

Impact of Quantitative Feeding on Nutritional Parameters of Fifth Instar Larvae of *Antheraea mylitta* Drury

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Abstract: Feeding is an important aspect of insect nutrition which in turn supports growth, development, reproduction and survival. The impact of quantitative feeding on nutritional parameters of fifth instar *Antheraea mylitta* larvae was studied by providing fresh leaves of *Terminalia tomentosa* for 1 to 4 times a day. All the parameters improved upon providing fresh diets more times over the single diet (1FD). The absolute body weight increased with increase in number of feeds/day significantly ($P < 0.001$). While the maximum weight recorded was 29.61 g in 1FD, the respective weights for two feeds/day (2FD), three feeds/day (3FD) and four feeds/day (4FD) were 39.41 g, 40.63 g and 42.66 g respectively. Feeding period and instar duration were declined ($P < 0.001$) and survival increased significantly ($P < 0.001$) upon increase in the number of diets. Nutritional indices like ingestion, digestion, relative consumption rate (RCR), relative growth rate (RGR) and gain in body weight increased significantly with the increase in number of feeds/day, but approximate digestibility (AD) and efficiency of conversion of ingested food (ECI) declined, while efficiency of conversion of digested food (ECD) did not change.

Key words: *Antheraea mylitta* • Quantitative feeding • Nutritional parameters

INTRODUCTION

Larval feeding in non-feeding adult insect is an active and dynamic process and the amount, rate and quality of food consumed directly affects the growth, development, final weight, survival and reproductive potential as well [1]. To achieve the growth target the insect has to reach the intake target in order to attain functional optima [2] and any change in intake and growth targets will affect the performance of the insect. Food deprivation had a multidirectional affect on the insect [3-5]. *Antheraea mylitta* is wild, polyphagous and autogenic in nature and the quantum food requirement by the larva for its ideal growth, survival, development and reproduction is not yet known. Earlier studies on food consumption and utilization revealed that the intake increased by 1.4 to 2 times and the growth by 1.8 to 2.1 times upon providing two feeds/day over one feed/day. Adult characters and reproductive potential excels when 4 diets a day are provided over single diet [6]. No literature is available on the effect of number of feeds/day on larval growth and nutritional parameters in *Antheraea mylitta* which is the aim of the present investigation.

MATERIALS AND METHODS

Freshly ecdysed 5th instar larvae obtained from the stock culture of *Antheraea mylitta* (Lepidoptera: Saturniidae) reared on the bush of *Terminalia tomentosa* outdoor [5, 7]. Fifth instar larvae were selected as the experimental animal owing to its higher consumption and utilization of food during the instar [8] where the effect of quantitative feeding can be studied properly. The silkworms (average weight 11 g) were shifted to indoor condition (temp. 26-30°C, 70-77% R.H. and 11L: 13D and fed on fresh and pre-weighted leaves of *T. tomentosa* [5]. The details of feeding treatments were: one feed/day = 1FD (where the whole quantum requirement of feed for the day was provided in one installment), two feeds/day = 2FD (where the whole quantum requirement of feed for the day was provided in two installments), three feeds/day = 3FD where the whole quantum requirement of feed for the day was provided in three installments) and four feeds/day = 4FD where the whole quantum requirement of feed for the day was provided in four installments).

There were 10 replications each with 25 larvae. Buffer stocks were also maintained for each feeding treatment to compensate mortality. The nutritional parameters were studied as per Waldbauer [9]: relative growth rate (RGR) = P/TA, relative consumption rate (RCR) = E/TA, approximate digestibility (AD%) = 100(E - F)/E, efficiency of conversion of ingested food (ECI%) = 100P/E, efficiency of conversion of digested food (ECD%) = 100P(E - F) [where A is the mean dry weight of larva during the feeding period; E, dry weight of food eaten; F, dry weight of faeces produced; P, dry weight gain of larva; and T, duration of feeding (days)].

