2</sup>	0.982
ECI%	-113.5697+3.9739x-0.0259x <sup>2</sup>	0.997
ECD%	-145.9612+4.6765x-0.0273x <sup>2</sup>	0.992
Larval duration (days)	-1.2760 1415.59x	0.963

\* All r<sup>2</sup> values are significant at 1% level (P<0.01), df=18

#### 4.1.2. Effect of leaf moisture on growth

Both absolute growth rate and final larval weight increased progressively with the rise in foliage water (Table 1). Regression characteristics of these two indices on the leaf moisture showed a distinct linearity (Figs 7 and 8). The pattern of increase in the growth rate in relation to the increasing water content was almost uniform, but the pattern of the same for larval weight was not uniform although. At the subsequent higher water levels beyond 65% the degree of growth was relatively low. But the  $r^2$  values were significant (Table 3).

#### 4.1.3. Effect of leaf moisture on larval duration

The duration of larval life became progressively shorter with the rise in water content of the leaf (Table 1). The relation between short larval duration and the rise in water levels was negative but highly significant. The regression characteristic was slightly curvilinear. The pattern of shortening was highest between 60 to 65%, then with the rise in water content it was relatively lower, the lowest being at the control leaf moisture (Fig.9).

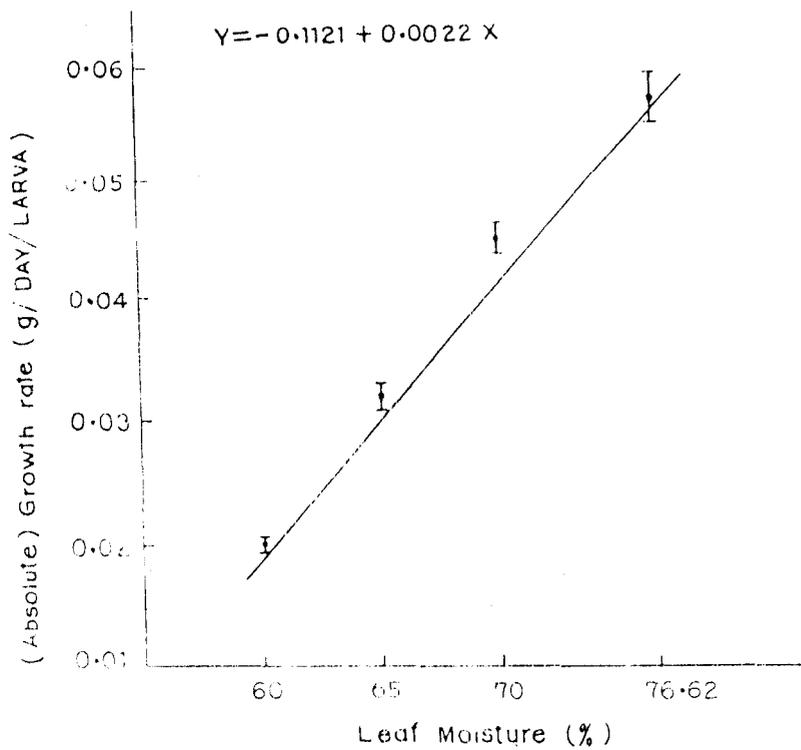


Fig. 7. Effects of leaf moisture on absolute growth rate ( $r^2 = 0.984$ ,  $df=18$ ).

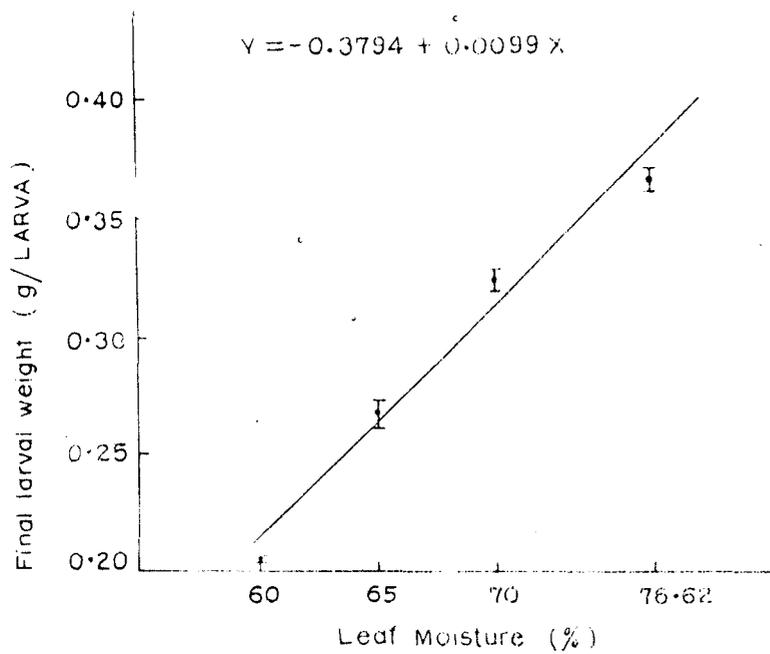


Fig. 8. Effects of leaf moisture on final larval weight ( $r^2 = 0.972$ ,  $df=18$ ).

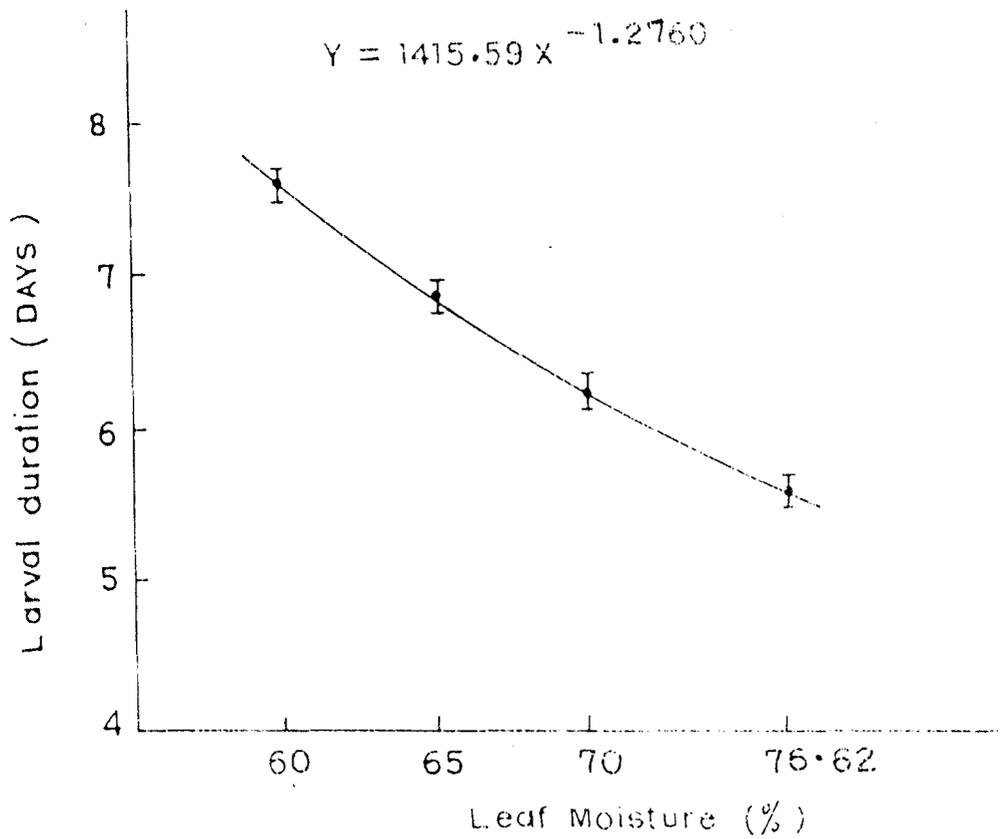


Fig.9. Effects of leaf moisture on larval duration ( $r^2 = 0.963$ ,  $df=18$ ).

#### 4.2. EFFICIENCIES OF CONVERSION OF CONSUMED LEAVES INTO COCOON AND SHELL

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The quantity of food ingested and digested ( chap. 4.1, Table 1), ECI% and ECD% for cocoon and total dry weight of cocoon and shell increased with the increase of leaf moisture upto the control level (Table 4). But the ECI% and ECD% for shell also increased upto 70% leaf moisture, then declined slightly at control water level (Table 4). A converse situation occurred for quantity of food required for the production of every gram of shell, which decreased with the rise of leaf water upto 70%, then increased at 76.62% (Table 4). A significant ( $P < 0.01$ ) positive correlation was observed between the leaf moisture and each of the parameters and among the parameters except the quantity of ingested food required for each gram of shell production which showed negative correlation. This is evident from the  $r$  values (Table 5).

From the regression characteristics regarding the relationship of all the parameters on leaf moisture linearity was observed for the quantity of food ingested (Paul et al., 1992), weight of cocoon (Fig.10) and shell (Fig.11) with high coefficient of determination values ( $r^2$ ) which indicated a rise of these values with increasing leaf moisture. The rate of increase of these values might had been lowered at any point with

Table 4. Observed and predicted (in parentheses) mean value of different food utilization indices at different levels of leaf moisture. C.D. was calculated for the observed values.

Parameters	Moisture in the leaves (%)				C.D. at 5% level of significant
	60±0.5	65±0.5	70±0.5	76.62±0.5 (Control)	
Wt. (g)/Cocoon	0.191 (0.205)	0.255 (0.246)	0.300 (0.286)	0.326 (0.340)	0.004
Wt. (g)/Shell	0.068 (0.073)	0.093 (0.088)	0.114 (0.104)	0.118 (0.124)	0.002
ECI(%) for cocoon	10.838 (10.837)	14.506 (14.430)	16.348 (16.417)	16.630 (16.579)	0.380
ECD(%) for cocoon	33.596 (33.540)	40.306 (39.807)	43.606 (43.723)	45.640 (45.293)	0.995
ECI(%) for shell	4.926 (4.681)	6.232 (6.260)	7.275 (6.993)	6.888 (6.664)	0.158
ECD(%) for shell	15.269 (15.155)	17.535 (17.988)	19.429 (19.417)	19.005 (19.147)	0.487
Wt. (g) of ingested food required/g shell production	20.312 (20.424)	16.047 (15.864)	13.748 (13.704)	14.522 (14.537)	0.479

Table 5. Correlation coefficients between leaf moisture and each of the parameters and between the pairs of different parameters

	Leaf moisture (%)	Quantity consumed (g)	Quantity digested (g)	Cocoon Wt. (g)	Shell Wt. (g)	ECI % for cocoon	ECD % for cocoon	Quantity of ingested food required/g shell production	ECI % for shell	
Quantity consumed (g)	0.993	1								
Quantity digested (g)	0.960	0.963	1							
Cocoon Wt. (g)	0.966	0.965	0.995	1						
Shell Wt. (g)	0.926	0.922	0.986	0.986	1					
ECI% for cocoon	0.898	0.895	0.971	0.977	0.983	1				
ECD% for cocoon	0.937	0.932	0.974	0.989	0.976	0.967	1			
Quantity of ingested food required/g shell production	-0.814	-0.808	-0.928	-0.931	-0.965	-0.978	-0.948	1		
ECI% for shell	0.807	0.798	0.923	0.922	0.968	0.965	0.931	-0.993	1	
ECD% for shell	0.830	0.818	0.924	0.932	0.973	0.966	0.945	-0.987	0.993	1

All are significant at 1% level ( $p < 0.01$ ),  $df = 18$

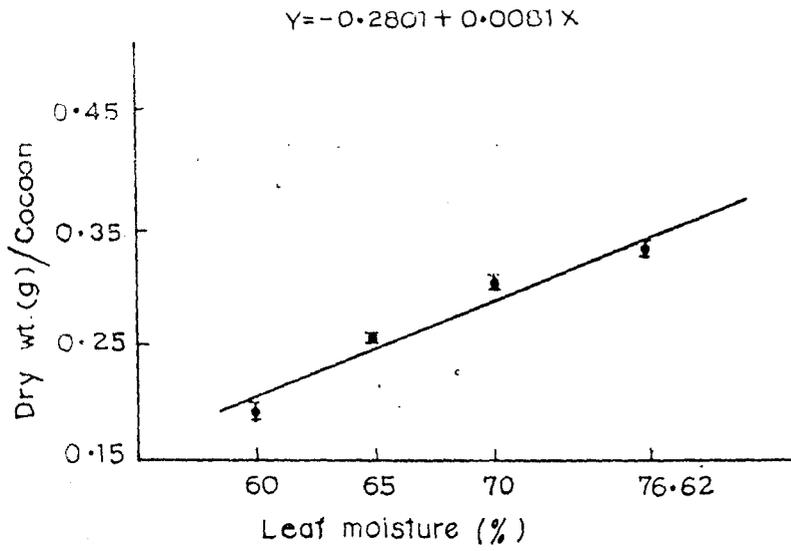


Fig.10. Impact of foliage water levels on cocoon weight ( $r^2 = 0.934$ ,  $df=18$ ).

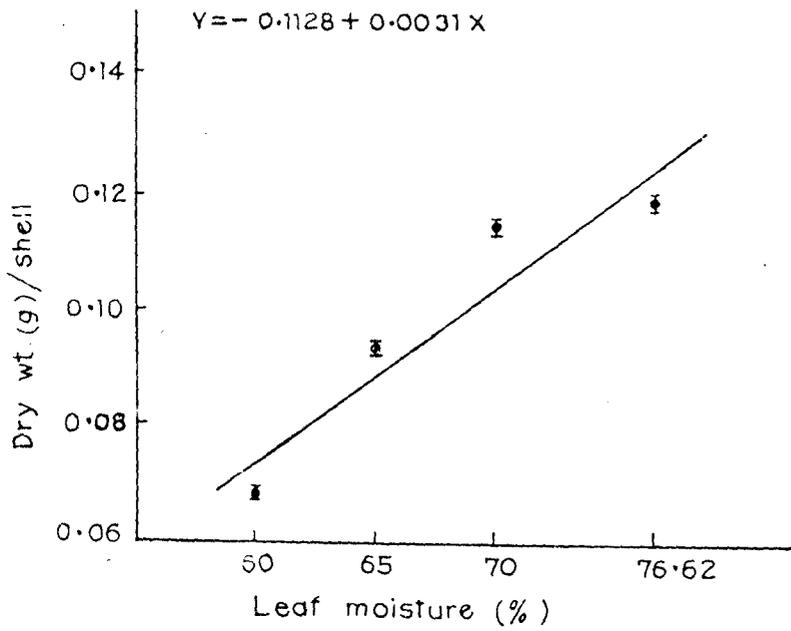


Fig.11. Impact of foliage water levels on shell weight alone ( $r^2 = 0.858$ ,  $df=18$ ).

further rise of water level beyond 76.62%. But for the quantity of food digested (Paul et al., 1992), ECI% for cocoon (Fig. 12) and ECD% for cocoon (Fig. 14) there were also an increase of values corresponding to the higher levels of leaf moisture; however, the ECI% and ECD% for shell (Figs. 13 and 15) increased with the rise of water upto 70%, thereafter the values decreased at the control water level (Table 4). The  $r^2$  values relating to all these parameters were also high (Table 6), and the relationship was curvilinear. The quantity of ingested food required for the production of one gram of shell with regard to different levels of dietary water, the relationship was negative and significant showing high  $r$  values (Table 5), but the regression was curvilinear (Fig. 16).

In order to test the effectiveness of regression equations of all the parameters on leaf water, the predicted values derived from regression equation were compared with the observed values (Table 4). The marginal higher or lower deviations of predicted values from the observed values emphasized the better goodness of fit of regression equation, as because the  $r^2$  values were also significantly high.

