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Developmental parameters and cocoon production by five silkworm, Bombyx mori L. *(Lepidoptera: Bombicidae)* hybrids at different feeding regimes

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Abstract

The developmental characteristics of five silkworm hybrids - J_2J_1, K_1W_1, W_1D_2, J_1C_1, and C_1J_2 – fed with mulberry leaves, 2, 3, 4 and 5 times per day were investigated in a 5x5 factorial arrangement fitted into a completely randomized design (CRD) with four replicates. The aim of the experiment was to identify the best of the four feeding regimes for cocoon production optimization. Each replicate consisted of one hundred silkworm larvae of each hybrid. Results showed that larval and pupal weights and fecundity were significantly jointly influenced by silkworm hybrid and feeding regime. Hybrid C_1J_2, when fed 3 times daily, produced the highest larval and pupal weights, and fecundity relative to other hybrids and feeding regimes, while higher fecundity was obtained at 4 and 5 feedings per day. Whereas larval developmental period was significantly influenced by silkworm hybrids, adult longevity was influenced by feeding regime. Mortality was higher with C_1J_2 at 1 feeding per day compared to other times of feeding, whereas adult longevity was influenced by feeding regimes. Shell weight and shell ratio were influenced jointly by silkworm hybrids and feeding regime. At feeding regime 1, significantly lower number and weight of cocoons were produced. Highest cocoon and shell weights were obtained from C_1J_2 at feeding regime 4 compared to other hybrids and feeding regimes.

Keywords: silkworm, *Bombyx mori*, , feeding, cocoon, larva

Introduction

The silkworm, *Bombyx mori* L. (Lepidoptera: Bombicidae), the insect producing mulberry silk, is a domesticated species of silkworm which has been exploited for over 4000 years (He, 2010). The insect takes necessary nutrients for its growth from the mulberry (*Morus alba* L.) leaves (Adolkar *et al*., 2007; Kanafi *et al.,* 2007). The growth rate, development of silkworm larvae and subsequent silk production are highly influenced by the nutritional quality of the mulberry leaves. In addition to leaf quality, feeding regime plays significant role in cocoon production (Machii and Katagiri, 1991). The number of feedings of silkworm instars influences production of high quality cocoons, and poor feeding could result into very poor quality cocoons or even total rearing failure (He *et al*., 1989).

The larva, which is the only feeding stage of the silkworm, feeds on the mulberry leaves to accumulate the proteins required for the production of cocoons. The proteins are secreted by a pair of large silk glands located on the ventral side of body cavity. Two major proteins are synthesized by the silkworm in the silk glands from the mulberry leaves for cocoon production - fibrion and sericin (Mahesh *et al*, 2010). Feeding influences the resistance to disease, the amount of silk produced, and the duration of each developmental stage. The larva feeds for 20-24 days after which it starts to spin cocoon.

Suitable leaves for silkworm must contain several nutrient constituents such as protein, carbohydrate, mineral, fat, amino acids, vitamins and water (Tribhuwan and Mathur, 1989). Such leaves must be favourably tender and thick so as to be attractive to the silkworm (Bahar *et al*., 2011). Factors that influence the nutrient quality of mulberry leaf include leaf maturity, variety, climate, season, exposure to sunlight, irrigation and soil type in which mulberry is grown (Bahar *et al*., 2011). Similarly, food consumption and insect fecundity vary with life style and feeding pattern of the insects (Muthukrishnan and Pandian, 1987).

Since the silkworm larva is the only feeding stage of the silkworm and it is the stage that produces the cocoon, feeding is therefore, a very important management practice in larval rearing and subsequent cocoon production. While proper feeding enhances optimum cocoon production, poor feeding can result into lower cocoon production and prolonged larval or pupal duration (Sarka and Absarm, 1995; Kherdekar *et al*., 2000; Rouhollah, 2010). Haniffa *et al*. (1988) reported that reducing the number of feedings from eight to one per day influence larval nutrition, survival growth and reproduction of *Bombyx mori*. In this work therefore, the influence of five feeding regimes on developmental characteristics of silkworm, cocoon yield and quality was investigated.