All data recorded were based on dry weight except the growth which was measured by both dry and fresh weight. Data were analyzed using ANOVA and correlation coefficient (r) to assess the effect of feeding treatment on the parameters. Regression coefficient of number of feeding per day on different parameters of growth and nutrition were studied along with the change RCR and RGR during 5th instar.

RESULTS

Regression characteristics of different parameters (except ECD) on different feeding treatments showed a distinct linearity and the R² values were also found highly significant (Table 1). The correlation coefficient between pairs of nutritional indices was found significant, but it was not significant when other parameters were tested with ECD (Table 2).

Leaf Moisture: Leaf moisture percentage tends to decline in all the leaves under different feeding conditions but the loss percentage decrease with increased in number of feeds/day (Fig. 1, Table 1, 2). Moisture loss percentage in the leaves of 1FD was found to be 18.67% while it was 5.11% in 4FD condition.

Larval Growth and Survivability: The silkworm gained weight rapidly throughout the feeding period irrespective of the number of feedings per day, but higher number of

Table 1: Regression coefficient of number of feeding per day on different parameters of growth and nutrition

Parameter	Regression coefficient	Standard error	F value	R ²
Moisture	y=21.725 - 4.417x	0.2274	377.393	0.90852*
Ingestion	y=22.5731 + 10.103x	0.6045	279.276	0.88023*
Digestion	y=11.8527 + 2.6699x	0.2823	89.4269	0.70179*
RCR	y=0.1759 + 0.1085x	0.0059	336.076	0.89842*
RGR	y=0.03568 + 0.0118x	0.0009	146.277	0.79379*
ECI	y=17.7271 - 1.0770x	0.0962	125.387	0.76742*
ECD	y=37.5612 + 0.2150x	0.3575	0.36166	0.00943 ^{NS}
AD	y=47.3538 - 3.3879x	0.2438	193.0313	0.83552*
Survivability	y=37.4200 + 9.9890x	0.9145	119.258	0.75840*
Final weight	y=27.9840 + 4.0373x	0.3876	108.4602	0.74050*
Instar duration	y=26.0560 - 2.3900x	0.2214	116.4589	0.75400*
Gain in weight	y=16.9840 + 4.0373x	0.3851	109.9325	0.74313*

*indicate significant at P<0.0001

Table 2: Correlation coefficient between pairs of nutritional indices

	Number of feeds per day	Loss in moisture (%)	Duration of feeding (days)	Instar duration (days)	Survival (%)	Final larval wt (g)	Food ingested (g)	Food digested (g)	RCR	ECI	ECD	AD	Gain in wt. (g)	RR
Number of feeds per day														
Loss in moisture (%)	-0.9532													
Duration of feeding (days)	-0.8268	0.8859												
Instar duration (days)	-0.8683	0.9016	0.9872											
Survival (%)	0.8708	-0.9445	-0.8802	-0.8701										
Final larval wt (g)	0.8605	-0.9244	-0.8605	-0.8528	0.9427									
Food ingested (g)	0.9382	-0.9718	-0.8877	-0.8994	0.9434	0.9423								
Food digested (g)	0.8377	-0.9085	-0.8325	-0.8229	0.8957	0.9025	0.9469							
RCR	0.9184	-0.9668	-0.9190	-0.9286	0.9309	0.9001	0.9601	0.8899						
ECI	-0.8760	0.8759	0.7288	0.7626	-0.7937	-0.7599	-0.9117	-0.8715	-0.8645					
ECD	0.0971	-0.0792	-0.1897	-0.1916	0.1618	0.1428	0.0206	-0.2101	0.1150	0.2359				
AD	-0.9141	0.9381	0.8635	0.8628	-0.9072	-0.9019	-0.9591	-0.8940	-0.9535	0.8724	-0.0308			
Gain in wt. (g)	0.8620	-0.9265	-0.8634	-0.8556	0.9433	0.9993	0.9442	0.9032	0.9015	-0.7638	0.1425	-0.9024		
RGR	0.8909	-0.9380	-0.9722	-0.9758	0.9223	0.8797	0.9296	0.8575	0.9621	-0.7919	0.1990	-0.8969	0.8838	