Analysis of regression figures revealed a differential rate of increase or decrease of the different parameters corresponding to the rise of leaf moisture from one level to the next higher level (Figs. 10 - 16, Table 4). A common trend of decrease in the

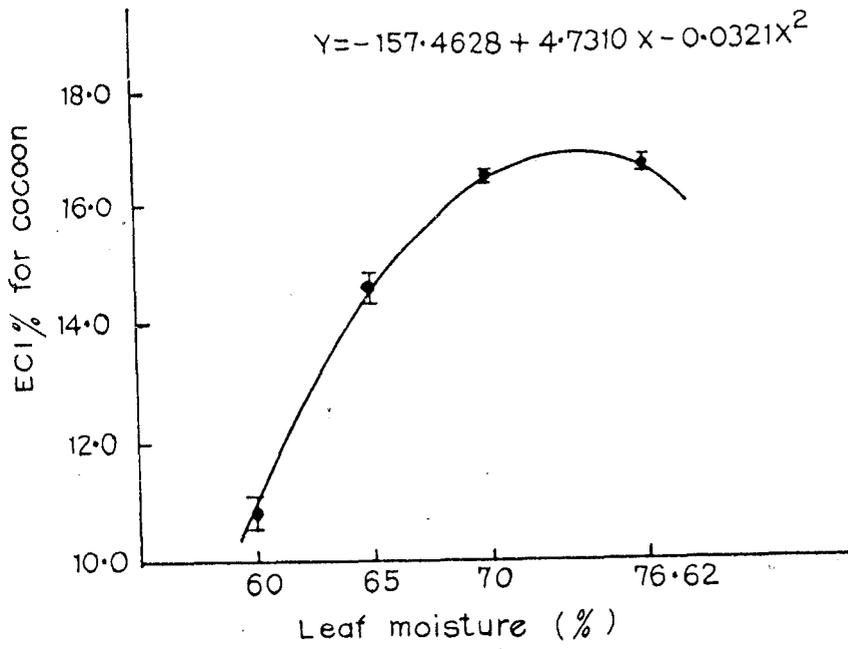


Fig.12. Impact of foliage water levels on ECI% for cocoon ( $r^2 = 0.990$ ,  $df=18$ ).

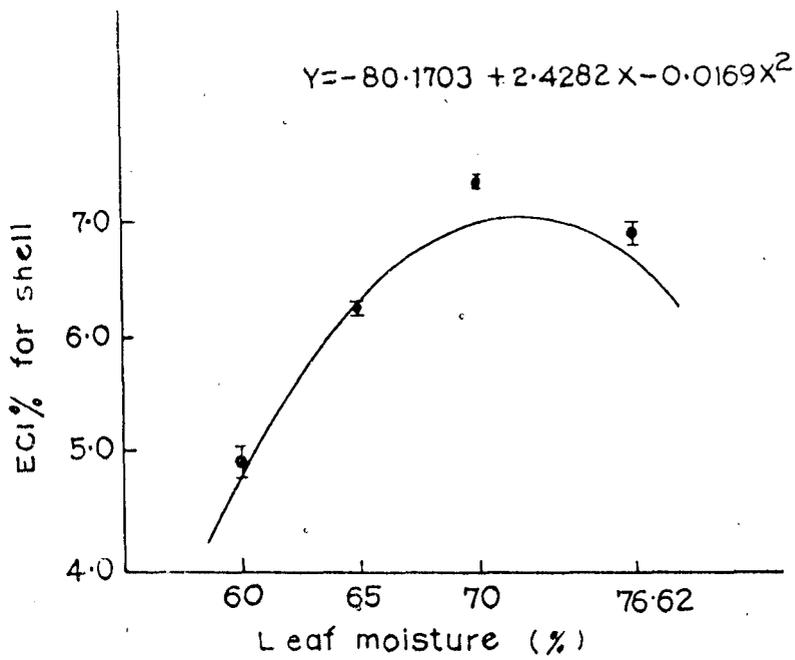


Fig. 13. Impact of foliage water levels on ECI% for shell ( $r^2 = 0.967$ ,  $df=18$ ).

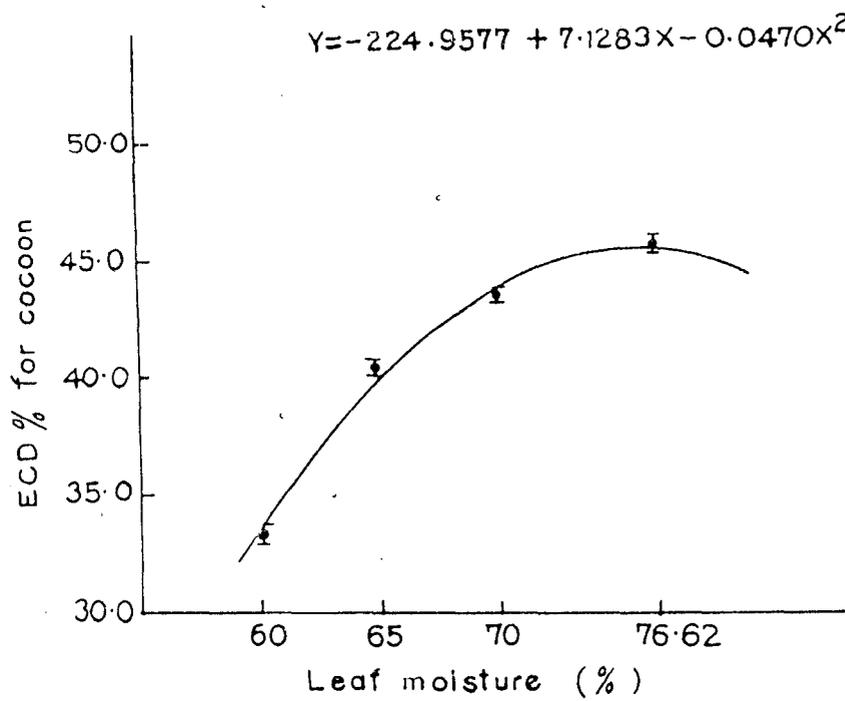


Fig.14. Impact of foliage water levels on ECD% for cocoon ( $r^2 = 0.985$ ,  $df=18$ ).

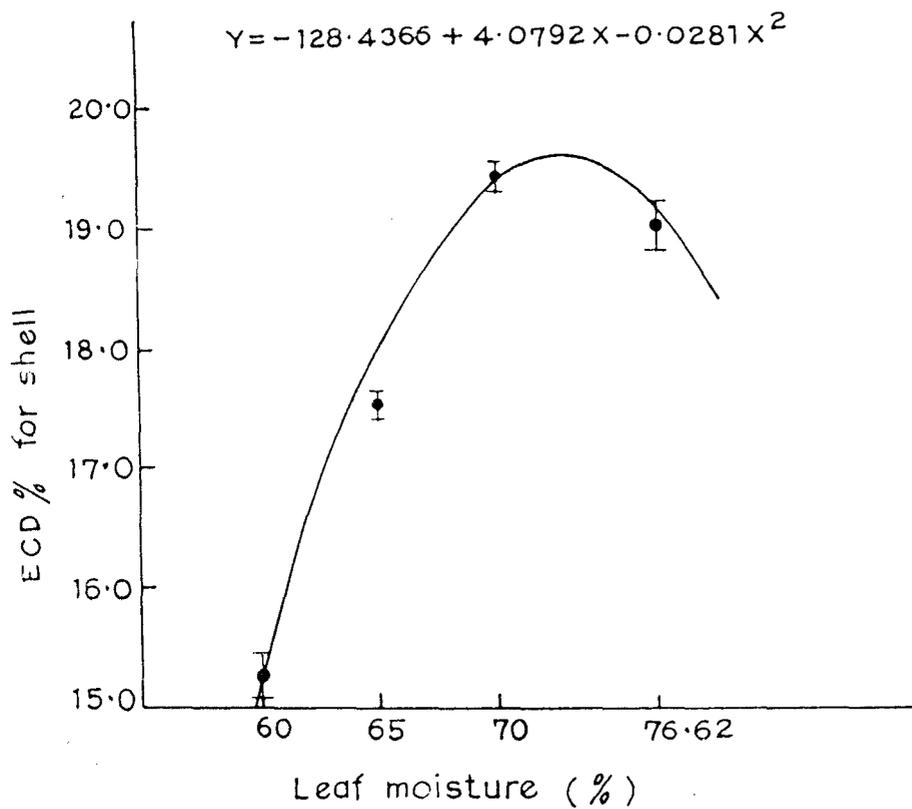


Fig.15. Impact of foliage water levels on ECD% for shell ( $r^2 = 0.943$ ,  $df=18$ ).

Table 6. Regression co-efficients of leaf moisture on different index parameters

Parameters	Regression equations(y)	Co-efficient of determination ( $r^2$ )
A. Linear Regression equations		
* Quantity consumed(g)	$0.1113+0.0208x$	0.986
Cocoon wt(g)	$-0.2810+0.0081x$	0.934
Shell wt(g)	$-0.1128+0.0031x$	0.858
B. Curvilinear Regression Equations		
*Quantity digested(g)	$-2.9827+0.0930x-0.0006x^2$	0.994
ECI% for cocoon	$-157.4628+4.7310x-0.0321x^2$	0.990
ECD% for cocoon	$-224.9577+7.1283x-0.0470x^2$	0.985
ECI% for shell	$-80.1730+2.4282x-0.0169x^2$	0.967
ECD% for shell	$-128.4366+4.0792x-0.0281x^2$	0.943
Quantity of ingested food required for/g shell production	$262.3448-6.9120x+0.0480x^2$	0.987

All  $r^2$  values are significant at 1% level ( $P < 0.01$ ),  $df=18$

\* Paul et al. (1992)

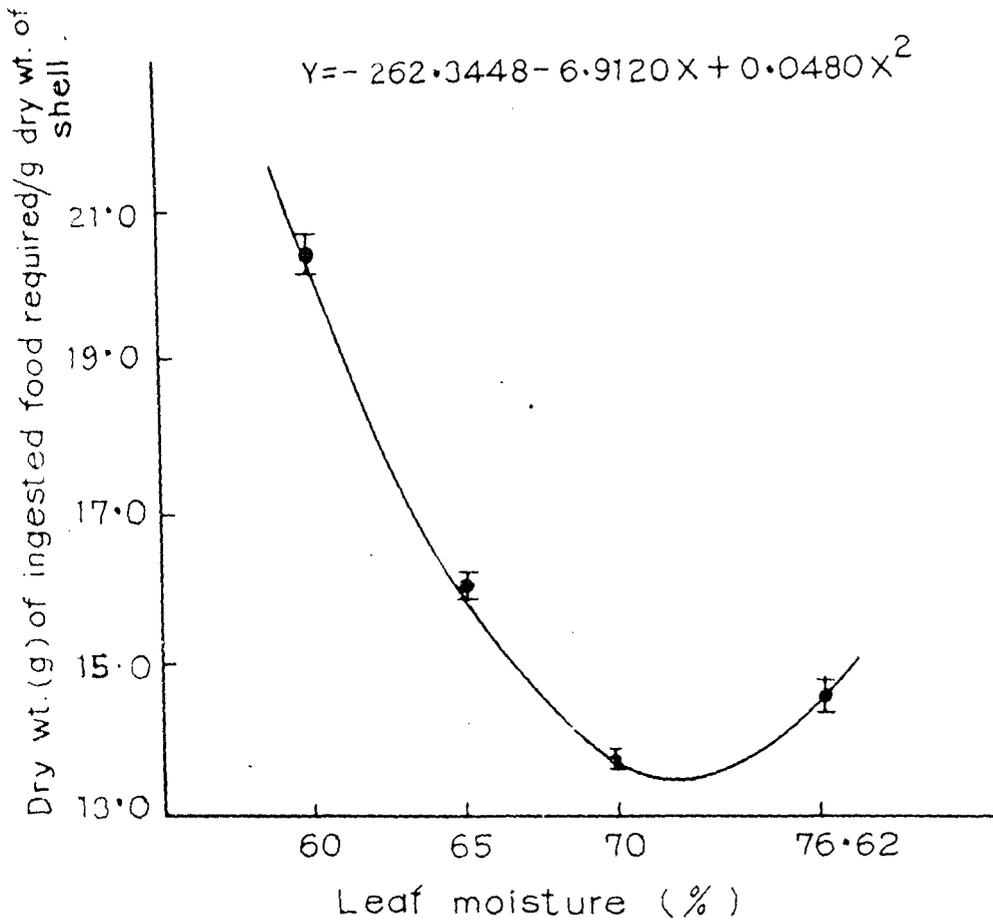


Fig.16. Impact of foliage water levels on the weight of ingested food required(g) for production of each gram of shell ( $r^2 = 0.987$ ,  $df=18$ ).

rate with the rise of water levels could be noticed for quantity digested, cocoon and shell weights and ECI% and ECD% for cocoon. Almost a similar trend was recorded for ECI% and ECD% for shell upto 70% followed by a sharp decline at the control water level. Finally, there was a decrease in the requirement of consumed food for the production of unit weight of shell upto 70% followed by an increase at the control moisture level.

#### 4.3. CONSUMPTION AND UTILIZATION OF LEAF NITROGEN FOR THE NITROGEN OF SHELL

##### 4.3.1. Ingestion, digestion and approximate digestibility of nitrogen

The percentage of nitrogen calculated for the control leaves and after drying for each category of leaves <sup>with depleted water</sup> was almost constant. It was about 3.724% on a dry weight basis.

Larvae ingested and digested higher amount of food with the rise of leaf moisture (Chap. 4.1, Table 1). Accordingly, the quantities of nitrogen ingested and digested were also high and showed significant differences ( $P < 0.05$ ) among the treatments and each of the treatments with the control values (Table 7). However, the approximate digestibility of nitrogen increased progressively upto 70%, but decreased at the control water level. The differences were all significant.

Table 7. Observed and predicted (in parentheses) mean value of different nitrogen utilization indices for shell production at different levels of leaf moisture. C.D. was calculated for the observed values.

Parameters	Moisture in the leaves (%)				C.D. at 5% level of significant
	60±0.5	65±0.5	70±0.5	76.62±0.5 (Control)	
Nitrogen consumed(g)	0.050 (0.050)	0.055 (0.054)	0.058 (0.058)	0.063 (0.063)	0.00094
Nitrogen digested(g)	0.024 (0.024)	0.029 (0.029)	0.034 (0.033)	0.035 (0.036)	0.00048
Approximate digestibility (%)	47.722 (46.948)	52.264 (53.715)	58.608 (56.982)	55.864 (55.923)	0.64886
ECI% for nitrogen of shell	24.040 (23.600)	30.218 (31.264)	35.017 (34.757)	33.338 (33.451)	0.92474
ECD% for nitrogen of shell	49.304 (49.296)	57.984 (57.620)	59.751 (61.420)	59.736 (59.489)	1.66089

The percentage of nitrogen in the faeces with relation to dietary water was in the order of 60 > 65 > control > 70% . The differences were also significant. The total amount of nitrogen per shell increased significantly with increasing dietary water. This was due to the production of correspondingly heavier shells (Table 8).

The nature of increase of nitrogen ingestion, digestion and approximate digestibility is presented by regression characteristics (Figs. 17-19). A linearity was observed for nitrogen ingestion, indicating a proportional higher intake with the rise of every 5% leaf moisture. The observed values were in close proximity to the regression line. The coefficient of determination( $r^2$ ) value was very high and significant (Table 9). Where as, for the quantity of nitrogen digested and approximate digestibility the nature of nitrogen utilization was curvilinear. The quantity of digestion showed an almost proportional rise upto 70%, thereafter the rise was slowed down at the control level. A non-proportional rise in approximate digestibility was noticed upto 70% and then declined. The  $r^2$  values for both the parameters were high and significant.

#### 4.3.2. Efficiency of conversion of leaf nitrogen into nitrogen of shell

The absolute quantity of nitrogen in each shell increased with

Table 8. Nitrogen in the faeces and in the shell at different levels of leaf moisture.

Parameters	Moisture in the leaves (%)				C.D.at 5% level of significant
	60±0.5	65±0.5	70±0.5	76.62±0.5 (Control)	
Nitrogen in faeces (%)	2.874	2.764	2.467	2.579	0.02579
Nitrogen in shell (g)	0.012	0.017	0.020	0.021	0.00037

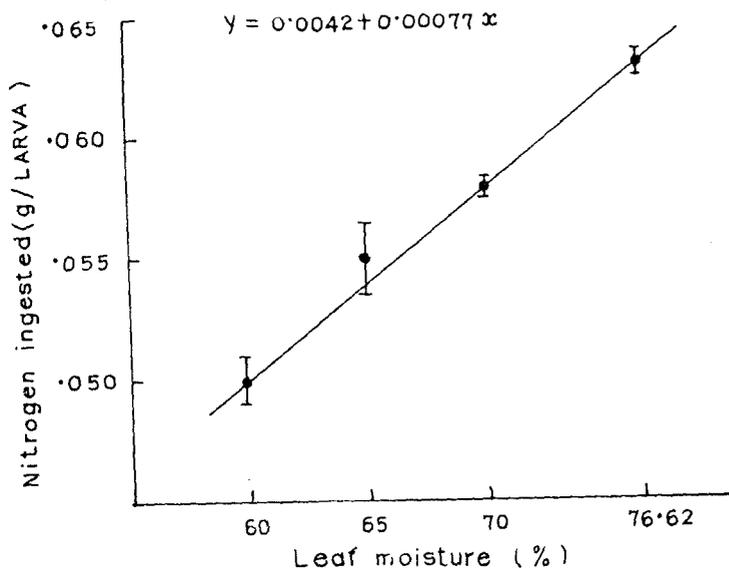


Fig.17. Influence of foliage water levels on quantity of leaf nitrogen consumed ( $r^2 = 0.992$ ,  $df=18$ ).

