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NUTRITIONAL EFFICIENCY AND ITS RELATION TO *Bombyx mori* L. PRODUCTIVITY UNDER DIFFERENT CONSTANT TEMPERATURES

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ABSTRACT

All experimental studies on *Bombyx mori* L. under suitable temperatures aim to improve silk, cocoons and eggs productions. The fifth larval instar was reared under three different constant temperatures (20, 23 and 26 °C) to determine the growth, dietary efficiency, silk production and egg yield. Rearing performance as larval weight, duration and growth rate, effective rate of rearing, disease incidence, pupal weight, pupation ratio, pupal duration, adult longevity, fecundity and fertility were recorded. Cocoon parameters as cocoon, cocoon shell weights, cocoon shell ratio and nutritional parameters like ingesta, digesta, and approximate digestibility percentage were calculated and recorded. Silk filament length and filament breaks were recorded during cocoon reeling. Significant variations in the performance of *B. mori* were noticed due to the influence of temperature.

Most of these studied parameters were significantly high for larvae reared at 20°C, as well as, the feed conversion efficiency parameters, ingesta and digesta were required to produce one gram of cocoon were higher in larvae reared at 20°C. Female moths were capable to mate with more than one male which could produce more egg laying and consequently fertile eggs.

INTRODUCTION

Bombyx mori L. is one of the most important domesticated insects, which produces luxuriant silk thread in the form of cocoon by consuming mulberry leaves during larval period. The late age of larval stage is the most active feeding stage during which the larva accumulates large quantity of fuel reserves in various tissues and is endowed with unique biochemical adaptations to conserve nutritional resources available during active larval stage of the silkworm (Etebari *et al*., 2007).

The relationship between the environment and genes is considered to be directed with food consumption efficiency on gene expression. This relationship varies depending on the genetic background of an organism and expressed in the physiological or nutritional unit in gene regulation mechanism (Milner, 2004; Kang, 2008; Ogunbanwo and Okanlawon, 2009). Hence, it obviously indicates at higher level, nutrition/physiology play an imperative role of genes expression in the genome of an individual and the impact of variations in genome on response to nutrition promises to improve insight into nutrient metabolism and revolutionize biomarkers development to utilize with the genome for its phenotypic expression (Ramesha *et al*., 2010). In sericulture, nutrition of silkworm larvae and its conversion efficiency contribute directly or indirectly on the cost benefit ratio of silkworm rearing. It was considered as an important physiological criterion for evaluating the superiority of silkworm breeds (Zhang *et al*., 2002).Because growth and development are of primary importance to all animals, insects may respond to such variation in temperatures by adjusting the amount of food eaten (Slansky and Scriber 1985). The consumption and utilization of food in insects facilitate the understanding of the adaptability of insects to the environment which influence directly the crop (Benchamin and Jolly 1986). The cocoon-related characters are influenced by ambient temperature, rearing seasons, quality and quantity of mulberry leaf, and genetic constitution of silkworm strains (Rajesh and Elangovan 2010). The optimal conditions were not necessary to be the same for all races and amongst these conditions, temperature is the most important (Ramachandra *et al*., 2001). The seasonal differences in the temperatures considerably affect the genotypic expression in the form of phenotypic output such as cocoon weight, shell weight, and cocoon shell ratio (Nacheva and Junka 1989).

Spinning of the silk which determine the cocoon traits e.g. cocoon reliability and quality depends not only on silkworm variety but also on temperature during cocoon spinning (Ramachandra *et al*., 2001). Fecundity and egg fertility can be enhanced by avoiding temperature and humidity fluctuations during larval rearing (Hussain *et al*., 2011). In this field of study, Kato *et al*., (1989) observed that resistance to high temperature is a heritable character and it may be possible to breed silkworm races tolerant to high temperature.

The aim of the study is to find out the optimum temperature for better rearing performance of a local univoltine commercial hybrid and less food consumption with higher efficiency conversion rate.

MATERIALS AND METHODS

Bombyx mori eggs were obtained from the Sericulture Research Department of Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC), Giza, Egypt. Rearing technique was done in the laboratory under the hygro-thermic conditions 28 ± 1 °C and 75 ± 2% RH, following the standard methodology of rearing in the Sericulture Department according to Krishnaswamy (1978). Larvae were fed on leaves of Kokuzo-27 mulberry variety.