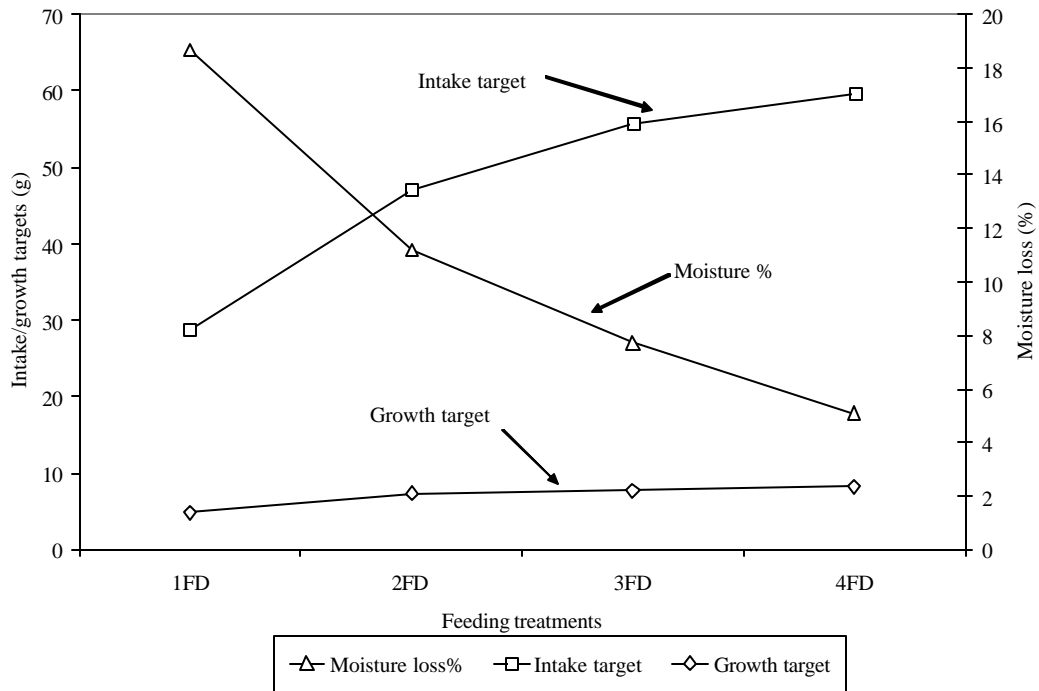


Fig. 1: Effect of different feeding treatment on leaf moisture loss, intake and growth targets in fifth instar larvae of *A. mylitta*. For detailed regression equation please refer Table 1