Materials and methods

Experimental set-up

The study was carried out in the Sericulture Laboratory, Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The eggs of the five silkworm species used were obtained from Ekiti State Sericulture Centre Ado-Ekiti, Nigeria, while the mulberry plant variety S30 accessioned in FRIN was used for the feeding of the silkworm species. The experiment was a 5 x 5 factorial arrangement fitted into a completely randomized design with 4 replications. The factors were: 5 silkworm hybrids- J_2J_1, K_1W_1, W_1D_2, J_1C_1, and C_1J_2, and 5 feeding regimes per day - 1 feeding (8.00 hrs), 2 feedings (8.00 hrs and 14.00 hrs), 3 feedings (8.00 hrs; 14.00 hrs and 27.00 hrs), 4 feedings (8.00 hrs, 11.00 hrs, 14.00 hrs, and 17.00 hrs) and 5 feedings (8.00 hrs, 11.00 hrs, 14.00 hrs, and 17.00 hrs and 16.00 hrs) local time. One hundred larvae were used as a replicate or each of the hybrids. The different larval instars were fed on leaf rations as follows: 1^{st} instar, 16 g; 2^{nd} instar, 80 g, 3^{rd} instar, 250 g, 4^{th} instar, 320 g, 5^{th} instar, 500 g per day per disease free laying (DFL) as recommended by Ullal and Narasimhanna (1987). The different feeding regimes were determined by dividing the recommended daily rations by the number of feedings being tested per day to obtain the desired weight of each ration.

Silkworm is known to react negatively to environmental conditions, especially temperature and relative humidity. These factors were closely monitored during the cause of these experiments and maintained at 23-26^{o}C and 73-86% RH using the standard suggested by Ullal and Narasimhanna (1987) and Suresh *et al*. (2007).

Preparation of eggs for hatching

Diapausing eggs of the five silkworm hybrids were preserved in refrigerator at 2.5-5^{o}C. The eggs were removed from the refrigerator as need arose. Prior to the removal of the eggs for rearing, the temperature of the refrigerator was raised from 5 to 15^{o}C for 3 hours to avoid shock. Thereafter, the egg cards were rinsed in 2% formalin solution for 15 minutes and later washed in running water. The egg cards were then kept between two large sheets of filter paper to dry and subsequently immersed in glass trough of Hydrochloric acid (HCl) (1.10 specific gravity) at 29^{o}C for 50 minutes (cold acid treatment) to break the diapause (Jolly, 1988). Thereafter, the eggs were removed from the acid and rinsed under running water for 5 minutes until the acid was completely washed off. For incubation, the egg cards were then turned upside down on large paper spread in a rearing tray on which old newspaper sheets were spread.

Twenty four hours later, previously disinfected foam pads were soaked in water and arranged at the four sides of the rearing tray before covering with paraffin or nylon paper. This was to maintain high humidity and temperature for 10-11 days, while the colour change of the eggs was observed. When eggs were freshly laid, they were yellow in colour but turned brown 24 hours later ushering in hibernation and it was at this stage they were acid-treated. When the egg colour turned blue or at the egg pigmentation day, the egg cards were kept in total darkness for 2 - 3 days, a process called 'black boxing'(Jolly, 1986). On the 11th day of incubation, the eggs were exposed to bright light early in the morning. This was done to achieve uniform hatching because darkness arrests hatching of the developed eggs and facilitates lagging embryo to reach hatching phase. The egg cards were spread uniformly in one layer on paraffin paper.

Brushing of newly hatched eggs.

After two hours of exposing the eggs to light in the morning, chopped leaves (0.5 cm x 0.5 cm) were sprinkled in a single layer over the hatched larvae for about 10-20 minutes to allow the larvae to crawl on the cut leaves. The egg sheets were turned upside down and held about 5 cm above the rearing tray with paraffin paper and taped gently to complete the process of transferring the larvae (brushing).

Preparing rearing house and equipment

Five days to the commencement of brushing, the rearing room, rearing trays, and all other equipment were washed, dried and disinfected with 3% formalin solution. After brushing, one hundred silkworm larvae were randomly selected from each of the five hybrids and fed according to the treatments highlighted above until they passed the five larval stages. Treatment trays were randomly distributed on the rearing racks, and thereafter, larval mortality was determined.