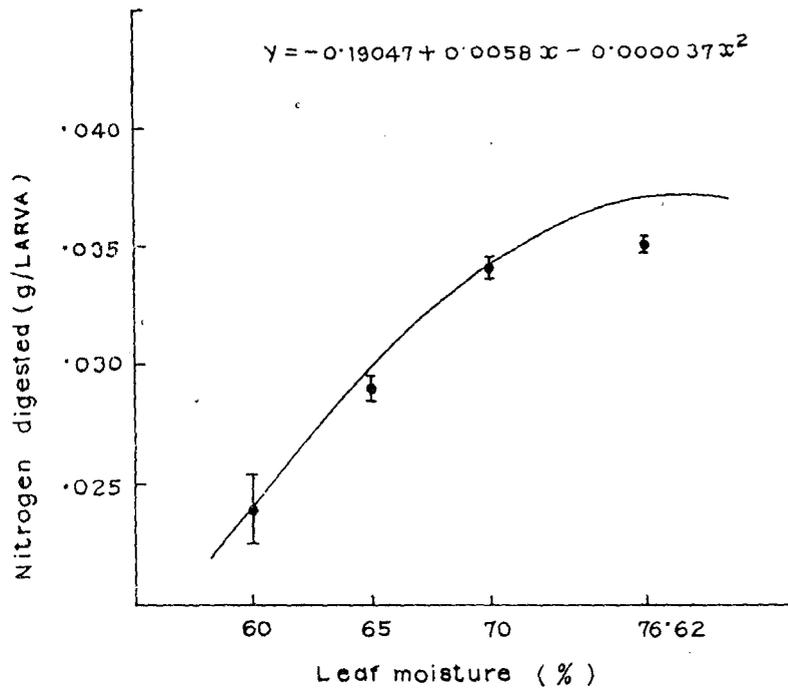


Fig.18. Influence of foliage water levels on quantity of leaf nitrogen digested ( $r^2 = 0.987$ ,  $df=18$ ).

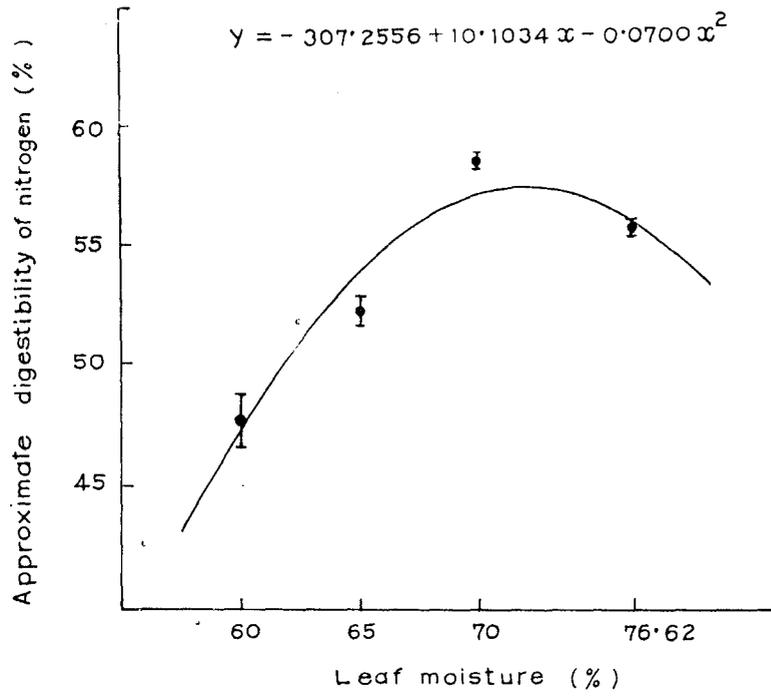


Fig.19. Influence of foliage water levels on approximate digestibility (%) of leaf nitrogen ( $r^2 = 0.959$ ,  $df=18$ ).

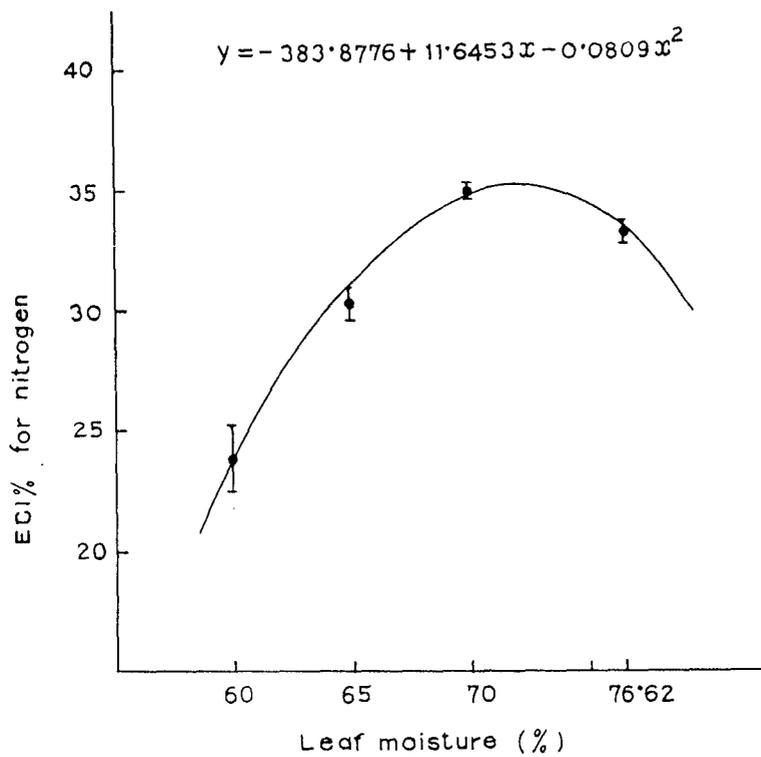


Fig.20. Influence of foliage water levels on ECI% for nitrogen of shell ( $r^2 = 0.980$ ,  $df=18$ ).

the rise of dietary moisture. But the value of ECI% and ECD% for nitrogen increased upto 70%, then at the control dietary moisture the ECI declined significantly (Table 7). The differences in values among the treatments and each of the treatments with the control were significant except for the ECD%, for nitrogen with respect to 70% and control level of leaf moisture. The regression characteristics (Figs. 20 and 21) revealed an increase in curvilinear pattern and was not proportional to the rise of moisture from 60% to the control level. The rise from 60 to 65% was much higher than that from 65 to 70% moisture. However, the  $r^2$  values were high and significant (Table 9).

Irrespective of treatments and control the percentage of nitrogen in the shell was the same, which was 17.90% .

#### 4.3.3. Interrelationship between leaf moisture and each of the parameters and between pairs of parameters in respect of nitrogen consumption and utilization

A significant positive correlation ( $P < 0.01$ ) was observed between leaf moisture and each of the parameters and between the pairs of parameters (Table 10). The highly significant values of correlation coefficient emphasized a strong influence of each parameter on the others. The leaf moisture also had strong impact on the parameters, high values were observed at higher water levels, and low values at lower dietary water.

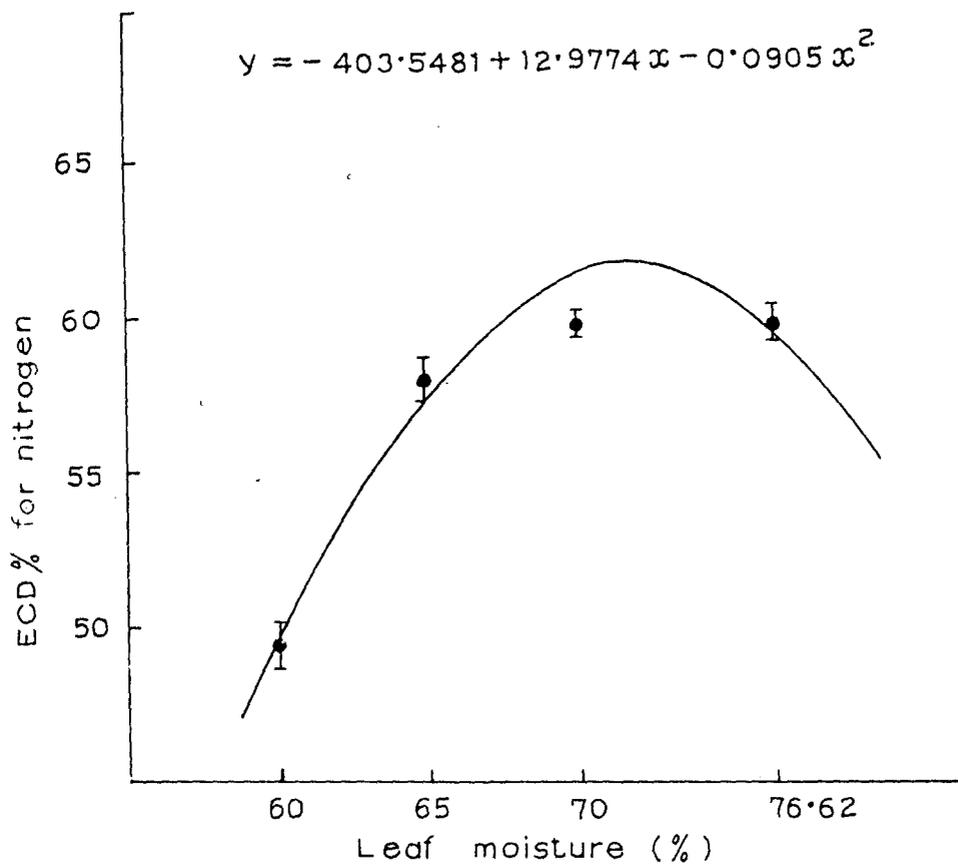


Fig.21. Influence of foliage water levels on ECD%  
 for nitrogen of shell( $r^2 = 0.979$ ,  $df=18$ ).

Table 9. Regression co-efficient of leaf moisture on different parameters

Parameters	Regression equations (Y)	Co-efficient of determination ( $r^2$ )
	(A) Linear Regression Equation	
Nitrogen ingested (g)	$0.0042 + 0.00077x$	0.992
	(B) Curvilinear Regression Equations	
Nitrogen digested (g)	$-0.19047 + 0.0058x - 0.000037x^2$	0.987
Approximate digestibility (%) of nitrogen	$-307.2556 + 10.1034x - 0.0700x^2$	0.959
ECI(%) for nitrogen of shell	$-383.8776 + 11.6453x - 0.0809x^2$	0.980
ECD(%) for nitrogen of shell	$-403.5481 + 12.9774x - 0.0905x^2$	0.979

\* All values are significant at  $P < 0.01$ ,  $df = 18$

Table 10. Relationship between leaf moisture and each of the parameters and between the pairs of parameters in respect of nitrogen consumption and utilization.

	Leaf moisture (%)	Nitrogen ingested (g)	Nitrogen digested (g)	Approximate digestibility (%)	ECI (%)
Nitrogen ingested(g)	+0.992				
Nitrogen digested(g)	+0.954	+0.954			
Approximate digestibility(%) of nitrogen	+0.799	+0.791	+0.937		
ECI(%) for nitrogen of shell	+0.802	+0.790	+0.925	+0.981	
ECD(%) for nitrogen of shell	+0.779	+0.777	+0.881	+0.916	+0.958

All are significant at  $P < 0.01$  level. d.f.18.

#### 4.3.4. Examination of effectiveness of the regression models

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The validity of regression models for the parameters on leaf moisture was examined by comparing the observed and predicted values (Table 7). The marginal deviation between the two values coupled with high  $r^2$  values confirmed the better goodness of fit of the regression equations. Even the same expected and predicted value was recorded for some parameters at some particular moisture levels. The highest difference of 1.669 was observed for ECD for nitrogen at 70% moisture.

#### 4.4. LARVAL BODY WATER AND FAECAL WATER WITH REFERENCE TO LEAF MOISTURE

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##### 4.4.1. Leaf moisture and larval body water

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With the progress of age of the 5th instar larvae and irrespective of treatment and control levels of leaf moisture, larval body water decreased very gradually but the decrement was relatively pronounced towards the late days (Fig. 22). Further, irrespective of leaf moisture, the larval body water on the final day i.e., the day of spinning was of little difference. The percent body water of this day was 78.31, 78.48, 78.67 and 80.21 for 60, 65, 70 and 76.62% dietary water respectively (Table 11), though the initial larval body water prior to the

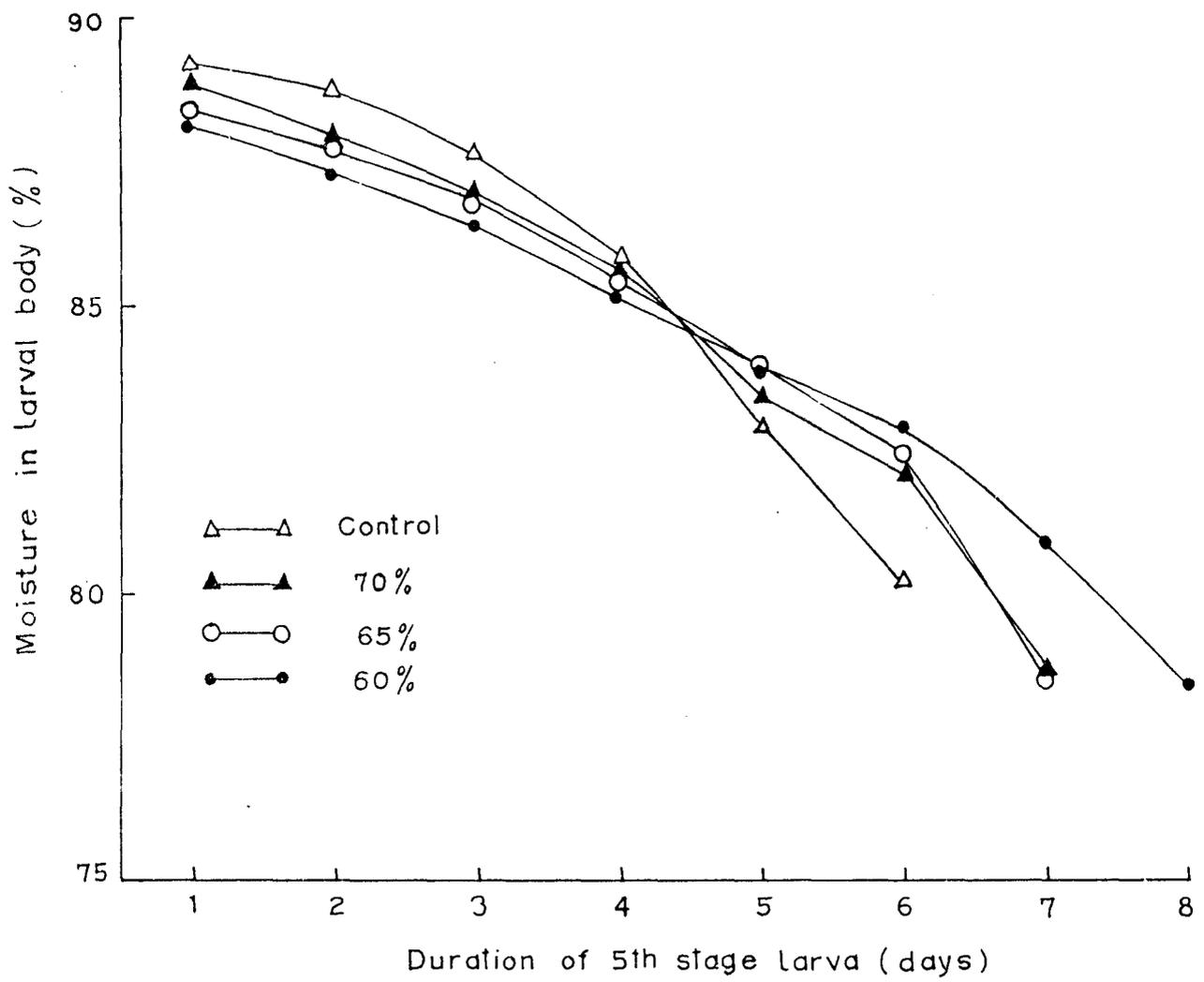


Fig.22. Larval body water with reference to dietary water and age of the larvae.