At the beginning of the 5^{th} larval instar, 80 larvae were kept at each temperature (20, 23 and 26 °C), with relative humidity 50 - 55% RH, darkness and standard recommended feed quantum. The larvae were divided into four groups (20 larvae / group); the first group, 20 larvae separated individually for 20 replicates and placed in plastic box (one larva/ one box) for food consumption estimation. The second larval group is stock with the same age and weight were maintained to compensate mortality. The third larval group were kept for recording the rearing performance. The fourth larval group were kept for cocoon reeling.

Sample of mulberry leaves used for each treatment was placed in a separate box (blank). The healthy larvae were counted daily in each replication, while the unhealthy, as well as dead larva were removed and replaced with another one. The excreta and left over leaf were manually separated and weighted daily.

Observation on larval weight, larval duration were recorded daily for dietary efficiency calculation. Fresh weight of left over leaf, excreta, larval weight gain and effective rate of rearing were recorded for all replications of each treatment.

Nutritional indices include growth rate, consumption indices, ingesta, digesta, excreta, approximate digestibility efficiency percentage of conversion of ingesta and digesta to larval mass and cocoon weight were worked out by gravimetric method as described by (Waldbauer, 1968; Scriber and Feeny, 1979). Data were subjected to analysis using statistical analysis system version 9.1 program proc. GLM (SAS Institute 2003).

1- Growth rate (GR)

It refers to larval gain biomass and indicates the efficiency of conversion of nutrition into larval biomass:

GR = G / D M

G = Gain weight during larval period (gm)

D = Duration of larval period (days)

M = Mean weight of larva during the same period (gm)

2-Weight of consumed food

(CF) = (1-A/2) X (1-R) + B X R)

Where:

$$A = \frac{\text{Weight of control food before} - \text{Weight of control food after}}{\text{Weight of control food before}}$$

$$B = \frac{\text{Weight of control food before} - \text{Weight of control food after}}{\text{Weight of control food after}}$$

I = Weight of introduced food (gm)

R = Weight of remaining food (gm)

3- Consumption Indices (CI):

Consumption indices related to the rate of food intake to the mean weight of the larvae during the feeding period with the high nutritional interest since, it measures the rate at which the nutrients enter the digestive system:

CI = CF / D M

CF = fresh weight of consumed food (gm)

D = Duration of feeding period (days)

M = Mean weight of larva during the same period (gm)

Ingesta: The total intake of the fresh weight of mulberry leaves by silkworm larvae during the 5^{th} instar up to spinning or ripening:

Ingesta = weight of leaf fed - weight of left over leaf

Digesta: Total assimilated food from the intake or ingesta of fresh weight of mulberry leaves by silkworm larva during the 5^{th} instar until spinning or ripening:

Digesta = weight of leaf ingested - weight of feces

Excreta: Excreta refer to the non-utilized mulberry leaves in the form of litter from the ingested mulberry leaves of a silkworm

Excreta = Ingesta - Digesta

3- Approximate digestibility (AD)

It directly indicated the assimilation efficiency of mulberry leaves and depends on the passage rate of food through gut in silkworm.

AD = (weight of food ingested – weight of feces) / wt of food ingested X 100

4- Efficiency of conversion of ingested food to body mass (ECI)

ECI = GR / CI X 100

5- Efficiency of conversion of digested food to body mass (ECD)

ECD = wt gain / (wt of food ingested – wt of feces) X 100

6-Ingesta per gram (I/g) cocoon:

This particular trait was the expression of total ingesta required for the production of one gram of cocoon.

$$\text{I/g cocoon} = \frac{\text{Fresh weight of ingesta}}{\text{Fresh weight of cocoon}}$$

7- Digesta per gram (D/g) cocoon:

$$\text{I/g cocoon} = \frac{\text{Fresh weight of digesta}}{\text{Fresh weight of cocoon}}$$

Pupal weight, pupation percentage, pupal duration, cocoon weight, cocoon shell weight, cocoon shell ratio %, adult longevity, number of deposited eggs, number of fertilized eggs were recorded.

Ten cocoons produced from each treatment were dried in oven at 70°C for 24 hours then reeled. Dry cocoon weight, filament length, number of filament breaks, filament weight and silk ratio for each group were recorded.