Cleaning and caring for the worms

To maintain hygiene in the rearing bed, dirt such as left-over or rejected leaves, fecal matters and carcasses of the third, fourth and fifth instar larvae were removed from the rearing trays on a daily basis, and covered with clean nets. Mulberry leaves were broadcast on the net after the cleaning procedures, making the larvae to climb onto the net from the trays and hang onto them while trying to reach and feed on the fresh leaves. The larvae on the net were then transferred into other clean trays or cleaned old trays, and thereafter, clean sheets of paper were spread on them. This procedure was not carried out on the first and second instars which were fragile so as not to lose them.

At the commencement of each moulting, the larvae were dusted with slaked lime ($CaCO_3$) to dry up the bed and leaves so that all the larvae could stop feeding. Dusting also reduced the chances of the growth of mould and other disease-causing micro-organisms.

Mounting of mature worms and cocoon processing

A day before the commencement of cocoon formation, fully grown silkworm larvae were later mounted on montages for cocoon spinning. All cocoons were harvested at six days old, out of which 20 were randomly selected from each replicate for the determination of pupal weight, shell weight and shell ratio. The pupae were subsequently kept until the adults emerged. Percentage adult emergence was calculated using the formula:

$$\frac{\text{Number of emerged adults}}{\text{total number of pupae.}} \times 100$$

Freshly emerged male and female adults were placed on rearing trays for mating for three hours according to the different treatments in four replications. The females were later separated and put in cellule on thick brown cardboard paper where they laid their eggs. After 24 hours, newly laid eggs were acid–treated and incubated, and the newly hatched larvae were brushed (Ullal and Narasimhanna, 1987)

The number of hatched eggs was determined from the number of egg shells recovered. After hatching, the egg shells became white, un-hatched eggs became black, while unfertilized eggs retained their yellow colour. The number of hatched eggs was therefore calculated as percentage of the total eggs laid. Other data taken were: larval mortality, larval developmental period, larval weight, pupal weight, percentage larval emergence, and fecundity, In order to determine longevity of adult silkworm, five pairs (males and females) were kept separately in Kilner jars till death, and number of days from adult emergence to death represented longevity. The following formulae were used to calculate the data taken:

Larval developmental period = No of days from hatching to cocooning;
Larval weight = average weight of 10 larvae;
Pupal weight = average weight of 10 pupae;
Percentage larval emergence =

$$\frac{\text{No of emerged larvae}}{\text{Total number of eggs,}} \times 100$$

Fecundity = Total no of eggs laid by one adult female;

All the data collected were subjected to analysis of variance (ANOVA) test at $P < 0.05$, and where there were significant differences, Duncan's New Multiple Range Test (DMRT) was used to separate them.

Results

Silkworm hybrid and feeding regime jointly influenced silkworm larva weight, pupal weight and fecundity significantly (Table 1). Fecundity, larval and pupal weights were significantly higher in C_1J_2, while feeding silkworm 4 and 5 times per day produced significantly higher fecundity in the five hybrids. However, irrespective of hybrids, feeding silkworm 5 times per day produced significantly higher pupal weight relative to other feeding regimes (Table 1).

Table 1: Larval and pupal weights and fecundity of five silkworm hybrids at different feeding regimes