Table 11. Percentage of moisture in larval body on different days at different levels of leaf moisture

Age of larvae in day	Moisture (%) in the leaves				C.D. at 5% level of significance
	60±0.5	65±0.5	70±0.5	control	
1st	88.11	88.39	88.79	89.20	0.06
2nd	87.26	87.69	87.91	88.72	0.05
3rd	86.27	86.82	86.97	87.53	0.06
4th	85.09	85.37	86.58	85.81	0.05
5th	83.76	83.88	83.36	82.84	0.05
6th	82.81	82.36	82.01	80.21	0.05
7th	80.88	78.48	78.67	-	-
8th	78.31	-	-	-	-
Mean body water spanning the entire 5th stage larval duration	84.06	84.71	84.90	85.72	

commencement of feeding was 90.15% at the beginning of 5th instar.

Considering the entire span of 5th stage larval life the average percentage of body water increased with the increasing moisture of leaves. The average values were 84.06, 84.71, 84.90 and 85.72% on leaves with 60, 65, 70 and 76.62% moisture respectively.

#### 4.4.2. Leaf moisture and faecal water

With the advancement of age of the larvae the faecal water content gradually increased and this increase was irrespective of diet water, but the propensity of increased faecal water was somewhat more towards the later days of larval life except for those maintained on 60% leaf moisture (Fig.23, Table 12). However, the average percentage of faecal moisture, covering all the days of larval life, was remarkably higher with the rise of diet water. The values are 53.88, 58.98, 64.69 and 72.59% respectively on diets with 60, 65, 70 and 76.62% water.

#### 4.4.3. Relationship of leaf moisture with larval body water and faecal water

The leaf moisture showed a significant impact on the larva body moisture as a high positive correlation ( $P < 0.01$ ,  $r = 0.965$

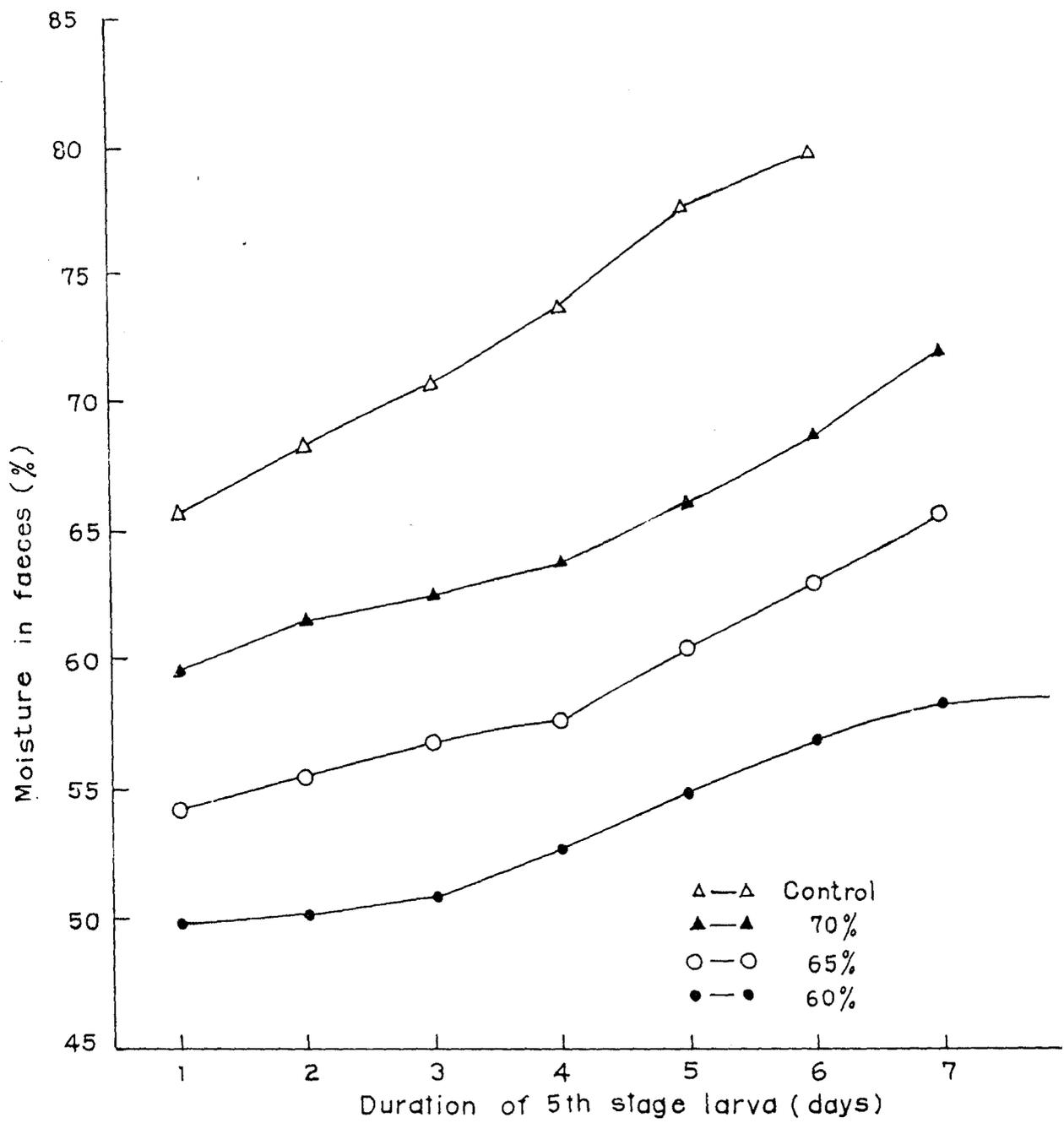


Fig.23. Moisture level in the faeces with reference to dietary water and age of the larvae.

Table 12. Percentage of moisture in faeces on different days at different levels of leaf moisture

Age of larvae in day	Moisture (%) in the leaves				C.D. at 5% level of significance
	60±0.5	65±0.5	70±0.5	control	
1st	49.73	54.25	59.56	65.61	0.45
2nd	50.06	55.52	61.30	68.26	0.48
3rd	50.78	56.71	62.47	70.54	0.54
4th	52.61	57.54	63.66	74.37	0.67
5th	54.77	60.41	65.78	77.43	0.77
6th	56.73	62.88	68.43	79.33	0.86
7th	58.07	65.52	71.67	-	-
8th	58.33	-	-	-	-
Mean of entire 5th stage larval duration	53.88	58.98	64.69	72.59	-

was obtained between the two.

The leaf moisture also had a good influence on the faecal water, since there was high positive correlation ( $P < 0.01$ ,  $r = 0.998$ ) between the two.

Further, the correlation between the average values of larval body water and faecal water obtained from the different treatments and control, was also positively significant ( $P < 0.01$ ,  $r = 0.963$ ).

#### 4.4.4. Relationship of larval age with body water and faecal water

The correlation between the larval age and body water and that of larval age and faecal water were highly significant as evident from  $r$  values. Irrespective of leaf moisture the  $r$  values between larval age and body water and between age and faecal water were  $-0.970$  and  $0.979$  respectively. Thus, the relation of age with the body water was negative and with the faecal water was positive.

### 4.5. REARING RESULTS

#### 4.5.1. Larval duration

The larval duration was prolonged in case of 60 and followed

by 65, 70 and control being the shortest (Table 13, Fig. 24a). The significant difference ( $P < 0.05$ ) was observed for larval duration among the treatments and each of the treatments with the control. In terms of percent there was extension of larval duration over that of control by 38.53, 29.36 and 11.01 respectively in 60, 65 and 70% leaf moisture (Table 14).

#### 4.5.2. Weight of 10 mature larvae

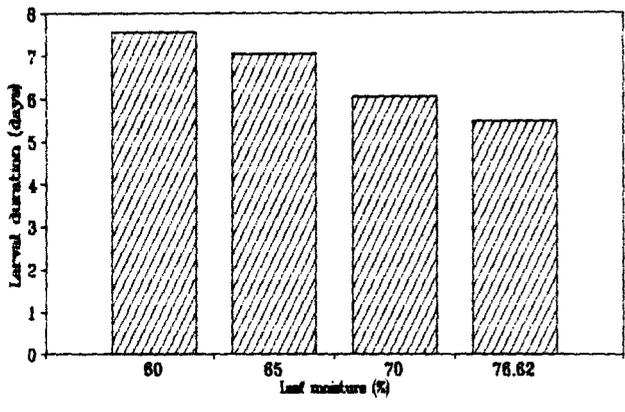
At the end of feeding period the larvae attained maximum weight and showed significant difference ( $P < 0.05$ ) among all cases. The maximum larval weight was recorded in case of control followed by 70, 65 and 60% dietary water (Fig. 24b). The loss over the control value were 2.33, 31.39 and 45.87% at 70, 65 and 60% water respectively.

#### 4.5.3. Effective rate of rearing by number (ERR No.)

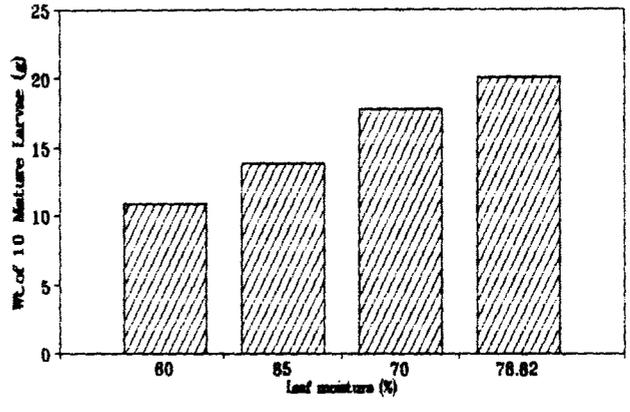
The high values of ERR No were recorded for 65 and 70% water levels, though their differences were not significant ( $P > 0.05$ ). A very poor value was obtained at 60% and an intermediate value at the control moisture (Fig. 24c). Compared with the control value, the values of each of the treatment differed significantly ( $P < 0.05$ ). Thus, at 60% water ERR No decreased by 36.04% from the control value and increased above the control values by 26.13 and 27.33% respectively at 65 and 70% moisture.

Table 13. Rearing result (Mean  $\pm$  SE in parenthesis)

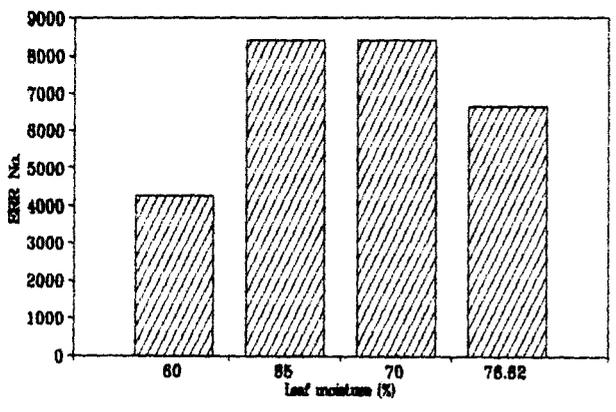
Leaf moisture (%)	Larval duration: days	Wt of 10 mature larvae (gm)	ERR No	ERR Wt (Kg)	Single cocoon Wt(gm)	Single shell wt(gm)	Cocoon:shell ratio (%)	Absolute silk content (gm)
60%	7.55 (0.05)	10.88 (0.10)	4260 (328)	2.68 (0.19)	0.646 (0.004)	0.071 (0.001)	10.92 (0.11)	301.1 (24.3)
65%	7.05 (0.05)	13.79 (0.15)	8400 (218)	6.90 (0.15)	0.869 (0.004)	0.104 (0.001)	12.00 (0.06)	875.0 (20.2)
70%	6.05 (0.05)	17.77 (0.09)	8480 (211)	8.40 (0.19)	1.008 (0.014)	0.130 (0.002)	12.86 (0.12)	1097.6 (20.5)
Control (76.62%)	5.45 (0.05)	20.10 (0.12)	6660 (257)	6.67 (0.24)	1.032 (0.009)	0.134 (0.001)	12.39 (0.11)	891.0 (47.9)
C.D.at 5%	0.15	0.35	771	0.59	0.028	0.005	0.31	91.37



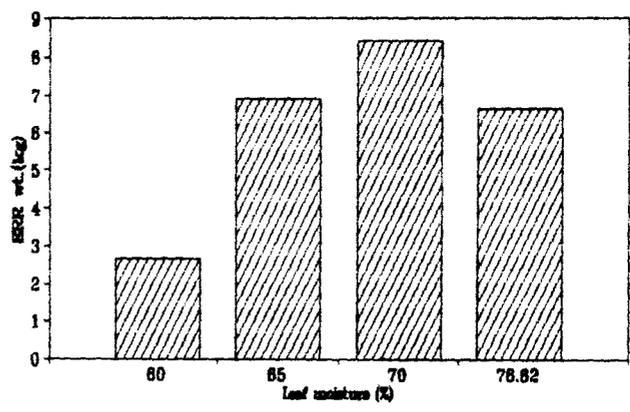
a



b



c



d

Fig.24. Rearing results for different characters on different dietary water levels  
a.Larval duration, b.Wt of 10 mature larvae, c.ERR No., d.ERR wt.

Table 14. Percentage gain over (indicated by +) and loss from (indicated by -) the control values on rearing result

Leaf moisture (%)	Larval duration	Wt of 10 mature larvae	ERR No.	ERR Wt.	Single cocoon Wt	Single shell wt	Cocoon shell ratio	Absolute silk content
60%	+38.53	-45.87	-36.04	-59.82	-40.29	-47.01	-11.86	-66.21
65%	+29.36	-31.39	+26.13	+3.45	-19.68	-22.39	-3.15	-1.79
70%	+11.01	-2.33	+27.33	+25.94	-6.84	-2.28	+3.79	+23.19

#### 4.5.4. Effective rate of rearing by weight (ERR Wt.)

Significantly the highest ERR wt. was recorded at 70 and the lowest at 60% (Fig.24d). But the difference between 65 and control was non-significant ( $P > 0.05$ ). Further, the percentage gain at 70 and 65% over the control values were 25.94 and 3.45 respectively.

#### 4.5.5. Single cocoon weight

The single cocoon weight showed significant difference among the treatments and each of the treatments with the control ( $P < 0.05$ ). The weight of single cocoon was highest at control followed by at 70, 65 and 60% (Fig.24e). The percentage loss over the control value were 6.84, 19.68 and 40.29 respectively at 70, 65 and 60% leaf moisture.

#### 4.5.6. Single shell weight

Significant gradual increase in shell weight was observed from 60 to 70%. Although the value was the highest in case of the control (Fig. 24f), the difference with the value at 70% was very little and non-significant ( $P > 0.05$ ). The percentage loss from the control value were 2.28, 22.39 and 47.01 respectively at 70, 65 and 60% dietary moisture.

#### 4.5.7. Cocoon shell ratio

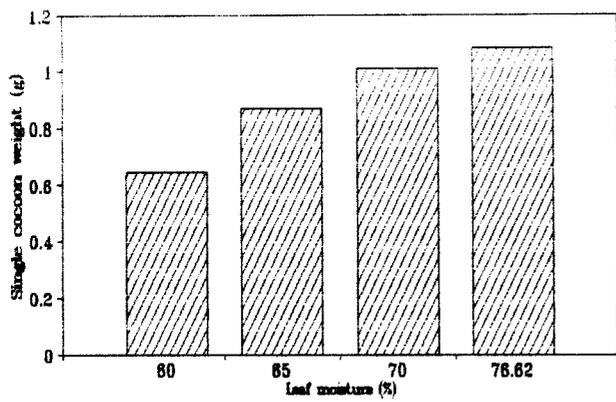
The difference in respect of cocoon shell ratio was significant among treatments and each of the treatments with the control ( $P < 0.05$ ). Further the value was highest at 70% followed by control, 65 and then at 60% (Fig. 24g). The percentage gain over the control value was 3.79 at 70% but the losses were 3.15 and 11.86% respectively at 65 and 60% water.