Silk ratio was calculated by the following formula:

$$\text{Silk ratio} = \frac{\text{Filament weight}}{\text{Dry cocoon weight}} \times 100$$

RESULTS AND DISCUSSION

The fundamental understanding on the nutrition - gene interactions and its effect on economic traits in silkworm is essential for evaluation of nutritionally efficient silkworm breeds. As dietary or nutrient factors and related metabolic interactions has direct and indirect influence on specific gene regulation and expression (Iftikhar and Hussain, 2002; Phillips *et al*., 2008). Such interactions and variations in the field of nutria-genetics could be applied to choose the silkworm breeds based on their nutritional efficiency parameters as biomarkers (Ramesha *et al*., 2010).

Temperature acclimation, physiological and behavioral thermoregulation allow individual insects to compensate to various degrees of changes in ambient temperature (Heinrich, 1981). The importance of temperature for growth of fifth larval instar is made apparent by comparison of the instar duration, growth rate (GR) and weight gain (Ahmed and kamal, 2001).

In the present study, results illustrated in Table (1) showed that larval duration was significantly long at 20°C (14.80 days) resulted in high larval weight (3.49 gm/ larva), while rearing efficiency recorded the highest value (69.56) comparing with the other two constant temperatures; 23 and 26°C and all the dead worms showed flacherrie disease symptoms. Death at 26°C temperature caused much early than silkworm kept at 20 and 23°C temperatures due to heat stress and dehydration. Larvae reared at 23°C recorded slightly higher growth rate (0.12/day). These results are confirmed by Rahmathulla *et al*. (2004) as they found that slightly lower temperature besides better aeration and sufficient quantum of quality leaf is recommended during later instars of development of silkworm larvae. It was suggested that the variation observed in relative growth rate between *B. mori* larvae in the present study was due to difference in larval duration among temperatures.

In sericulture, nutritional requirement and its conversion efficiency contribute directly or indirectly on the cost benefit ratio of silkworm rearing. The feed utilization study was confined to 5th instar larvae as 80-85% of the total leaf is consumed in this instar (Rahmathulla *et al*., 2005). The significance in food utilization among the three rearing temperatures was discussed in Table (2). Larvae reared at 20°C consumed the highest quantity of food (28.91gm) and consequently showed the better performance in ingestion and digestion (9.54 and 12.95, respectively), while the approximate digestibility (75.13) was not significantly deviated from the other two constant temperatures which being 74.98 and 73.73 at 23 26 °C, respectively.

In this field of study, Rahmathulla *et al*., (2003) suggested that increase in ingestion and digestion suggests the possibility of increase in the accumulation of organic constituents in the body tissue of the silkworm as biomass. The passage of food through gut became slow when consumption index decreases to facilitate increased digestion and assimilation with ultimate result of improved approximate digestibility and corresponding traits which ascertained in this study.

Efficiency of the nutrition almost nullified by the increase in consumption resulting in increased production of cocoon, shell and understood that dietary factors and related metabolic interactions has direct and indirect influence on specific gene expression. Feed conversion efficiency contributes directly and indirectly to the major chunk of the cost benefit ratio of silkworm rearing.

Table (1): Effect of different constant temperatures on *B. mori* 5th larval instar.

Temp. (°C)	Larval duration (days)	Larval weight (gm)	Growth rate/ day	Effective Rate of Rearing (ERR %)
20	14.8 ± 0.84 a	3.49 ± 0.27 a	0.1 ± 0.01 b	69.56 ± 1.79 a
23	11.8 ± 0.72 b	2.62 ± 0.49 b	0.12 ± 0.01 a	69.23 ± 3.04 a
26	10.75 ± 1.93 b	2.57 ± 0.3 b	0.11 ± 0.01 ab	59.52 ± 4.76 b
LSD 1%	2.85	0.62	0.02	5.41

Means in the column with the same letters are not significantly different

Table (2): Effect of different constant temperatures on *B. mori* 5th larval instar food utilization.