Variables	Silkworm hybrid	Feeding regime (per day) 1	2	3	4	5	Mean
Average Laval Weight(g)	J_2J_1	1.99g	2.62b-e	2.84ab	2.57b-f	2.31f	2.46
	K_1W_2	1.83gh	2.56b-f	2.51d-f	1.45j	2.54c-f	2.42
	W_1D_2	1.92gh	2.79a-d	2.53c-f	2.59b-f	2.38ef	2.44
	J_1C_1	1.64h-j	1.51ij	1.68h-j	1.76g-i	1.89gh	1.70
	C_1J_2	2.53c-f	2.84ab	3.00a	2.98a	2.81a-c	2.83
	Mean	1.98	2.46	2.52d	2.88	2.38	
		SXF*					
Average Pupal Weight(g)	J_2J_1	0.71f	0.82c-f	0.90a-c	0.87b-d	0.86b-e	0.83
	K_1W_2	0.78d-f	0.88a-d	0.85c-e	0.75ef	0.77d-f	0.81
	W_1D_2	0.82c-f	0.90a-c	0.87b-d	0.90a-c	0.87b-d	0.87
	J_1C_1	0.86b-e	0.87b-d	0.86b-e	0.90a-c	0.80c-f	0.86
	C_1J_2	0.80c-f	0.73f	0.99a	0.97ab	0.97ab	0.92
	Mean	0.79	0.84	0.89	0.90	0.96	
		SXF*					
Fecundity	J_2J_1	405.0d-g	389.3d-h	413.0c-g	414.3c-g	421.3c-f	408.6
	K_1W_2	364.0f-i	392.8d-g	307.8i	389.5d-h	447.5b-d	380.3
	W_1D_2	325.0hi	379.3e-h	401.8d-g	397.3d-g	352.3g-i	371.1
	J_1C_1	440.5b-e	419.5c-f	504.0ab	506.0ab	350.0g-i	480.0
	C_1J_2	493.3ab	415.7c-g	447.3b-d	476.5bc	549.0a	496.4
	Mean	405.6	419.3	414.8	436.7	460.0	
		SXF*					

Values are means of four replicates. S, Silkworm hybrids; F, Feeding regimes SxF= Interaction between Silkworm hybrid and Feeding regimes* = significant; ns= not significant at $P<0.05$

Similarly, mortality was jointly influenced by silkworm hybrids and feeding regime (Table 2). At 1 feeding a day, mortality in hybrid C_1J_2 was highest and was not significantly different from the values obtained when the same hybrid was fed twice and four times a day. The lowest mortality was however obtained in W_1D_2 at feeding five times per day (Table 2).

Larval developmental period in hybrids C_1J_2 and J_1C_1 was significantly longer (25 days) than in the other three hybrids which was 23 days (Table 2). Longer ($P < 0.05$) developmental period was observed in C_1J_2 and C_1J_1 irrespective of number of feedings per day (Table 2).

Table 2: Mortality, developmental period and longevity of five silkworm hybrids at different feeding regimes.

Variables	Silkworm hybrid	Feeding regime (per day) 1	2	3	4	5	Mean
Mortality (%)	C_1J_2	66.25a	55.75a-c	42.25c-g	58.50ab	50.00b-d	55.15
	J_1C_1	27.25g-j	43.00b-f	44.25b-e	43.50b-f	50.50b-d	41.70
	J_2J_1	40.75c-h	29.50e-j	23.00ij	26.50h-j	37.25d-i	31.40j
	K_1W_2	43.50b-f	28.50f-j	35.50d-j	34.00e-j	44.25b-e	37.15
	W_1D_2	37.00d-i	29.00e-j	25.00ij	32.50e-j	20.50j	28.80
	Mean	42.95	37.15	34.60	39.00	40.50	
		SXF*					
Developmental Period (days)	C_1J_2	25.00a	25.00a	25.00a	25.00a	25.00a	25.00
	J_1C_1	25.00a	25.00a	25.00a	25.00a	25.00a	25.00
	J_2J_1	23.00c	23.00c	23.00c	23.00c	23.00c	23.00
	K_1W_2	23.00c	23.00c	23.00c	23.00c	23.00c	23.00
	W_1D_2	23.00c	23.00c	23.00c	23.00c	23.00c	23.00
	Mean	23.80	23.80	23.80	23.80	23.80	
		SXF=ns					
Adult Longevity	C_1J_2	4.75c	4.25de	4.00e	4.00e	4.25de	4.25
	J_1C_1	4.00e	5.25ab	4.75c	3.50f	4.25de	4.35
	J_2J_1	5.00bc	4.00e	4.75c	4.00e	4.75c	4.50
	K_1W_2	5.00bc	4.00e	4.75c	4.25de	5.50a	4.70
	W_1D_2	5.00bc	4.00e	4.75c	4.25de	4.25de	4.45
	Mean	4.75	4.30d	4.60	4.00	4.60	
		SXF=ns					