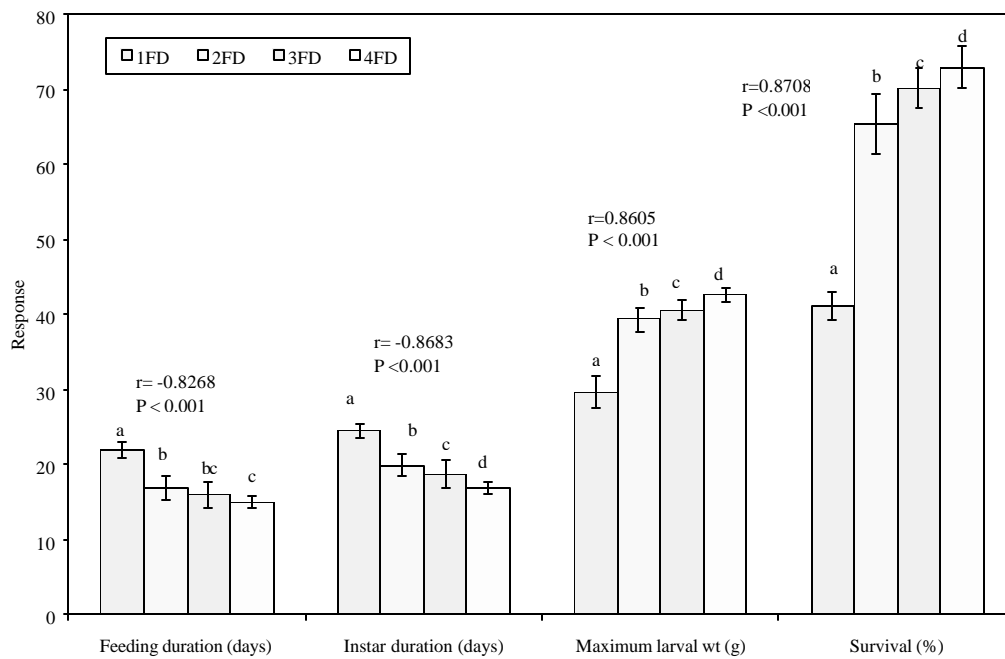


Fig. 2: Effect of quantitative feeding on feeding duration, instar duration, larval weight and survival of fifth instar *A. mylitta* larvae. Values are means \pm SD. Different alphabets show significance among feeding treatments (one-way ANOVA at 5%, df. 36). r-values along with level of significance are cited above the bar graphs

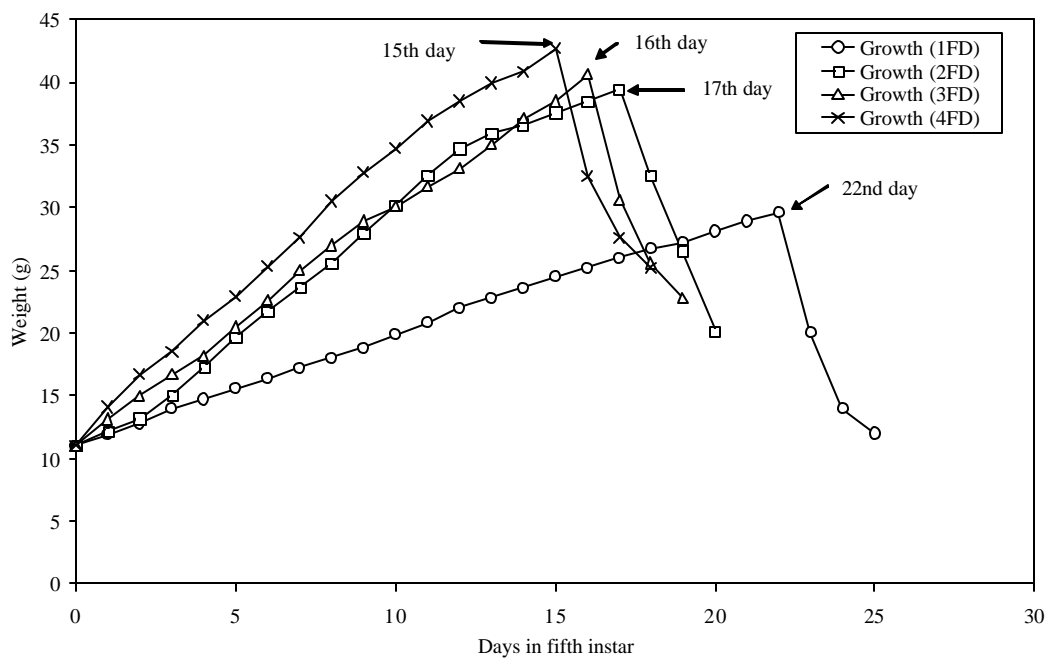


Fig. 3: Growth in *A. mylitta* larva during fifth instar development under different feeding treatments. Arrow marks indicate the day on which the larva attains maximum weight during the particular feeding treatment