Temp.(°C)	CF	CI	AD	ECI	ECD
20	28.91 ± 0.09 a	1.03 ± 0.06 b	75.13 ± 2.45 a	9.54 ± 0.51 a	12.95 ± 0.94 a
23	25.49 ± 0.13 b	1.35 ± 0.18 a	74.98 ± 6.05 a	9.29± 0.77 a	12.47 ± 1.91 a
26	25.14 ± 0.28 b	1.44 ± 0.27 a	73.73 ± 3.52 a	7.5 ± 1.15 b	10.04 ± 1.93 ab
LSD 1%	2.99	0.25	7.15	1.43	2.78

Means in the column with the same letters are not significantly different
CF = Consumed Food (gm) **CI= Consumption Indices**
AD = Approximate digestibility
ECI = Efficiency of Conversion of Ingested food to body mass
ECD = Efficiency of Conversion of Digested food to body mass

Data shown in Table (3) represented the amount of ingested and digested mulberry leaves by grams to produced one gram of cocoons under the influence of each temperature. It was showed that larvae reared at 20°C required to ingest and digest about (14.2 and 9.44 gm, respectively) to produced one gram of cocoon.

Table (3): Effect of different constant temperatures on 5th larval instar conversion of ingested and digested food to one gram cocoon.

Temp. (°C)	Ingesta/Co	Digesta/Co
20	14.2 ± 0.63 a	9.44 ± 0.92 a
23	14.47 ± 0.94 a	9.64 ± 1.55 a
26	18.3 ± 2.24 b	14.35 ± 2.13 b
LSD 1%	3.91	4.15

Means in the column with the same letters are not significantly different

The obtained results are in accordance with those published by (Ueda and Suzuki 1967; Junliang and Xiaoffeng 1992) they reported that with the increase in temperature (20–30°C) leaf-silk conversion rate decrease. Muniraju *et al*. (1999) noticed that, low temperature throughout the rearing period favored higher silk conversion with better survival in bivoltine silkworm. However, even silkworm from the same genetic stock found to exhibit varied response when fed on the mulberry leaves of different nutritional quality, its growth being dependent on the efficient utilization and conversion of nutrition into silk substance as suggested by Hamano *et al*. (1986).

Among the total cost of cocoon production, more than 60% of contribution is associated with mulberry cultivation itself in sericulture industry. Therefore, silkworm breeds with lower ingesta and higher conversion efficiency of nutrition into cocoon and shell attracts the attention of the silkworm physiologist and breeder in evolving nutritionally efficient breeds. Narayana *et al*. (1985) demonstrated that, the nutritional indices parameters like ingesta, digesta, approximate digestibility, and reference ratio were superior under optimum temperature (23–25°C). In the present study, the best results were recorded under range of temperatures between 20 and 23°C and maximum excreta which recorded at this range indicate that temperature at 20°C was more efficient on biomass conversion of ingested and digested food. Similar results were reported by Gokulamma and Reddy (2005).

Some of the earlier studies addressed the selection of silkworm breeds in respect of thermotolerance by identifying thermotolerant silkworm breeds. However, a clear understanding of the genetic basis and variability in the expression of quantitative and qualitative genetic traits during exposure to different temperatures is an important step for the selection of potential thermotolerance parental resources for breeding programs.

The cocoon formation process is greatly influenced by fluctuating conditions of environment, particularly temperature and humidity (Ramachandra *et al*., 2001). It was observed that, 20°C significantly increased the cocoon weight (1.57 gm). While, in both cocoon shell weight and cocoon shell ratio, the increment was not significant (0.29 gm and 19.03) comparing with the other two temperatures. For reeling parameters 20°C recorded significantly the longest silk filament length which recorded 993.8 m and silk ratio was 49.19, while the number of filament breaks was the same as at 23°C (0.33).

Table (4): Effect of different constant temperatures on *B. mori* cocoon and silk filament parameters .

Temp (°C)	Co. wt (gm)	Co. Sh. Wt (gm)	Co. Sh. Ratio	Filament length (m)	filament breaks	Silk ratio
20	1.57±0.2 a	0.29±0.04 a	19.03±3.54 a	993.8±391.64 a	0.33±0.45 a	49.19±9.1 a
23	1.40±0.15 ab	0.26±0.05 a	18.62±3.12 a	717±23.52 ab	0.33±0.52 a	40.90±2.42 ab
26	1.22±0.13 b	0.28±0.02 a	20.47±1.99 a	450.3±180.2 b	4.83±1.32 b	32.49±7.66 b
LSD 5%	0.26	0.06	4.63	373.82	2.103	11.08

Means in the column with the same letters are not significantly different
Co Wt = Cocoon weight Co. Sh. Wt. = Cocoon shell weight
Co. Sh Ratio = Cocoon shell ratio

Gowda and Reddey (2006) concluded that, the adverse effect of high temperatures was higher on rearing and spinning of silkworm larvae than lower, which was clarified in the present study. High temperature affects nearly all biological processes including the rates of biochemical and physiological reactions ultimately affecting the quality and quantity of cocoon crops (Hazel, 1995).