#### 4.5.8. Absolute silk content

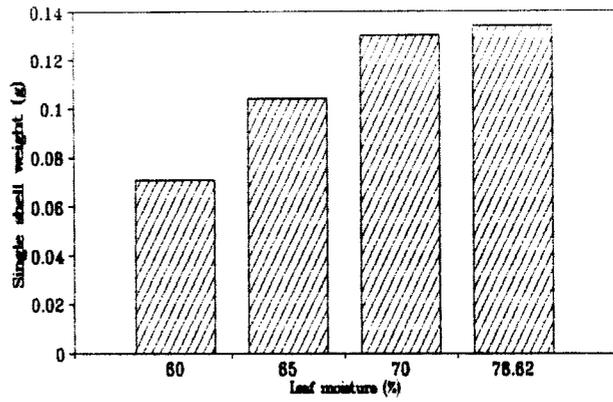
Significant ( $P < 0.05$ ) difference was observed among the treatments and each of the treatments with the control except between 65 and control where the difference was non-significant ( $P > 0.05$ ). The highest value was observed at 70% followed by control, 65 and 60% (Fig. 24h). The percentage gain at 70 over control value was 23.19.

#### 4.6. COCOON MELTING

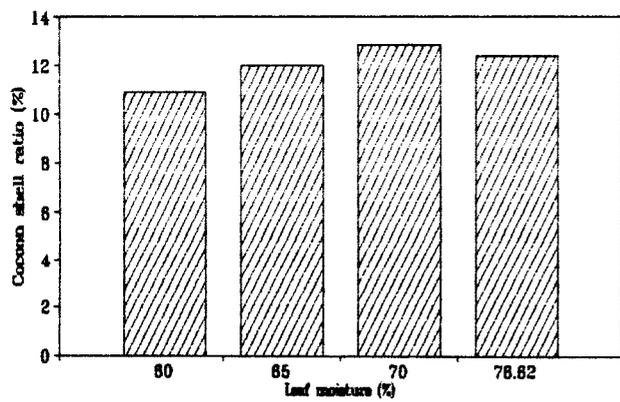
The mean number of cocoons obtained from every 300 larvae (Chapter 3.9) was lowest at 60% and highest at 70% dietary water. Out of the total cocoons the percentage of cocoon melting was low and did not differ significantly among those raised on 60, 65 and 70% leaf moisture, the percentage ranged from 8.984 to 9.928. But the melting percentage was the highest (16.680%) at



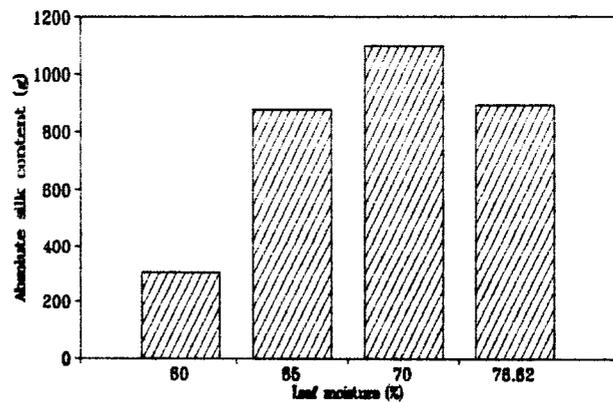
e



f



g



h

Fig.24. Rearing results for different characters on different dietary water levels  
 e.Single cocoon wt, f.Single shell wt, g.Cocoon shell ratio (SR%),  
 h.Absolute silk content

control level of dietary water. The percentage was almost twice that of rest of the treatments. A highly significant difference was recorded between the population of control water level and that of all the three depleted water levels(Fig. 25).

#### 4.7. REPRODUCTIVE PERFORMANCE

With the increase of leaf moisture there was gradual increase in both female and male pupal weight (Table 15, Fig. 26a,b). The differences in the weights among the treatments and each of the treatments with the control was significant ( $P < 0.05$ ), the highest value was obtained for the control and the lowest in 60% water content. The total number of eggs laid by each female moth also showed increasing trend with the rise of moisture in the leaf (Fig. 26c) and the differences among the treatments and each of the treatment with the control were significant. But the rate of increase in egg numbers with the increase of moisture was not uniform. It was 88 eggs more in 65% when compared with 60%, also 114 eggs more in 70% when compared with 65% and only 54 eggs more in control when compared with 70%. The gradual increase of leaf moisture resulted in corresponding increase in egg vigour (Table 15, Fig. 26d). The differences among the treatments and each of the treatments with the control were significant ( $P < 0.05$ ). However, no significant difference was observed in respect of hatching percentage among all cases though higher value was

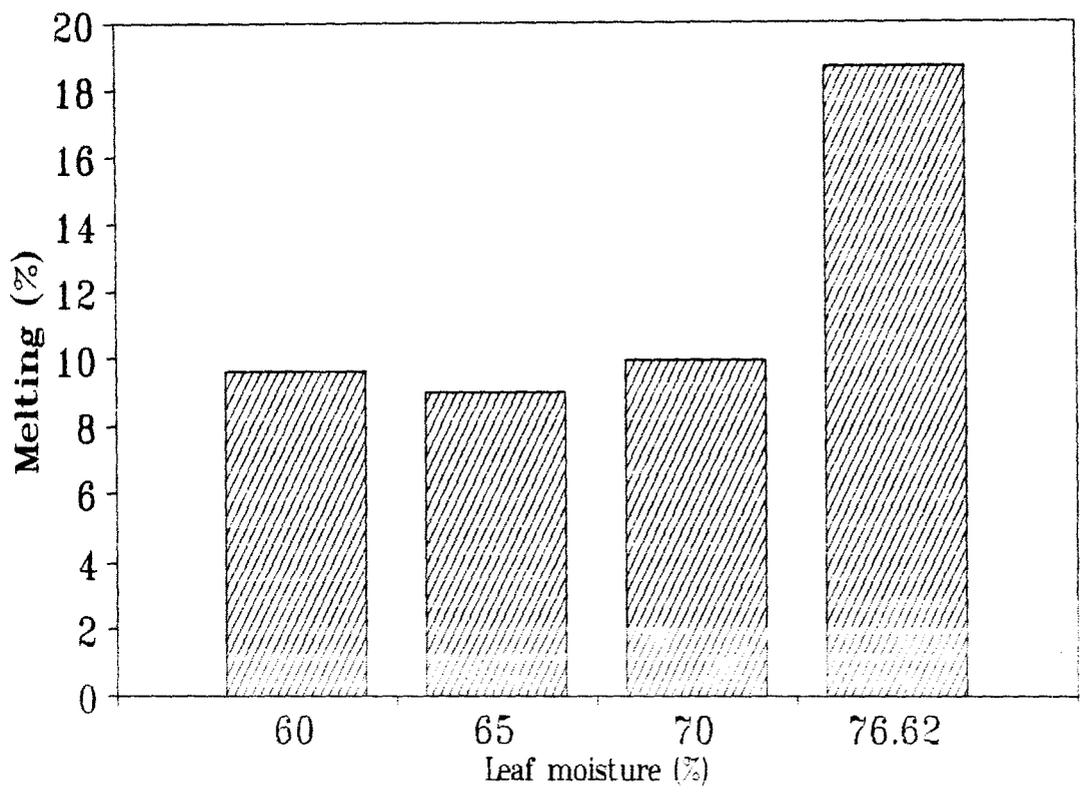
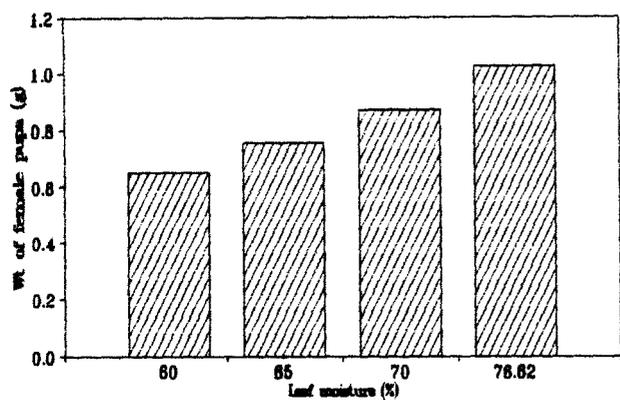


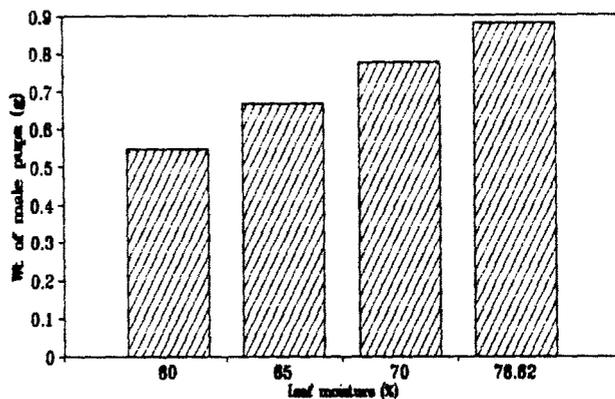
Fig.25. Incidence of cocoon melting with reference to leaf moisture.

Table 15. Pupal growth, fecundity, fertility and egg vigour

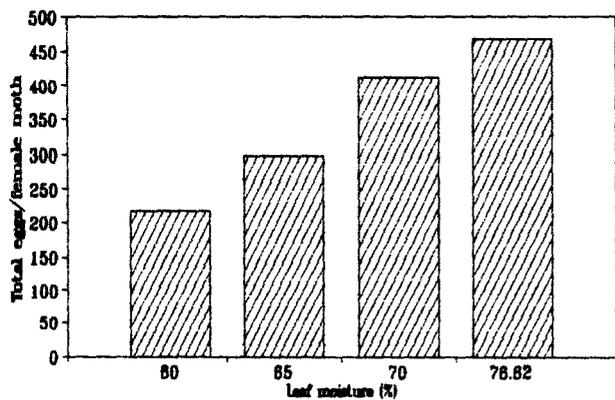
Treatment	Wt of Fe- male pupa (g)	Wt of Male pupa (g)	Total eggs/ female moth (No)	Egg vigour (Wt/100 eggs)(mg)	Hatching (%)
60%	0.649	0.544	218.133	42.128	94.248
65%	0.752	0.667	298.400	44.120	93.500
70%	0.873	0.774	412.333	45.918	91.101
Control	1.023	0.879	466.400	47.322	91.762
C.D. at 5%	0.026	0.022	21.116	0.151	N.S.



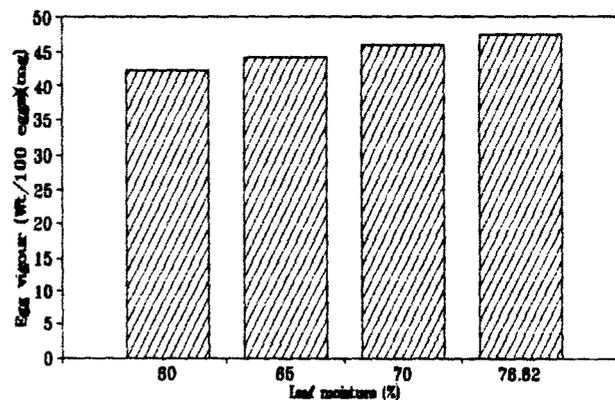
a



b



c



d

Fig.26. Characters related to reproductive performance due to different dietary moisture levels. a.Wt of female pupa, b.Wt of male pupa, c.Total no.of eggs/female moth, d.Wt of 100 eggs.

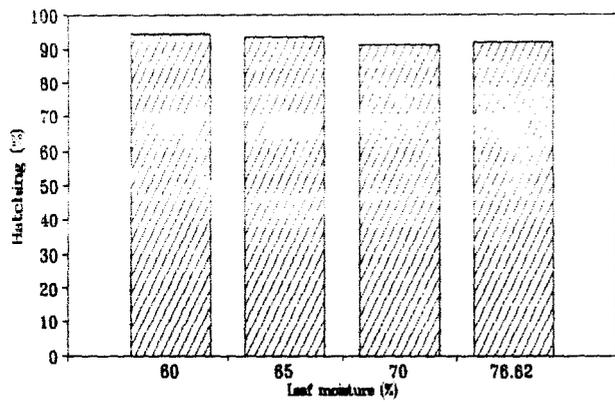
observed at 60% (Fig. 26e). The percentage of mating success (Table 16) was significantly lowest (Fig. 26f) in the control but without any difference among the treatments. The percentage of oviposition success was the lowest in control (Fig. 26g) and differed significantly with each of the treatments. But the values among the treatments though differed slightly, the differences were non-significant.

The physiological efficiency of egg production per unit weight of pupal mass was calculated. The number of eggs were 336.1, 396.8, 472.3 and 455.9 produced per gram of pupal mass on 60, 65, 70 and 76.62% dietary water respectively (Fig. 26h). The highest efficiency was obtained at 70% water and the lowest on the driest leaves.

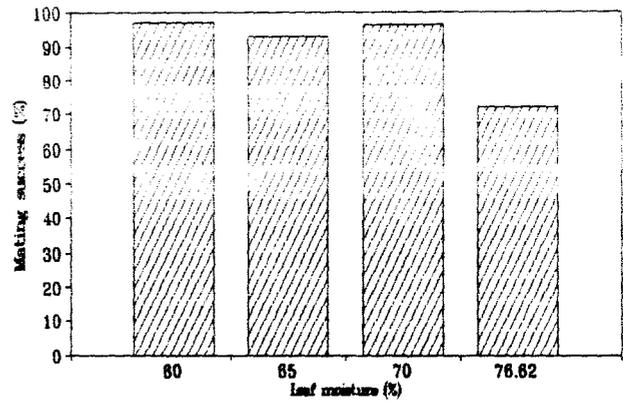
#### 4.8. REELING CHARACTER OF COCOON AND SILK FILAMENT CHARACTER

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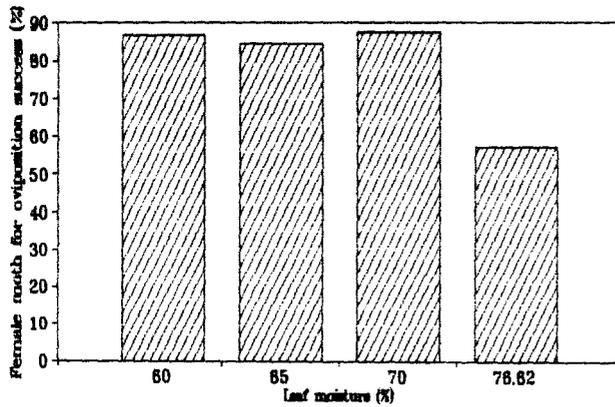
The reeling character of cocoon was considered on the basis of mean no. of breaks/cocoon during reeling process. The breaks occurred for all the treatments, but in case of control cocoons the no. of breaks was remarkably high (Fig. 27a). The differences among the three depleted water levels was not significant, but the differences with each of these three categories and the control value were highly significant (Table 17).