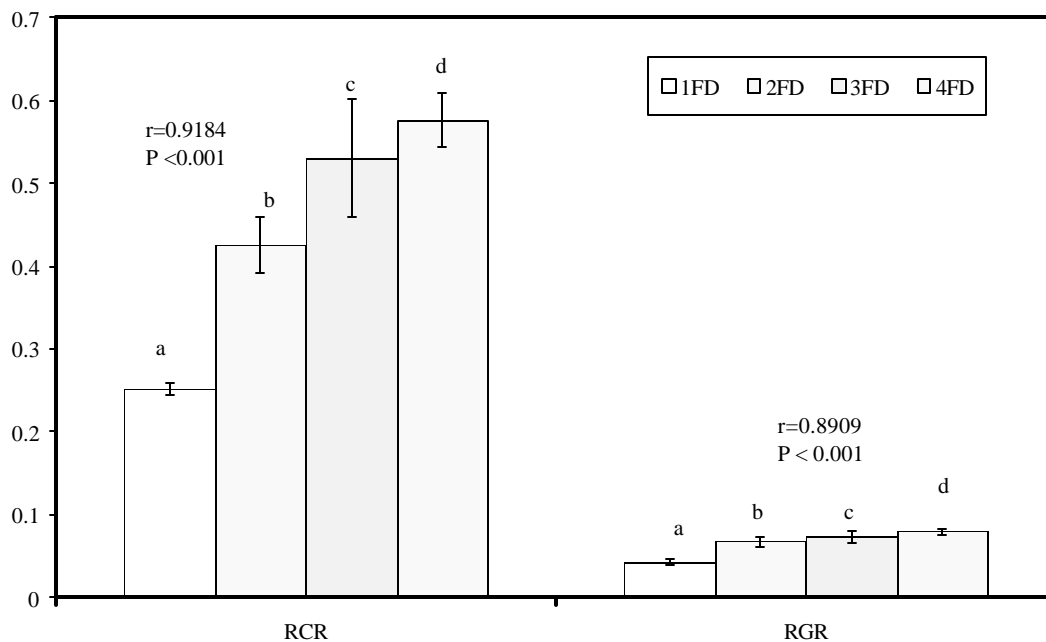


Fig. 4: RCR and RGR in fifth instar *A. mylitta* larvae following different feeding treatments. Values are means \pm SD. Different alphabets show significance among feeding treatments (one-way ANOVA at 5%, df. 36). r-values along with level of significance are cited above the bar graphs.

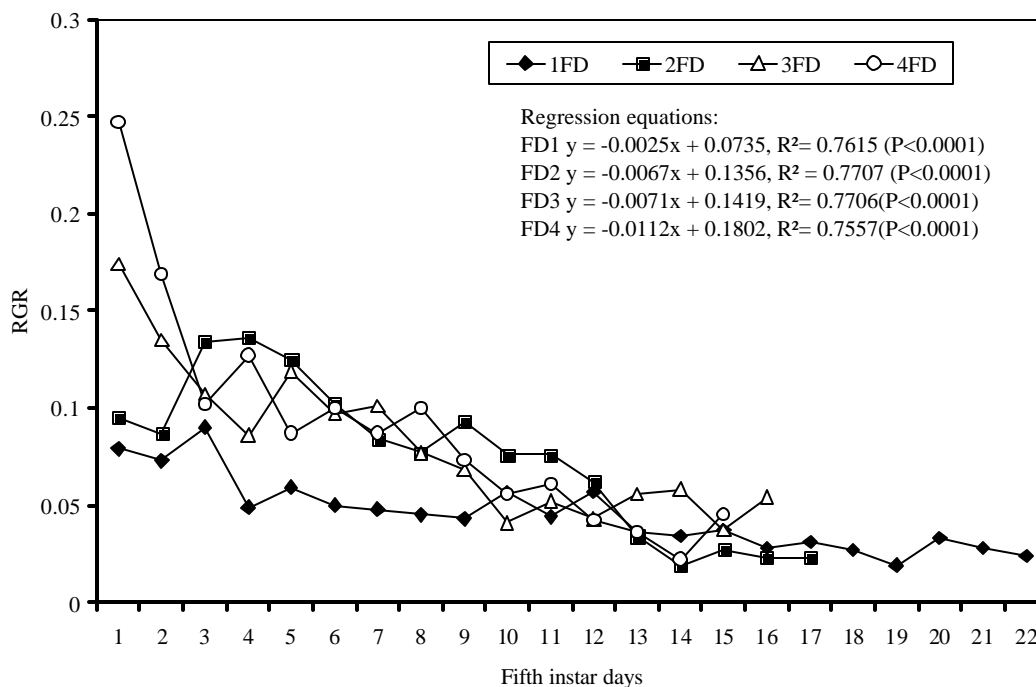


Fig. 5: Change in RGR during development of fifth instar larvae of *A. mylitta* following different feeding treatments