The cocoon weight and reproductive characters were greatly influenced by different temperature regimes (Singh and Samson 1999). Results shown in Table (5) represented the pupation ratio under the influence of 20, 23 and 26°C. The highest pupal percentage was recorded at 23°C (77.78%), the longest pupal duration was recorded at 20°C (22.5), while the pupal weight was not significantly affected under the influence of the three temperatures. On the other hand fecundity and fertility were not significantly high at 23°C (541 and 399 egg/female).

Table (5): Effect of different constant temperatures on *B. mori* pupa and egg yield (Mean±SD).

Temp (°C)	Pupation %	Pupal duration (days)	Pupal wt. (gm)	Adult longevity (days)	Fecundity no. egg/ female	Fertility no. egg/ female
20	75.02±3.05 a	22.5±1.12 a	1.27±0.19 a	11.65±2.75 a	511±71.92 a	371±30.5 a
23	77.78±3.46 a	18.75±0.5 b	1.13±0.14 a	8.56±1.73 a	541±25.5 a	399±103.5 a
26	64±3.1 b	17.5±0.58 b	1.05±0.04 a	3.25±0.95 b	204±88.25 b	11±3.04 b
LSD5%	7.91	1.43	0.23	4	265	149

Means in the column with the same letters are not significantly different

The data in the present study disagree with Penzaman and Jagdeeshkumar (2010) as they concluded that good quality cocoons are produced within temperatures ranged between 22 and 27 °C and level above this range makes cocoon quality worse. However temperature at 20°C was slightly lower than recommended but was not significantly affect reproductive behavior and fecundity and cause less number of egg laying in silkworm, female moth capable of mate with more than one male which resulted in more egg laying and consequently fertile eggs.

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الكفاءة الغذائية وعلاقتها بإنتاجية فراشة الحرير التوتية تحت تأثير درجات حرارة مختلفه

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معهد بحوث وقاية النباتات – مركز البحوث الزراعية – مصر

تهدف كل التجارب المعملية على فراشة الحرير التوتية إلى تحسين مواصفات خيط الحرير و كذلك زيادة إنتاجية الشرانق و البيض .في هذا البحث تم تربية يرقات العمر الخامس في ثلاث درجات حرارة مختلفة هي ٢٠ ، ٢٣ ، ٢٦ درجة مئوية لدراسة نمو الحشرة و الكفاءة الغذائية و تسجيل مختلف القياسات البيولوجية و الإقتصادية . خلال فترة العمر اليرقي تم تسجيل وزن اليرقة - طول العمر اليرقي – معدل نمو اليرقة – كفاءة التربية و نسبة الموت و الإصابه . خلال فتره التعذير تم تسجيل وزن العذراء – طول فترة التعذير – نسبة التعذير . بالنسبة للحشرة البالغة فقد تم تسجيل عمر الفراشة – عدد البيض الكلي – عدد البيض المخصب . بالنسبة للشرنقة فقد تم تسجيل وزن الشرنقة - وزن غلاف الشرنقة - نسبة المحتوى الحريري للشرنقة . تم حساب كفاءة التغذية لليرقه من خلال حساب كمية الغذاء الذي ابتلعته اليرقة و الغذاء الذي هضمته و نسبة الهضم التقريبي . تم تسجيل طول خيط الحرير و عدد مرات القطع خلال عملية الحل.

أظهرت النتائج وجود اختلافات معنوية في أداء الحشرة تحت تأثير درجات الحرارة المختلفة . وجد أن اليرقات التي تربى عند درجة ٢٠ درجة مئوية تستهلك أعلى كمية غذاء مقارنة بالدرجتين ٢٣ و ٢٦ درجة مئوية لإنتاج جرام واحد من الشرانق . أحسن خيط حرير أنتجته اليرقه عند درجة ٢٠ مئوية . تسببت درجة حرارة ٢٠ درجة مئويه إلى إطالة عمر الفراش مما ساعد الفراشة الأنثى إلى التزاوج أكثر من مره مما ساعد على زيادة عدد البيض المخصب .