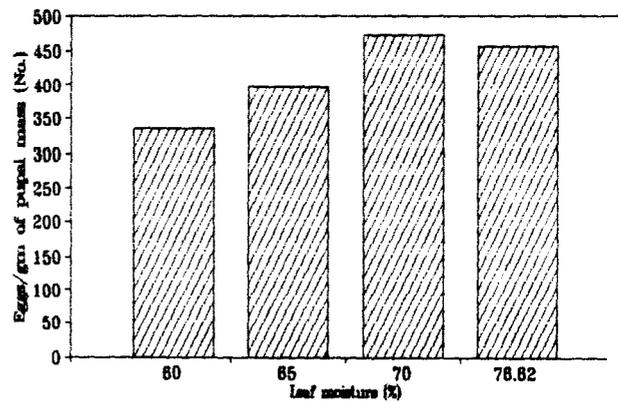
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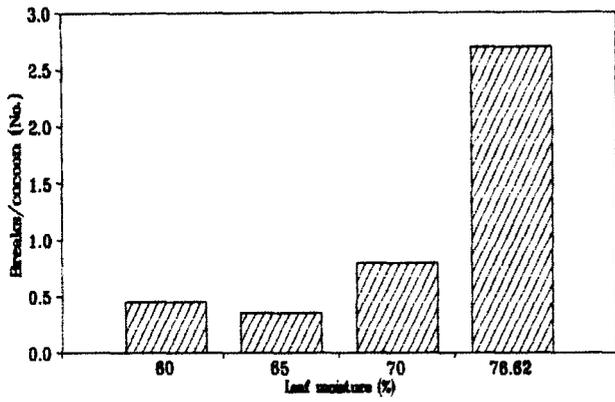


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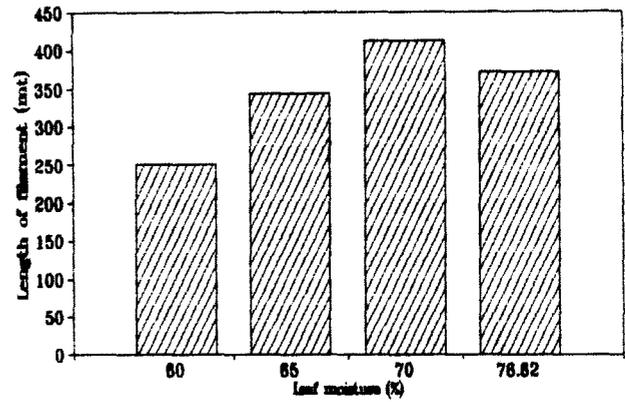
Fig.26. Characters related to reproductive performance due to different dietary moisture levels. e.Hatching percentage, f.Mating success, g.Oviposition success of female moths, h.No.of eggs produced/g pupal mass.

Table 16. Mating and oviposition success  
 (figures in the parenthesis represent  
 transformed values)

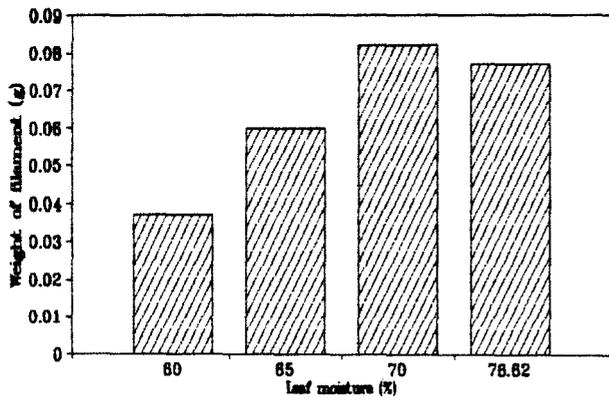
Treatment	Mating Success(%)	Female moth for Oviposition success (%)
60%	96.89 (79.84)	86.54 (68.47)
65%	92.57 (74.18)	84.20 (66.57)
70%	96.17 (78.72)	87.36 (69.17)
Control	72.30 (58.25)	56.94 (49.00)
C.D. at 5%	12.88	8.99



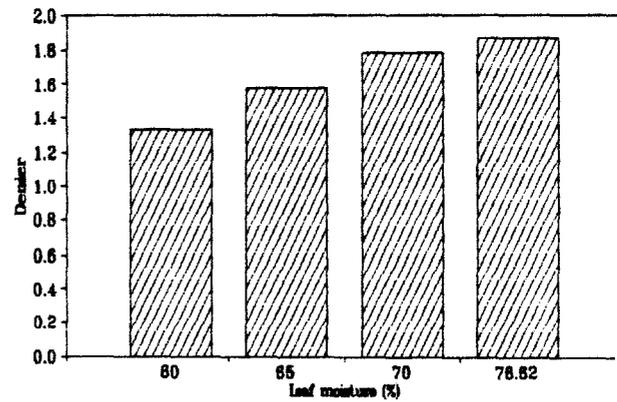
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d

Fig.27. Reeling and filament characters of cocoons. a.No.of breaks/cocoon, b.Length of filament, c.Wt of filament, d.Denier.

Table 17. Reeling and filament characters of cocoons

Leaf moisture (%)	Number of breaks/cocoon	Length of filament (m)	Weight of filament (g)	Denier
60	0.450	250.400	0.037	1.331
65	0.350	343.850	0.060	1.578
70	0.800	411.700	0.082	1.785
Control	2.700	371.450	0.077	1.867
C.D. at 5%	0.784	19.886	0.004	0.028

The filament length and weight were considered for the net filament obtained after reeling. The two characters differed significantly among the treatments and each of the treatments with the control. Both the characters increased significantly upto 70% water but at the control amount of water these were lowered significantly (Fig. 27 b,c). Thus, these two characters could be arranged in the order of 70% > Control > 65% > 60% (Table 17).

However, the denier value increased significantly, with the rise of leaf water from 60% to control level (Fig. 27 d). The value was highest in case of cocoons of control set.

## DISCUSSION

## 5. DISCUSSION

### 5.1. CONSUMPTION AND UTILIZATION OF MULBERRY LEAVES,

#### LARVAL GROWTH AND DURATION

The 5th instar larvae of B. mori within a span of less than a week consume about 87% of the total food consumption by all the larval instars (Matsumura and Takeuchi, 1950). This high rate of food consumption is translated into rapid growth due to greater efficiency of conversion of consumed food to larval biomass. Though rate of food consumption increases with a rise in temperature (Legay, 1958), dietary water appears to have an overall influence on the nutritional performances of B. mori and consequently on the larval growth and duration. Within the experimental range of dietary water levels, the performance of the 5th stage larvae in respect of all the parameters except approximate digestibility has been higher at high-water levels than with low-water content. Approximate digestibility, however, increased upto the 70% dietary water level but decreased significantly at the control level.

The quantity of food consumed by the control larvae increases to 20.47, 13.78 and 8.09% by comparison with the larvae reared on leaves with 60, 65 and 70% water respectively. ECI% and ECD% also decrease with the decreasing diet water. The influence of dietary water on the conversion efficiency of food to larval

biomass has been emphasized in the herbivorous insects by Soo Hoo and Fraenkel (1966) and Feeny(1975). Most of the studies emphasize the performance of the same insect species on different food plants or different insect species on the same food plant. Apart from water, the influence of protein and fibre contents has also been analysed. The present findings strongly corroborate the observations of Scriber (1977) in Hyalophora cecropia and Martin and Van't Hof (1988) in Manduca sexta. In the 5th stage larvae of B. mori a positive correlation exists between the dietary water and all of the nutritional indices as it is revealed from correlation coefficient values between the leaf water and each of the parameters and between the pairs of parameters (Table 2).

In this study all factors influencing growth and nutrition of B. mori remain constant for both experimental and control larvae apart from leaf water. Hence, whatever effects have been observed should be due to differential dietary water. Even, under water stressed condition additional protein cannot be utilized because of excessive metabolic load in handling the resultant nitrogenous excreta (Scriber, 1977; Schroeder, 1986). Further, for producing metabolic water essentially required for building up of new hydrated tissue or for resorbing greater amount of water from the hindgut under low dietary water, the larvae are to spend much energy (Scriber and Slansky, 1981; Martin and Van't Hof, 1988; Timmins et al., 1988; Slansky and Wheeler, 1989). All

these impediments lead to reduced larval growth when reared on low-water leaves leading to a lower final larval weight, though the ambient temperature and relative humidity were favourable for speedy growth of larvae. Even the prolongation of larval feeding duration could not compensate for impaired final larval weight. Thus, the present observation strengthens the need of optimum amount of leaf moisture for optimum nutritional performances of B. mori larvae. A prolongation of larval life under depleted dietary water also corroborates the observation of Van't Hof and Martin (1989) in Manduca sexta.

Theoretically the voraciously feeding 5th stage larvae of B. mori should consume a higher quantity of food during an extended life. Moreover, a long larval life span means a high maintenance cost. Both should force a higher quantity of food to be consumed. But a reverse situation has been encountered. In spite of prolongation of feeding period, the quantity of food consumed by the larvae reared on 60% dietary water has been lowered by 20.47% relative to the larvae reared on the control diet. This implies that water imposes a limiting factor for food consumption and subsequent utilization for larval biomass. A low final larval weight even after prolongation of life span may further be due to a high rate of respiratory metabolism which compensates for water deficiency by supplementing metabolic water.

During wet part of the summer, although the prevailing

factors ensure speedy growth of silkworm, yet there is a high mortality of fifth-instar larvae and pupae. Hence, the season is considered unfavourable for the silkworm. This is possibly due to high water content of mulberry leaves (76.62%) 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. Thus, control level and 60% leaf moisture appear to be the cause for 'lethal wetness' and 'lethal dryness' respectively. 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 the mortality to be the lowest. About 70% water in the mulberry leaves seems to be the optimum level.

## 5.2. EFFICIENCIES OF CONVERSION OF CONSUMED LEAVES INTO COCOON AND SHELL

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The silk glands of B. mori develop very rapidly during the fifth larval life, particularly during the late part of this instar. The glands become about 70 times bigger at the end of this instar than at the beginning (Horie and Watanabe, 1983 a). This happens due to greater amount of food resources allocation and their rapid rate of incorporation to the silk gland biomass (Inagaki and Yamashita, 1983). For this purpose there occurs in

the fifth stage larvae a metabolic shifting from lipogenesis at the early phase for reproductive allocation to glycogenesis at the late phase for silk protein allocation (Inagaki and Yamashita, 1986). The fifth larval stage demands for high quantity of food ingestion and digestion, and ECI% and ECD% for cocoon and shell. But the dietary water plays a role of significant limiting factor for nutritional and conversion efficiencies in a folivore like B. mori. This is why a highly significant positive correlation has been found between leaf water and each of the consumption and conversion efficiency indices. The conversion efficiencies of ingested and digested food into larval biomass decrease with the fall of dietary water also in other lepidopterans (House, 1965; Feeny, 1975; Scriber, 1977; Martin and Van't Hof, 1988). In the present study the leaf water has significantly influenced the ECI% and ECD% for shell in spite of the amount of nitrogen percentage in the mulberry leaves even after depletion to different levels of water remaining the same (about 3.87%). This corroborates that the growth rates and conversion efficiencies of lepidopteran folivores are strongly correlated with the nutritional quality of foliage, usually expressed in terms of water and nitrogen contents (Scriber and Feeny, 1979). In B. mori about 70% of shell protein is obtained directly from the mulberry leaves and 30% from the proteins of tissues and body fluid (Fukuda, 1960). Again, approximately 46 and 70% of ingested and digested leaf nitrogen are converted

respectively to shell protein in male worms and 43 and 63% respectively in female worms. In the present study, low - water diet impairs the efficiency of nitrogen utilization causing reduced shell weight. Additional support to this inference is that the percentage of nitrogen in the faeces has been very high with decreasing dietary water( Chaps. 4.3). Thus, low - water leaves impose a limitation for nitrogen utilization and conversion. Concomittently, the number of eggs and egg vigour of the resultant female moths have also been reduced(Chaps.4.7).

The highest values of ECI% and ECD% for shell have been recorded at 70% leaf water which appears to be the optimum level during wet part of summer(July-August) in the moist tropical areas like the plains of West Bengal. At high naturally occurring leaf moisture (control amount of 76.62% ) during this period though the quantity of ingestion and cocoon weight are high, but the SR% (shell wt/cocoon wt x 100) is low. This clarifies that at water level higher than 70% the food is utilized for building the pupal body than contributing to the shell. Hence, the cocoon weight becomes high. Also for the same reason the ECI% for shell is the highest at 70% while ECI% for cocoon is the highest at 76.62% water level.

From the commercial stand point too, 70 % appears to be the optimum leaf moisture during the period in concern. Because at this level least amount of dry matter is required for the

production of per gram of shell. Following Martin and Van't Hof (1988) possibly this water level satisfies non-feeding respiratory rate without excess maintenance cost, and is also optimum for reproductive and shell resources allocation and storage. The various abatements for sericulture during the wet part of summer such as high larval and pupal mortality, high ECI and ECD% for cocoon but relatively low ECI% and ECD% for shell, are considerably compensated at 70% leaf moisture.

### 5.3. CONSUMPTION AND UTILIZATION OF LEAF NITROGEN FOR THE NITROGEN OF SHELL

From the commercial view point the success of the 5th instar larvae of silkworm depends on the quantity of nitrogen consumption and its efficient conversion to the nitrogen of shell. The silkworms, particularly the 5th stage larvae, have the inherent ability for very efficient utilization of ingested nitrogen for silk protein synthesis. Though the nitrogen budget of the Nistari race has not yet been worked out, a general quantification has been made for bivoltine races (Horie and Watanabe, 1986). Over 60% of ingested nitrogen is digested and about 65% of digested nitrogen is utilized for silk - protein synthesis (Horie et al ., 1978). Further, about 70% of silk protein is produced directly from the nitrogen of mulberry

leaves, and 30% from larval body fluid and products of tissue lysis (Fukuda, 1960).

In the present investigation the quantity of nitrogen ingested by a larvae of Nistari has been determined by the quantity of mulberry leaves consumed. Since the leaf consumption increases linearly with the rise of leaf moisture (Paul et al., 1992) the quantity of nitrogen ingestion has also been responded accordingly (Fig. 17, Table 7). Therefore, the impact of foliar water is on the efficiency of nitrogen utilization for silk protein synthesis.

Compared to the bivoltine races, both ingestion and digestion of leaf nitrogen by the Nistari are low which is evident from the control values. The utilization efficiencies of nitrogen such as approximate digestibility, ECI% and ECD% values for shell nitrogen in Nistari are also quite low in comparison to these values of bivoltine races (Hiratsuka, 1917; Horie et al., 1978; Horie Watanabe, 1986). The low values of Nistari justifies the inherent low silk-yielding ability of this race. SR% of Nistari is about 11-12%, of Indian bivoltines is about 18-20% while that of recently evolved commercial breeds of Japan is 24-25%.

Maintenance of the same percent of nitrogen in the shell in all the treatments and control results is an indication that the silkworms do not sacrifice the quality of silk fibre in respect

of nitrogen content under constraints, rather the sacrifice is at the quantitative level.

In the present finding the differential nitrogen utilization efficiencies of Nistari might have been affected principally by dietary water, because the leaf moisture is the only variable, other conditions are constant. The approximate digestibility and ECI% and ECD% values for the nitrogen of shell have increased at a pronounced rate with the rise of water from 60 to 65%, then at a relatively low rate upto a rise of 70% water. Above 70% the approximate digestibility and ECI % have been decreased significantly but having almost no effect on the ECD%. That the efficiency of conversion of leaf nitrogen into shell nitrogen is the best at 70% water is further evident from the least elimination of nitrogen in the faeces. Maximum wastage of nitrogen through the faeces is found on the driest food, the values at both 65% and control water levels were higher than the value obtained at 70%. Thus, it is imperative that 70% or around 70% leaf water is the optimum level for the Nistari during wet part of the summer for highest efficiency for conversion of leaf nitrogen into silk protein.

The reasons for low nitrogen utilization efficiency on low-water leaves in the present investigation may be the same as ascribed by different authors for almost similar observation in some other lepidopteran larvae in relation to over all nutritional efficiencies. The various explanations given are : low nitrogen utilization may be an escape from increased metabolic load imposed on the larvae for nitrogen excretion and concomitant osmotic imbalance (Scriber, 1977; Schroeder, 1986), increased metabolic cost for producing metabolic water required for the synthesis of new hydrated tissue or for resorption of water from the faeces for water conservation (Scriber, 1977, 1979; Scriber and Slansky, 1981; Martin and Van't Hof, 1988; Timmins et al., 1988; Slansky and Wheeler, 1989). Martin and Van't Hof (1988) recorded a 16% increase of non-feeding respiratory rates for increased metabolic cost. The authors are of the opinion that the reduced efficiency for the conversion of digested nitrogen to larval biomass in Manduca sexta on low-water diet is not due to high food processing cost but due to the limitation of water available for the synthesis of new hydrated tissue. However, the results obtained from the present investigation is insufficient for arriving at a conclusive explanation for the low nitrogen utilization efficiency from leaf to shell by the larvae maintained on leaves with moisture lower than 70%.