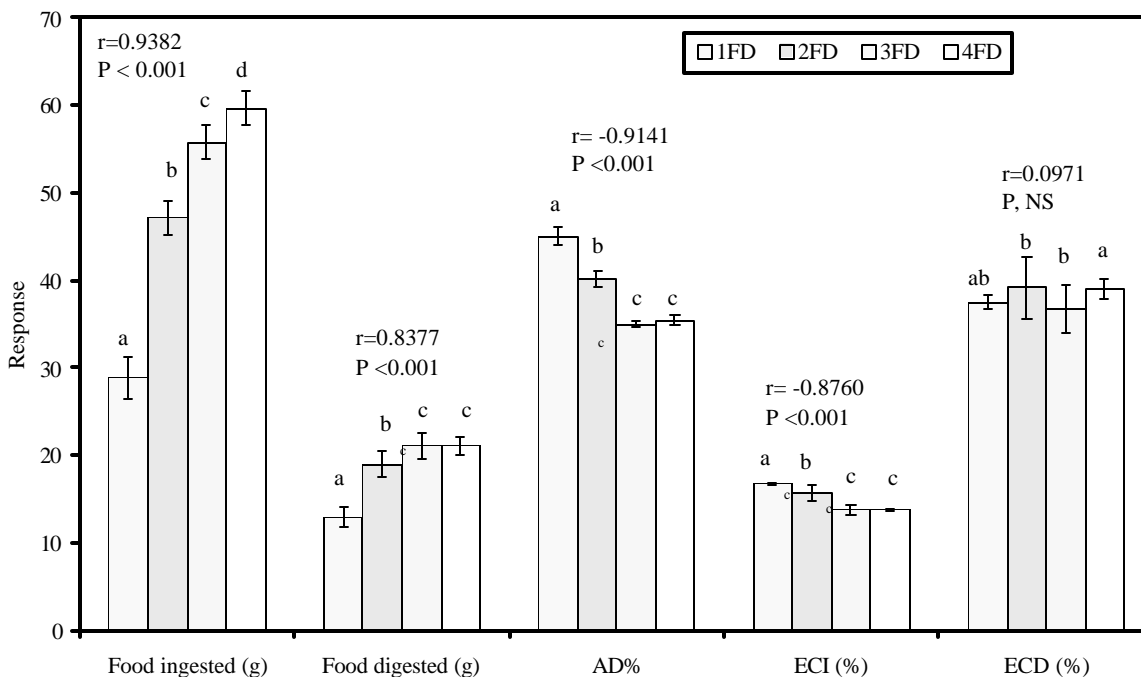


Fig. 6: Nutritional parameters in fifth instar *A. mylitta* larvae following different feeding treatments. Values are means \pm SD. Different alphabets show significance among feeding treatments (one-way ANOVA at 5%, df. 36). r-values along with level of significance are cited above the bar graphs