Values are means of four replicates. S, Silkworm hybrids; F, Feeding regimes SxF=Interaction between Silkworm hybrid and Feeding regime; * = significant; ns= not significant at P<0.05

At feeding regime 1, significantly lower number of cocoons was produced, except for J_1C_1 which produced the highest number of cocoons, compared to other regimes (Table 3). Weight of cocoons was also significantly lower at feeding regime 1, irrespective of silkworm hybrid relative to other feeding regimes. Highest cocoon weight was obtained from C_1J_2 at feeding regime 4; this value was significantly higher than those obtained from other silkworm hybrids or feeding regimes. Also silkworm hybrid C_1J_2 produced significantly higher cocoon shell weight at feeding regime 4 compared to other hybrids and feeding regimes, followed by W_1D_2. Irrespective of feeding regimes, W_1D_2 produced significantly higher shell ratio, but highest shell ratio was obtained from K_1W_2 at feeding regime 4.

Table 3: Silkworm cocoon yields of five silkworm hybrids at different feeding regimes

	Silkworm	Feeding regime (per day)					
Variable	hybrid	1	2	3	4	5	Mean
Number of cocoons	J_2J_1	47.3b-f	56.3a-d	61.8a	58.8ab	50.3a-e	54.9
	K_1W_2	35.3f-h	57.0a-c	51.5a-d	52.8a-d	44.5c-g	48.2
	W_1D_2	50.5a-d	56.8a-d	60.0ab	54.0a-d	61.3a	56.5
	J_1C_1	60.0ab	45.5c-g	44.5c-g	45.0c-g	37.8e-h	46.6
	C_1J_2	27.0h	35.3f-h	43.5c-g	33.0gh	36.0f-h	35.0
	Mean	44.0c-g	50.2a-e	52.3a-d	48.7b-f	46.0c-g	
		SXF*					
Single cocoon weight (g)	J_2J_1	0.89i	1.01f-h	1.09d-h	1.05d-h	1.05d-h	1.02
	K_1W_2	0.96hi	1.09d-h	1.03e-h	0.96hi	1.03e-h	1.02
	W_1D_2	0.99g-i	1.14c-f	1.05d-h	1.11c-g	1.06d-h	1.07
	J_1C_1	1.04d-h	1.13c-g	1.07d-h	1.07d-h	1.05d-h	1.07
	C_1J_2	1.16b-e	1.24bc	1.29ab	1.42a	1.18b-d	1.26
	Mean	1.01	1.12	1.11	1.12	1.07	
		SXF*					
Shell weight (g)	J_2J_1	0.17c	0.19c-e	0.20c-e	0.20c-e	0.20c-e	0.19
	K_1W_2	0.20c-e	0.21c-e	0.19c-e	0.21c-e	0.21c-e	0.20
	W_1D_2	0.21c-e	0.25b	0.22b-d	0.21b-e	0.20c-e	0.22
	J_1C_1	0.18de	0.20c-e	0.19c-e	0.18de	0.21c-e	0.19
	C_1J_2	0.23bc	0.25b	0.25b	0.30a	0.20c-e	0.24
	Mean	0.20c-e	0.22b-d	0.21c-e	0.22b-d	0.21c-e	
		SXF*					
Shell Ratio	J_2J_1	19.5a-f	18.8c-g	18.8c-g	19.3a-g	19.8a-f	19.2
	K_1W_2	20.0a-e	18.8c-g	18.0e-g	22.0a	20.3a-e	19.8
	W_1D_2	21.0a-d	21.8ab	21.0a-d	18.8c-g	19.0b-g	20.3
	J_1C_1	17.8c-g	18.3d-g	18.0efg	16.5g	20.0a-e	18.1
	C_1J_2	19.0b-g	20.0a-e	19.8a-f	21.3a-c	17.0fg	19.4
	Mean	19.5	19.5	19.	19.6	19.2	
		SXF*					

Values are means of four replicates. S, Silkworm h ybrids; F, Feeding regimes SxF= Interaction between Silkworm hybrid and Feeding regime ; * = significant; ns= not significant at P<0. 05

Discussion

Results obtained indicated that interaction effect between the silkworm hybrids and feeding regime was significant for larval weight, pupal weight, and fecundity, as well as number of cocoons, single cocoon weight, shell weight and shell ratio which are the major economic indices of cocoon production. Also cocoon weight shell weight increased with increase in number of feedings per day. These findings suggest that feeding plays significant role in the development of silkworms and in the quality of cocoons. The non-significant interaction between silkworm hybrids and feeding regimes with respect to developmental period and adult mortality suggest that the parameters were not influenced jointly by the two factors.