Equally it will be premature to arrive at a cause for the reduced efficiency of conversion at dietary water above 70%. However, the observation of Timmins et al (1988) may be compared to that of the present observation. The authors attributed lower conversion efficiency at high dietary water to increased metabolic costs required for resorption of salts. The present result differs from that of Slansky and Wheeler (1989) on a noctuid caterpillar. The authors observed a low nitrogen consumption and elimination through faeces when the larvae fed on diet diluted with water. The low consumption was compensated by increased nitrogen utilization efficiency.

Whichever may be the reason, the 70% moisture in the mulberry leaves appears to be the optimum level for the best utilization efficiency of leaf nitrogen into silk nitrogen by the Nistari race during the concerned season.

#### 5.4. LARVAL BODY WATER AND FAECAL WATER WITH REFERENCE TO LEAF MOISTURE

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Since the ambient temperature and r.h. during the rearing have been the same for all cases, the variations observed for moisture situations in respect of larval body and faeces are simply the impact of water levels of mulberry leaves. Irrespective of stressed, comfortable and surplus water levels of diet the larvae of Nistari race have exhibited an inherent

ability for the maintenance of final amount of body water at the onset of spinning or pupal programme. Beginning with an average of 90.15% body water in the freshly moulted 5th stage larvae, the percentage of water in the pre-spinning larvae of all categories has been reduced to an almost the same level, the difference between the larvae supported on driest and wettest leaves is only 1.90%. Thus, the Nistari race possesses the inherent property of dehydration during the 5th larval life similarly as in the case of bivoltine races (Hiratsuka, 1917; Udea and Suzuki, 1967). Even the difference between the average moisture of the entire 5th larval life on driest and wettest leaves is also very little, being only 1.66%. Further, analysing the daily body water components *interestingly* it has been revealed that during the early days the body water differs parallelly with the intake amount. But this situation tends to be reversed towards the late ages mainly after the 4th day. However, in all the cases maximum dehydration occurs towards the concluding day of larval life. This may be due to no need for additional amount of water for food processing or for hydration of new tissues in the pre-spinning larvae.

Fluctuations of body water due to any of the diet water levels are not proportional on different days of 5th larval life, which justifies the statement of Edney (1977) that the water content of body is variable from individual to individual and

within the same individual from time to time.

Regarding the release of water through the faeces, the amounts are higher correspondingly with increased diet water levels. The average amount of loss spanning the entire larval life shows that with reference to 60% diet water the loss is 5.10% higher at 65%, 10.81% higher at 70% and 18.71% higher at the control diet water level. This justifies the highly significant positive correlation between the larval body water and loss of water through the faeces. Thus, higher leaf-moisture leads to higher body water and consequent loss of higher percentage of water through the faeces. Such water regulation strategy corroborates the observation of Pathak and Vyas (1988) in B. mori and the same trend in other lepidopteran insects (Krishna Rao and Goel, 1987).

With the progress of larval age specially after the 4th day relatively increased percentage of water has been passed out through the faeces by almost all categories of larvae. Even there seems to have a parity in the increased rate of water elimination with the progress of larval age.

Insects maintain water balance strategy depending on the ambient situation and the state of hydration in the body (Chapman, 1972). During the rainy season the larvae of B. mori eliminate water through the faeces depending on the intake along with the leaves.

Further, the difference in the body water between the larvae fed on leaves with 70% and 76.62% on the final day i.e., just before the onset of spinning is only 1.54% and between 60% and 76.62% leaf-moisture is 1.90%. In spite of these little differences, the larval green weight is to be taken into consideration. The difference of final green weight of the larvae on wettest food is nearly twice that of the larvae on driest food. Therefore, the amount of excess water/larvae of control category is considerable for accounting the excess urination during spinning and absorbed in cocoon shell. The excess urination has also been reported by Pathak and Vyas (1988), Kumararaj (1969) and Kumararaj (1972) during rainy season.

Though there is higher amount of diuretic hormone secretion in B. mori during the rainy season (Pathak, 1991) yet the larvae on wettest leaves cannot bring down the body water level to the optimum value before spinning. Thus, the retention of excess body water ~~althrough~~ the 5th stage larval life render the larvae physiologically weak in resisting diseases caused by micropathogens under high humidity (Yokoyama, 1962) particularly the flacherie, a bacterial disease (Chigasaki, 1937).

Compared with the control larvae, at 70% diet water larval survivability is relatively high, rearing bed humidity is congenial and there is less amount of urination, hence the

production of less number of yellow- strained cocoon shell.

#### 5.5. REARING RESULT

Larval duration increases significantly with the drying of leaves. In contrast, larval weight increases with the wetting of the leaves. These reflect a retardation of growth of 5th stage larvae with decreasing dietary water, corroborating earlier observations on other lepidopteran larvae (Scriber, 1977, 1979; Reese and Beck, 1978; Slansky and Scriber, 1985; Martin and Van't Hof, 1988; Timmins et al., 1988) and also on the 5th instar larvae of other races of B. mori (Narayanaprakash et al., 1985). The resources stored largely by the 5th stage larvae of B. mori are finally converted to economic characters. Food rationing and regulated feeding experiments conducted by several investigators have shown a growth impairment of larvae and consequently the economic characters (Muthukrishnan et al., 1978; Radhakrishnan et al., 1985; Radhakrishna and Deivi, 1987; Haniffa et al., 1988; Paul et al., 1990). But the final larval weight and concomitant pupal weight and silk yield potentials depend on larval nutritional efficiencies (Sunioka et al., 1982; Tanaka, 1988). A poor nutritional efficiency has also been exhibited by the Nistari race under moisture stress condition ((Paul et al., 1992). The overall poor rearing result has been obtained from the diets with 60 and 65% water. Higher

values for the weights of larvae, cocoon and shell obtained at control water are quite in agreement with the findings of Giridhar *et al.* (1991 a) during the rainy season.

The ERR No simply represents the number of larvae capable of surviving and successfully transforming into cocoon, while the ERR Wt qualifies for the economic pursuits to be expressed later on. The economic characters pertaining to rearing result such as larval wt, ERR Wt, ERR no, Cocoon Wt, shell wt, cocoon shell ratio and absolute silk content have the lowest values when the larvae were supported on 60% leaf water. Compared with the control, these values are lower by 45.87, 36.04, 59.82, 40.29, 47.01, 11.86 and 66.21% respectively. Except for the ERR no and ERR wt, the values of other characters obtained from 65% leaf-water are also less than those of control but higher than the values at 60% water. The value of ERR No is notably and of ERR Wt is slightly higher over the control ones. 70% diet water has resulted in a little lower value from the control in respect of weight of larvae, cocoon and shell, but considerably higher values for the remaining economic characters (Table 2). These comparative losses and gains with reference to control values express that the driest food is most unsuitable, food with 65% water is also unsuitable, because the gains in ERR No and ERR Wt cannot mitigate the high percentage of losses for other characters. At control water level though the larval duration is

the lowest and with accompanying highest values for larval wt, cocoon wt and shell wt. these apparent advantages may not be of highest commercial benefit because of low values of ERR No, ERR wt, cocoon shell ratio and absolute silk content when compared with these values obtained from 70% leaf water. Further, a higher larval weight particularly during humid condition causes high larval mortality in the Nistari race (Subba Rao et al., 1991). Cocoon weight within optimum weight range is always preferable, higher weight than the optimum range produce higher percentage of unfertilized egg-laying moths (Tanaka, 1988).

Thus, compared with the control values even with a little sacrifice of cocoon wt, shell wt, and a little longer larval duration the much higher values of important economic characters, particularly the ERR No, ERR Wt, SR% and absolute silk content at 70% leaf-moisture qualifies that this amount of dietary water economically viable for the Nistari race during the wet part of summer in the tropical humid plains such as in West Bengal. At this moisture level too, the use of mulberry leaves have been most economically used by the larvae and best output *has been obtained*

#### 5.6. COCOON MELTING

Cocoon melting due to death followed by decomposition of pupa within the shell has been recorded to be high during rainy

season (Giridhar et al., 1990). Its incidence is common both in bivoltine and multivoltine races. The extent of cocoon melting in bivoltine races has been quantified both in India and abroad. However, no well-documented information is available on the extent of this phenomenon in the multivoltine 'Nistari' race, particularly during the rainy season.

The combined effect of high temperature and high humidity may be the indirect cause inducing melting (Krishnaswami, 1986; Ullal and Narasimhanna, 1987). The high moisture in the leaves coupled with high humidity preventing transpiratory loss, may result in excess body water in the larvae. Such larvae, particularly at the fifth instar stage may become physiologically weak to resist bacterial and viral diseases as evident from the low ERR No for control larvae (Chap. 4.5). Early larval instars are relatively less susceptible to the diseases even under high temperature and humidity. However, the diseases carried over from larvae to the pupae cause cocoon melting.

The high incidence of pupal melting in general has been obtained at all the dietary water levels. This corroborates the report of Giridhar et al. (1990) during rainy season. Significantly, the melting percentage is almost double in the control set when compared with other three treatments. This highest incidence of melting among the control larvae apparently seems to be due to high intake of water through

diet and consequent maintenance of high body water even after elimination of excess amount along with faeces (Chap. 4.4.). But such a claim fails when the incidence of almost the same percentage of melting at all the three depleted water levels is looked into. Thus, either the excess body water or combined effect of high temperature and humidity alone cannot be linked as the sole cause of cocoon melting. Rather, the indirect but combined impact of climatic conditions and excess larval body water may explain for the high incidence of cocoon melting during wet part of summer. The factors may favour in some unknown ways the population growth of micropathogens or render the silkworm larvae and pupae relatively weak to resist the diseases and lead to their death and decomposition.

#### 5.7. REPRODUCTIVE PERFORMANCE

In B. mori the growth and maturation of oocytes are completed during the pupal life. After emergence the moths mate and the females usually lay almost all the mature eggs within 24 hrs. The nutrition and storage of resources during the larval life, particularly during the 5th stage, influence the egg production (Mathavan et al., 1987; Haniffa et al., 1988). Further, the fecundity depends on the resource accumulation and allocation during the early days of 5th larva (Inagaki and Yamashita, 1983). Growth and final larval weight determine the pupal and

adult vigours which have a direct bearing on the fecundity (Pillai and Krishnaswami, 1989).

With the rise of leaf water there has been corresponding increase in food consumption and larval biomass production. The resultant pupae have also been heavier accordingly. The number of egg laid by the females is also correspondingly high. Thus, the present results support the hypothesis of 'reciprocal interaction between food consumption and egg production' (Engelmann, 1970; De Wilde and De Loof, 1973 a,b; Walker, 1976). Larval nutrition not only determines the number of egg production by a female but also the weight of a single egg in B. mori (Legay, 1958). The egg vigour showed an increasing trend with the rise of leaf moisture. The differences in the egg vigour is the impact of nutritional efficiency at different moisture levels. That the pupal weight is directly related to the number of egg production in B. mori (Rahman et al., 1978; Gowda et al., 1988; Jayaswal et al., 1991) corroborates the present result.

However, the percentage of hatching is almost the same under different water levels. Thus, nutrition impairment has no effect on hatching as has been reported by Haniffa et al. (1988). In spite of almost the same hatching percentage, it is of no practical value in case of the moisture —stressed treatments, because the number of eggs/female is very poor at 60% and 65% diet water.

The lower percentage of mating success in case of control moths may reasonably be the effect of carried over diseases as has been evident from the external symptom, the blackening of body particularly the abdomen of the victim adults. Even after mating some females fail to oviposit and die within 24 hours, though the life span of both mated and virgin females is about 8 days (Paul et al., 1993). All these results support the data at farmer's level regarding the recovery of laying (considered as the number of eggs obtained out of a number of cocoons of both the sexes) in grainage. The recovery of laying is very low during the unfavourable season (July-September). The seasonal impact on fecundity of B. mori has been emphasized by Tazima (1958). Again, the number of egg production/insect does not necessarily reveal the real reproductive potential, it is a cumulative effect of both extrinsic (such as seasonal factors) and intrinsic factors (Engelmann, 1970). This is also reflected in the present study.

A physiological efficiency for the production of number of eggs per unit pupal biomass has been expressed by Pillai and Krishnaswami (1989). The highest efficiency has been expressed in case of 70% diet water followed by control, 65% and 60%. This differential efficiency supports the argument that optimum range of cocoon weight results in the highest efficiency of egg production. A higher or lower weights than the optimum range show

reduced efficiency (Narasimhanna, 1988; Tanaka, 1988). This is why Watanabe (1961) from his observation on various breeds of B. mori used the term 'luxuriance' for the relation of <sup>higher</sup> pupa weight to the egg production efficiency.

#### 5.8. REELING CHARACTER OF COCOON AND SILK FILAMENT CHARACTER

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At the onset of spinning, the larvae of B. mori reduce their body water through urination. This is essential for maintaining the physiological integrity during pupal life (Arlian and Verselica, 1979; Bradley, 1985). Some quantity of urine so discharged was found to absorb in shell. In spite of having well-balanced water regulation mechanism, the larvae reared on control leaves have retained a little more water which has resulted in the relatively higher amount of final urination. As a result, yellow-stained cocoons have been produced at higher frequency. Such cocoons have suffered from higher frequency of breaks of filament during reeling. Because, the alkaline urine dissolves the outer sericin coating of filament at many points leading to dissociation of two fibroin brins in the core (Kumararaj, 1972). The highest incidence of yellow-stained cocoon and consequent break of filaments in the control set corroborate the earlier observation during rainy season (Kumararaj, 1969). In addition to urination, absorption of atmospheric moisture by both sericin (Hu et al., 1987) and

fibroin (Tsukuda *et al.*, 1989) also decreases fibre strength promoting the breaks. This is why breaks have been recorded during wet part of summer in all categories of treatments.

The breaks cause increased droppings and release of filaments in lump during reeling. All these impediments results in increased quantity of waste silk and net loss of reeled fibre. In the present investigation the shell weight has increased significantly with the rise of water, the highest being at control water (Chap. 4.2). The length and weight of filament obtained from a cocoon after reeling have also increased with the rise of leaf moisture, but up to 70%. At control water level, however, these two values have been lowered due to excessive loss during reeling.

The denier of silk filament has gone up with increasing leaf moisture. This is due to higher nutritional efficiency and efficiency of conversion of nutrients to silk yield under increasing dietary water. Parallelism of silk yield to high nutritional efficiency has also been advocated by Sikdar (1993).

Considering all aspects of up and down, 70% leaf moisture appears to be moderate during the wet part of summer for reeling and silk filament characters.

#### 5.9. RANKING OF THE TREATMENTS FOR COMMERCIAL SUITABILITY

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In an experiment involving several treatments and many parameters often makes it difficult to draw conclusion about any specific treatment in respect of its superiority over the other as the same treatment may not show always better result in respect of all the parameters. In the present investigation under 4 treatments (inclusive of control) 20 parameters having commercial importance under rearing, melting, reproduction and reeling have been considered for ranking the treatments. None of the treatments show consistently desired result in respect of all the 20 parameters studied. So the data (mean value) of the 20 parameters under 4 treatments have been subjected to analysis by using the computer programme called 'Selection Index Programme' (Barreto et al., 1991) for ranking the treatments (Table 18). The programme selects a specific number (4) of genotype (here treatments) based on two selection parameters defined as (i) selection objective and (ii) selection intensity. In operational terms, it computes a variable called 'INDEX', which mathematically incorporates all the characteristics desired in a single numerical value. This 'INDEX' variable represents the 'Euclidean' distance of the set of variables to the objective desired. The lower the value of the INDEX the closer is the treatment for the desired objectives. Thus, a treatment is considered as superior on the basis of

Table 18. Analysis of the data for ranking the 4 leaf moisture levels in order to determine commercial compatibility of the particular moisture level.