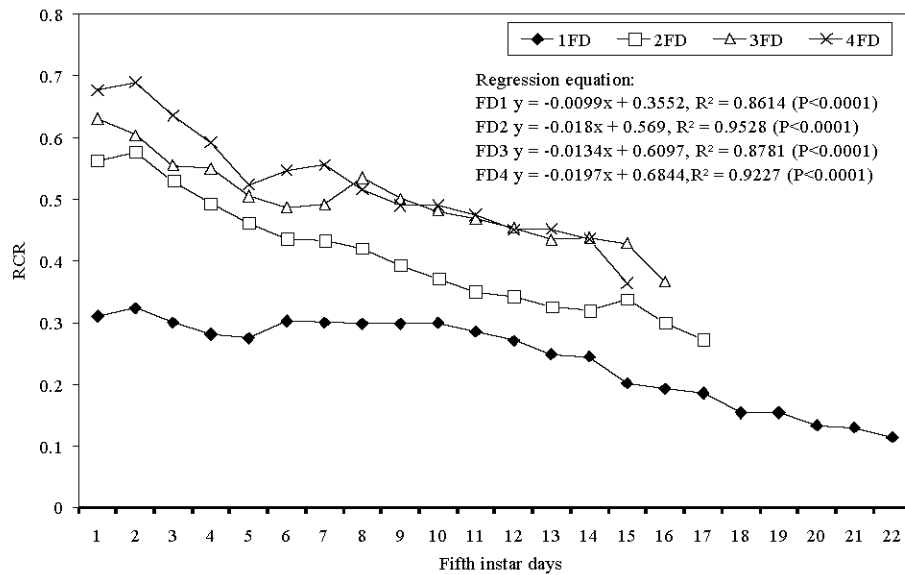


Fig. 7: Change in RCR during development of fifth instar larvae of *A. mylitta* following different feeding treatments.

feeds per day had an immense effect on larval growth in *A. mylitta*. The final weight increased from 29.61 g (in 1FD) to 39.41 g in 2FD, 40.63 g in 3FD and 42.66 g in 4FD (Fig.2, Table 1, 2). The larvae in 4FD condition were reached maximum weight on 15th day but it was delayed by one day in 3FD (on 16th), two days in 2FD (on 17th), five days in 1FD (on 22nd) (Fig. 3). After this loss in larval body weight was observed, which is associated with the emptying of the gut (Fig. 3). Relative growth rate, weight gain and final weight of the larvae increased significantly ($P < 0.001$) with the increase in number of feeds/day. These parameters also exhibit significant difference among the treatment groups. The daily average increase in weight of larva was found to be highest (2111 mg/day) in 4FD condition among others (846 mg, 1671 mg, 1852 mg and 2111 mg in 1FD, 2FD, 3FD and 4FD conditions respectively). Though the RGR registered an increased by 88% in 4FD over 1FD the gain in body weight increased by 70% (Fig. 4).

A gradual but significant decline in RGR during 5th instar development was recorded irrespective of feeding treatments (Fig. 5). This leads to delay in attaining the maximum weight by the larva when the number of feeds per day was reduced. The decline in phagoperiod and instar duration were found directly correlated with the number of feeds/day ($P < 0.001$). The phagoperiod declined by 7 days, so also the instar duration by 7.6 days in 4FD over 1FD (Fig. 2).

Larval survivability increased significantly upon increase in number of feeds/day from 41.2% in 1FD to 65.4%, 70.10% and 72.9% in 2FD, 3FD and 4FD

respectively. Differences in survivability percentage among the treatment groups were also found significant (Fig. 2).

Food Consumption, Digestion and Utilization (AD, ECI and ECD): The parameters studied (except the ECD) had a direct relationship with the number of feeds/day and were found significant ($P < 0.001$) (Fig.4 and 6, Table 1, 2). Food ingestion, digestion and RCR increased with increase in number of feeds/day while the AD and ECI declined.

Increase in feeds enhances the RCR from 0.251 in 1FD to 0.576 in 4FD which helps in food ingestion that rose by 63.66% in 2FD, 93.51% in 3FD and 106.94% in 4FD over 1FD. An increase in digestion by 46.52% in 2FD, 62.98% in 3FD and 63.29% in 4FD over 1FD was also observed. Significant differences in these parameters were observed among treatments with an exception in food digestion, AD and ECI which did not differ among 3FD and 4FD conditions (Fig. 6). A gradual but significant decline in RCR during 5th instar development was recorded irrespective of feeding treatments (Fig. 7).