Similarly, the reduction in fecundity from 1 feeding per day compared to 4 or 5 feedings may be attributed to the fact that at 4 or 5 feedings, leaf rations were spread and given fresh over a period of time. This is contrary to 1 feeding where the bulk of leaves packed at once for the silkworm would have lost moisture over time and wilted as a result of high evaporation rate under the tropical climatic condition. Moisture is very crucial for insect body metabolism and development. Feeding phytophagous insects with wilted leaves produced adverse effects in the insect metabolism (Friend, 1958; Waldbauer, 1968). Since silkworms derive their moisture from leaves, the leaves must be kept and given fresh (Esfandarani *et al*., 2002). The importance of leaf moisture content in relation to the performance of silkworm, growth rate per day and general performance of silkworm have been reported by Paul *et al*. (1992). Hanifa *et al*. (1988) reported that one feeding per day resulted in extended larval period, high mortality, delayed oviposition and decreased fecundity, while increase in number of feeding enhanced larval weight, as well as assimilation and metabolism. Mathavan *et al*. (1987) observed that the length and biomass of the ovary steadily increased with the number of feedings per day. Similarly, He *et al*. (1989) reported higher cocoon and shell weights with increase in the number of feedings.

Another reason that may be attributed to the observed poor cocoon production and development of the larval and pupal stages of the silkworms at 1 feeding per day is that the leaves were likely to have been contaminated with faecal materials since they remain with the silkworm for a long period. This may result in silkworm rejecting the leaves thus leading to poor feeding, starvation, slow growth, development and lower fecundity compared with other feeding regimes.

Generally, for all the five hybrids viz J_2J_1, K_1W_1, W_1D_2, J_1C_1 and C_1J_2, and at all feeding regimes (1, 2, 3, 4 and 5 feedings per day), cocoon yields, shell weight, shell ratio and fecundity, which form the major economic indices of the silkworm, fell within acceptable international standard. The standard shell weight for Japanese pure breeds is 300 – 400 mg, while those of hybrids from the same country are between 250 mg and 300 mg (Rangaswami *et al*., 1976; Mendoca *et* al., 2010) Also, in India, the standard shell weight for multivoltine pure breed is betweem 150 mg and 180 mg; multivoltine hybrid is 150 mg while univoltine hybrid is 200 mg - 300 mg (Rangaswami *et al*., 1976; Raju and Krishnamurtthy, 1993). This implies that all the tested hybrids can still be recommended for commercial cocoon production in a tropical environment. The likely reason for high performance of these hybrids was that they were sourced from southern part of India which share similar tropical climatic condition with the location of this experiment.

The significant interaction between silkworm hybrid and feeding regime on some biological parameters and cocoon production reveals that the performance of the tested silkworm hybrids depended on number of times the worms were fed. When fed once daily, silkworm hybrid J_1C_1 produced the highest cocoon number of 60 and single cocoon weight of 1.04 g. This suggests that the hybrid is tolerant to restricted or staggered feeding. Hybrid C_1J_2 recorded the largest larval weight which resulted in high single cocoon weight and shell weight; the hybrid also produced the highest number of eggs (fecundity) compared to other hybrids. This implies that this hybrid produced high quality cocoon and is better under the tropical environmental condition. Silkworm hybrids C_1J_2 and W_1D_2 ranked best in terms of cocoon yield and quality because they produced the highest weight per cocoon and shell weight. These yield parameters are the most important economic characteristics in cocoon production because they indicate the quantity of reelable silk per cocoon. In commercial production of silk, the silk shell weight is an important factor in silk content of the cocoon, and it influences the value of its production (Mendonça *et al*., 2010).

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