Parameters	Moisture (%) in the leaves				Target	Intensity	
	80±0.5	85±0.5	90±0.5	98.62±0.5		Uniform	Variable
(Larval duration(days)	7.55	7.05	6.05	5.45	-3	10	4
(Wt of 10 mature larvae(g)	10.88	13.19	17.77	20.10	+3	10	5
(ERR NO.)	4280	840+0	8480	6680	-3	10	10
(ERR Wt. (kg.)	2.68	6.90	8.40	6.67	+3	10	10
(Single Cocoon Wt(g)	0.646	0.866	1.008	1.082	+3	10	7
(Single Shell Wt(g)	0.077	0.104	0.130	0.134	+3	10	9
(SR(%))	10.92	12.00	12.86	12.39	+3	10	9
(Absolute silk content(g)	307.10	875.0	1087.6	891.0	+3	10	10
(Female pupal wt(g)	0.635	0.745	0.942	1.088	+3	10	6
(Male pupal wt(g)	0.519	0.669	0.737	0.807	+3	10	2
(Total egg/female(No)	219.133	298.400	412.000	466.400	+3	10	6
(Wt of 100 eggs(mg)	42.128	44.120	45.918	47.322	+3	10	2
(Hatching (%)	94.25	93.50	91.10	91.76	+3	10	3
(Mating success(%))	94.4	90.8	90.6	71.6	+3	10	8
(Oviposition success (%)	96.0	95.2	98.4	98.8	+3	10	8
(Cocoon melting(%))	9.63	8.98	9.82	19.68	+3	10	9
(Filament length(m)	250.400	343.850	411.700	371.450	+3	10	7
(Filament Wt(g)	0.037	0.060	0.082	0.077	+3	10	10
(No. of breaks/cocoon	0.450	0.350	0.900	2.000	+3	10	9.5
(Denier	1.331	1.578	1.793	1.867	+3	10	1.5
Intensity (Uniform)							
INDEX	54.25	43.34	35.45	41.48			
RANK	IV	III	I	II			
Intensity(Variable)							
INDEX	44.42	35.17	28.08	35.23			
RANK	IV	II	I	III			

desired characteristics. The programme calculates the original mean of the population and the mean of the selected fraction to obtain the selection differential in real and standardized units.

There are two criteria values mentioned which are to be specified for each parameters. For selection objective -3.0 to 3.0 (rounded off to one decimal point) specifies the selection target or goal to be achieved. For example in case of single cocoon weight, selection normally aims at identifying the segment of the population with high value so that selection objective shall be + 3.0. A negative selection objective like -3.0 is used when low values of a parameter are intended as in case of larval duration. The value like 0.0 is recommended for those parameters (here none) for which selected treatments are not needed to be different from original population. While the selection intensity reflects the relative importance of different parameters to be used in the selection. The programme accepts values from 0.0 to 10.0 (rounded off to one decimal point) to represent the intensity depending upon the importance. The greater the intensity value (like 10.00 for E.R.R.) implies the greater the weightage given to the parameter in question. Simultaneously the 'INDEX' value has also been calculated based on uniform intensity (equal weightage) for all the 20 parameters.

Accordingly, under both variable and uniform intensity 70%

treatment ranks 1st, and 60%, ranks 4th. The lower value of the 'INDEX' implies that the result achieved against 70% found to be closer towards the objective whereas higher 'INDEX' value in 60% implies that the result is far away from the objective. For the rest of the cases under uniform intensity control ranks 2nd and 65% ranks 3rd while under variable intensity 65% ranks 2nd and control ranks 3rd.

In fine, the ranking clarifies that there are some positive effect of high moisture content (control level) in the leaf during rainy season. But those positive effects are suppressed by many negative effects which are very crucial in determining the success of the crop during unfavourable season (rainy). From the overall observation of the data in the present investigation it is revealed that considerable success can be achieved by using the mulberry leaf containing 70% moisture during the rainy season under agro-climatic condition that prevails in the plains of West Bengal.

## SUMMARY

## 6. SUMMARY

1. During the rainy season in West Bengal relatively high temperature, excessive humidity and luxuriant mulberry leaf growth are very favourable for the quick growth of larvae of B. mori. But under this ambient situation larvae and pupae face high rate of mortality because of their higher susceptibility to micropathogenic diseases. Further, over all reproductive performance, rearing output and reeling of the cocoons suffer badly during the season.

2. In order to reduce the consequent economic loss in sericulture attempt had been made to investigate the general nutritional and conversion efficiencies of food to the economic characters, reproductive performance, rearing result and silk filament characters of the locally adapted multivoltine 'Nistari' race of B. mori using mulberry leaves with artificially depleted moisture content of 60, 65 and 70% and the natural amount of 76.62% moisture which was considered as control.

3. The investigation was started with the fifth instar larvae which actually suffered badly from diseases.

4. Regarding general nutritional efficiency, the absolute consumption and growth rates/day/larvae increased with increasing leaf moisture. The quantity of consumption and digestion, ECI%, ECD% and final larval weight also increased

with increasing water. The approximate digestibility, however, increased up to 70% leaf moisture, but reduced at the control water level. larval duration was prolonged under low water content but without a corresponding increase in the quantity of consumption. ECI% and ECD%, hence larval growth was impaired. The shortest larval duration was recorded in case of control level of moisture.

5. Cocoon and shell weight, ECI% and ECD% for cocoon increased with the rise of leaf moisture. Whereas, ECI% and ECD% for shell increased only upto the 70% water content, then declined at the control level of water. Quantity of dry matter of leaf required for the production of each gram of shell decreased with increasing moisture also upto 70%. again increased slightly at the control level.

6. Because of higher amount of leaf mass consumption and digestion with the increasing water contents, the nitrogen consumption and digestion also increased correspondingly. The values for the approximate digestibility of nitrogen and ECI% and ECD% of leaf nitrogen to shell nitrogen also increased upto 70% moisture content, then declined at the control level. However, the difference in respect of ECD% of leaf nitrogen to shell between 70% and control level of moisture contents was non-significant. Faecal nitrogen content was in the order of 60 > 65 > 76.62 > 70%. ECI% and ECD % of leaf nitrogen to the

nitrogen of shell were highest at the 70% dietary water.

7. Larval body water levels decreased with the progress of age and correspondingly eliminated at increased amount of water through the faeces. Irrespective of the 4 different dietary moisture levels the final amount of body water between the pre-spinning larvae supported on wettest and driest leaves differed by only 1.90% and between 70% and 76.62% the difference was 1.54%. On the other hand the difference in respect of mean faecal water spanning the entire 5th stage larval life between the wettest and driest leaves was 18.71%. Considering the green weight of the pre-spinning larvae, 1.54% difference in respect of body water between the sets of 70% and 76.62%, appeared to be of significance for the higher proportion of yellow-stained cocoon production by the control larvae due to excessive urination .

8. At the control level of moisture content the highest larval, cocoon and shell weights and shortest larval duration were recorded. All these characters were considered beneficial from the stand point of commercial rearing. But in respect of larval survivability (ERR No.), ERR Wt, SR% and absolute silk content the highest values were obtained at the 70% leaf water. Thus, slightly lower dietary water than the control level ultimately ensured higher yield with a little sacrifice for cocoon and shell weight and a slightly longer larval duration.

Too much low leaf moisture of 60% caused very poor rearing results.

9. Mulberry sericulture suffers most from cocoon melting during the rainy season. Significantly, at the control level of dietary water the melting percentage was the highest (18.680%), whereas at the depleted water levels it ranged from 8.984 to 9.928% and showing no significant differences.

10. Accompanied with the increasing dietary moisture levels were the increased pupal weight and correspondingly the number and weight of eggs, and without a significant impact on the hatching. On the other hand, the percentage of mating and oviposition success were the lowest at the control level, but there was no significant difference for these characters among those reared on the three depleted water contents. Thus, some beneficial effects due to the highest moisture content were suppressed by the poor mating and oviposition successes. Accordingly, more number of successful layings were obtained with a little sacrifice in the number of eggs per laying in case of 70% moisture content of leaves, that ultimately ensured the highest number of egg production if considered with relation to a particular number of cocoons on large scale basis. This was considered an essential criterion for silkworm seed production.

11. Excess urination by the spinning larvae of control set

induced highest number of breaks during the silk filament reeling, the lowest number was observed for the cocoons obtained from 60% moisture content. This reeling property differed significantly between the control and all the three low-water levels, but not among the low-water levels themselves. The filament length and its weight were in the order of 70 > 76.62 > 65 > 60%. This proportion indicated the impaired ~~reel~~reliability of cocoons of control set when compared the same with that of 70% moisture set.

12. In order to assess the suitability of a particular leaf moisture level in respect of economic characters of the Nistari race of B. mori during the rainy season a ranking analysis was employed giving equal weightage (Uniform intensity) and differential weightage (Variable intensity) to all the 20 parameters. 70% moisture content ranked first and 60% the last for both the intensities. Whereas, 65% leaf moisture ranked second and third for uniform and varibale intensities respectively.

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ANNEXURE

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

D. C. PAUL,<sup>1</sup> G. SUBBA RAO<sup>1</sup> and D. C. DEB<sup>2,\*</sup>

<sup>1</sup>Central Sericultural Research and Training Institute Berhampore, West Bengal, 742101 and  
<sup>2</sup>Department of Zoology, University of North Bengal, Darjeeling, West Bengal, 734 430, India

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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 the 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.

### 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

\*To whom all correspondence should be addressed.

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<sub>1</sub>* 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 further 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 losing 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

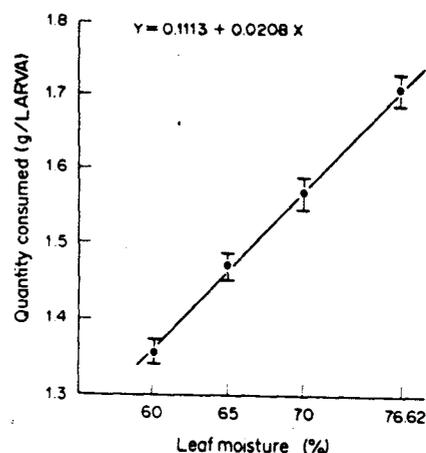


Fig. 1. The effects of leaf moisture on dry weight of food consumed ( $r^2 = 0.986$ ,  $df = 18$ ).

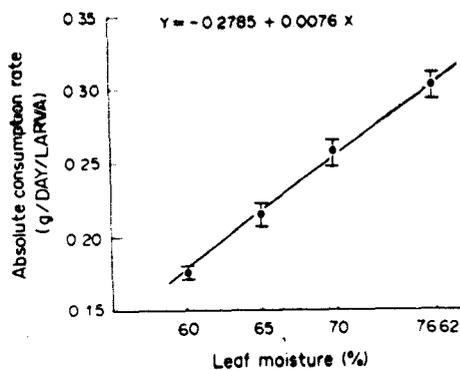


Fig. 2. The effects of leaf moisture on absolute consumption rate ( $r^2 = 0.984$ ,  $df = 18$ ).

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).

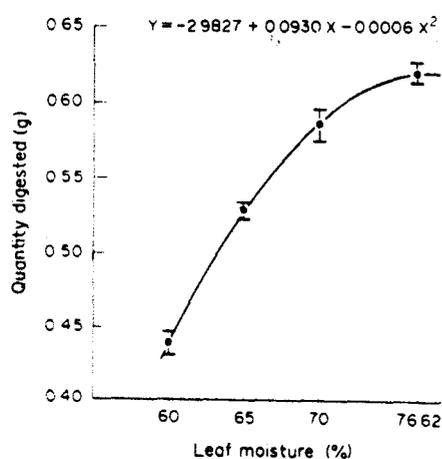


Fig. 3. The impact of foliage water levels on quantity of leaf dry matter digested ( $r^2 = 0.994$ ,  $df = 18$ ).

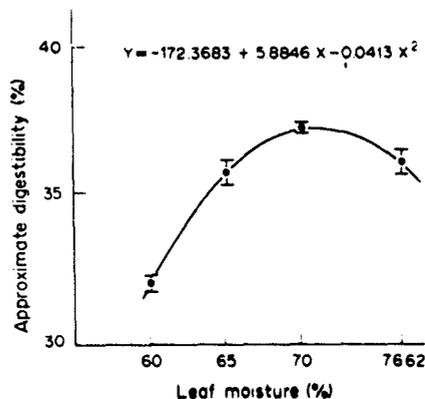


Fig. 4. The effects of leaf water content on larval approximate digestibility (%) ( $r^2 = 0.982$ ,  $df = 18$ ).

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 significant 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

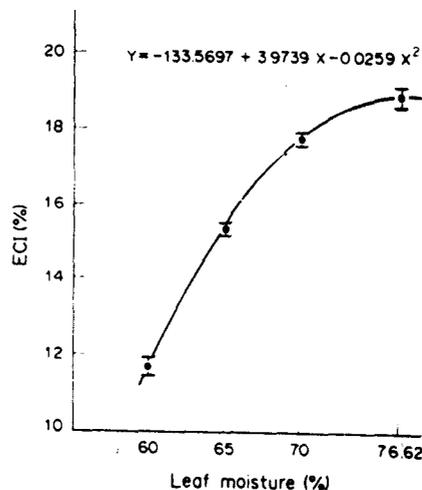


Fig. 5. The effects of foliage water levels on efficiency of conversion of ingested food (ECI%) ( $r^2 = 0.997$ ,  $df = 18$ ).

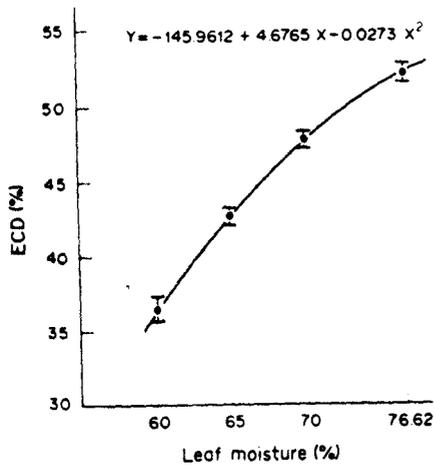


Fig. 6. The impact of foliage water levels on efficiency of conversion of digested food (ECD%) ( $r^2 = 0.992$ ,  $df = 18$ ).

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

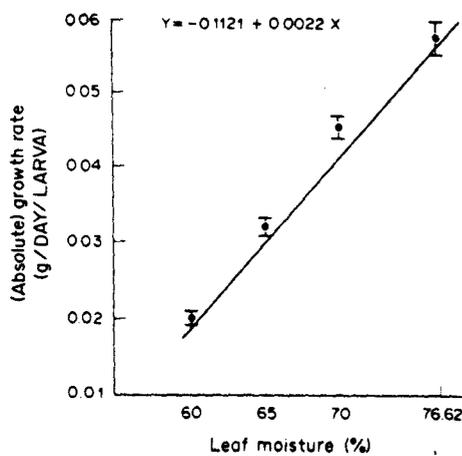


Fig. 7. The effects of foliage water levels on absolute growth rate ( $r^2 = 0.984$ ,  $df = 18$ ).

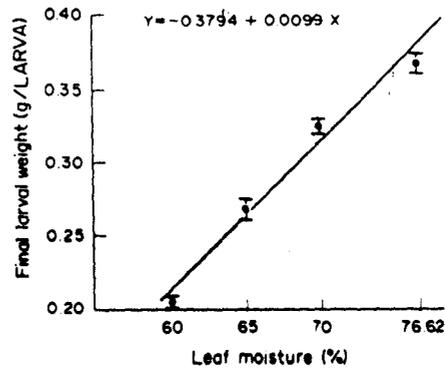


Fig. 8. The influence of foliage water levels on final larval weight ( $r^2 = 0.972$ ,  $df = 18$ ).

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

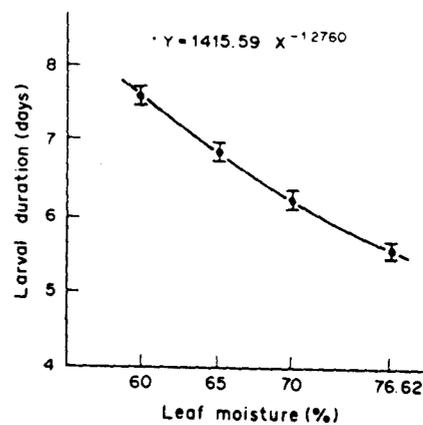


Fig. 9. The impact of foliage water levels on larval duration ( $r^2 = 0.963$ ,  $df = 18$ ).

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 relative 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 ( $r^2$ ) <sup>*</sup>
(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)	$-2.9827 + 0.0930x - 0.0006x^2$	0.994
Approximate digestibility (%)	$-172.3683 + 5.8846x - 0.0413x^2$	0.982
ECI%	$-133.5697 + 3.9739x - 0.0259x^2$	0.997
ECD%	$-145.9612 + 4.6765x - 0.0273x^2$	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 perform-

ances are likely to be better and mortality to be lowest. Such an investigation is under progress.

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