The intake target of the wild silkworm was found to change with the number of feeds/day which had a direct bearing upon the moisture loss associated with it affecting the growth target considerably. In the present experiment the intake target was changed significantly with feeding conditions (28.81 g for 1FD, 47.15 g for 2FD, 55.75 g for 3FD and 59.62 g for 4FD) which eventually culminated to lower growth 4.84 g in 1FD, 7.39 g in 2FD, 7.7 g in 3FD and 8.23 g in 4FD (Fig. 1).

DISCUSSION

Quantitative feeding has a great impetus in larvae which are dynamic feeders and tend to support proper growth and keeps potentially fit the insect for its reproductive behaviour [6]. The insect in fact tries to reach its intake target to attain its growth target for its physiological potential [2].

The present study revealed that the dietary water which is very closely associated with number of feeds/day (as moisture loss was higher in 1FD) plays a crucial role in the growth in *A. mylitta* as in other herbivorous larval Lepidoptera [1, 10]. Consumption of food of lower water content can result in significant reduction in RGR and efficiencies of conversion of digested food [11-15]. Since growth requires the allocation of significant quantities of water for the hydration of newly synthesized tissues, water can be a growth limiting nutrient for larvae on low-water diets. It might be due to the increased metabolic cost for processing the low-water diets (as in 1 FD condition) as observed in *M. sexta* [13]. Thus reduced growth rate of *A. mylitta* on a low-water diet is due to the consequences of slower growth imposed by nutrient limitation [16].

An extension of larval period have been reported in many insects including sericigenous species following food deprivation and/or by reduction in number of feeds/day [4, 5, 17-20] corroborate our present observations where phagoperiod was extended by 7 days when 1FD condition was maintained over 4FD. Prolongation of larval life under depleted dietary water has also been observed by Van't Hof and Martin [16] in *M. sexta*. Extension of larval period seems to be necessary to reach the intake and growth targets in order to attain functional optima [2].

Larval survivability increased significantly upon increase in number of feeds/day are in accordance with the findings in *B. mori* where the survivability of larvae declined to zero in 1 feed/day from 97% in 8 feeds/day [20].

The absolute increase in ingestion with increase in number of feeds/day might be due to higher water content in the leaves which implies that water imposes a limiting factor for food consumption, utilization and its efficient conversion to larval biomass.

Increase in number of feeds/day had an immense effect on larval growth in *A. mylitta*. The dry biomass gain rose from 4.84 g in 1FD to 8.23 g in 4FD where the daily average increase in weight of larva recorded were 846 mg, 1671 mg, 1852 mg and 2111 mg in 1FD, 2FD, 3FD and 4FD conditions respectively. Similar results are also reported

in *B. mori* where the larval weight of 770 mg in 1 feed/day rose to 2641 mg in 8 feeds/day condition confirms our findings [20].

Haniffa *et al.* [20] reported the energy budget as a function of feed number in *B. mori*. The increases in feeding and assimilation rates in *B. mori* are in confirmation with the findings in *A. mylitta*. The AD% in *B. mori* registered a decline of 12% and 13% in 6 feeds/day and 8 feeds/day respectively corroborate our findings. Higher moisture loss in 1FD renders the leaves less palatable and thus ingestion and RCR were declined which leads to longer retention of food inside the gut to maximize the digestion and hence a significant higher AD% in 1FD was recorded than others. While in *A. mylitta* ECI declined significantly with increase in number of feeds/day, a rise has been reported for *B. mori* [20]. The value of ECD in *B. mori* increased from 17.8 to 20.9 while it did not change in *A. mylitta* with the increase in number of feeds/day. These differences between the two sericigenous species are might be due to their ethology, where *A. mylitta* is wild and is not that much efficient in converting the ingested and digested food like that of *B. mori* which is a completely domesticated one. *B. mori* larvae expends about 54-55% of total metabolizable energy while, *A. mylitta* expends about 88% for various activities other than silk production, egg production and residual energy [21, 22].

The study reveals that frequency of feeding may be increased to get healthier larvae in order to achieve required functional status of the insect. An earlier study also revealed that an enhance the reproductive fitness in *A. mylitta* can be achieved through an increase in number of feeds/day [6].

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