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COMPARATIVE GROWTH ANALYSIS: LENGTH, WEIGHT AND BREADTH RELATIONSHIP OF DEVELOPMENTAL INSTARS OF TASAR AND MULBERRY SILKWORMS (LEPIDOPTERA: BOMBYCOIDEA)

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ABSTRACT

The present paper deals with the weight-length-breadth relationships of different larval instars of mulberry *Bombyx mori* Linn. (Lepidoptera: Bombycidae) and tasar silkworms, *Antheraea mylitta* Drury (Lepidoptera: Saturniidae) for knowing the growth pattern and prediction of silk yield. It was observed that mulberry silkworm's attained maximum length and breadth in late 5th larval instar and values recorded were 65.50 mm and 5.388 mm respectively. In mulberry silkworms the maximum growth in breadth occurred in 3rd instar, whereas in tasar silkworm's maximum growth occurred in 4th instar larval stage. The regression analysis of weight-length-breadth relationship was worked out showing progressive growth pattern with direct correlation existing therein indicating favourable physiological machinery for silk yield.

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INTRODUCTION

Growth is useful integrated index of the physiological status of an animal, which is influenced by several physical and biological parameters. It may be affected by changes in food supply, ability to acquire food, or changes in behavioural and physiological status of the animals. The information on length, breadth and weight may serve as an index of the growth of animals. Studies on the weight, length and breadth relationships of the larval stages of silkworms may be a significant tool for their culture both in outdoor and indoor rearing programmes. Prediction of the potential silk yields and its quality are directly dependent upon pattern of growth of the silkworms (Gowda *et al*, 1989).

Considerable works have been done on the growth analysis of the silkworms (Mathur *et al*, 1989; Rajanna and Puttaraju, 2000; Sarkar and Fujita, 1994). The relationships of the weight, length and body composition was analysed by Kaufman and Bayers (1972) and Koilpillai (1995). The insect growth rate is related to the capacity of food intake and the growth at different leaf maturity (Gaberiel and Rapsuas, 1976).

Rajanna and Puttaraju (2000) gathered information on food utilization, growth and relative rate in the lines of silkworms, *Bombyx mori* Linn. Selected for pupal weight. Ueda *et al*. (1974), Ueda(1982), Prabhakaram *et.al*. (1995) stated the theory of the growth of silkworm larvae and its application. Practically no information is available on the comparative growth pattern of tasar and mulberry silkworms. The objectives of the present study is to know the growth pattern in terms of measurement of length-weight-breadth relationships of mulberry and tasar silkworms for prediction of potential silk yield and its quality. Considering such objectives, the present study on the length-weight-breadth relationships of mulberry and tasar silkworms has been undertaketn.

MATERIALS AND METHODS

The Disease free layings (DFLs) of silkworms were collected and the crops were harvested in the laboratory. In the experiment 10 males and 10 females larvae were used. The leaves were placed in such a way that the maximum portion of the edge of each leaf was available to the larvae for feeding. All selected larvae were kept under the laboratory conditions for measuring the size of length, breadth (diameter) and weight. The weight was taken with Electronic Balance Machine. The rearing was carried out under proper hygienic conditions to maintain the normal surrounding conditions. The young larvae 1st to 3rd instars were reared at 27-29°C with 85-90% relative humidity. The prediction of potential silk yield and its quality was determined. The larvae were fed tender leaves three times a day. The weight of silkworm larvae of each instar were recorded daily as well as the size of length and breadth were also measured in scale for growth analysis. The process was revised till last (5th) instars of larvae. Food eaten was determined by subtracting the weight of uneaten food from the weight of the food provided. Litter were separated from the uneaten food and weighed. The average weight of each instar of larvae were calculated as sum of the weight of beginning and the weight of the end of instars

and was divided by the duration of each instar. The calculated data has been analysed in allometric logarithmic regression scale. The rearing was done as suggested by Krisnaswami *et al*, (1973). and Jolly *et al*. (1979).

Allometry is the body size relationship which set the parameters for scaling relationship with measurement of size and biomass content. Scaling is important for leaf moisture both in palatability and assimilation of nutritive components.

Leaf Diet

Freshly plucked leaves/twigs containing a few leaves were washed with tap water and blotted before use as diet for the larvae.

Larvae

Larvae and excreta of every instar were collected for wet weight measurement. Freshly hatched larvae were utilized to start the study and newly molted larvae of every instar were taken for further observations.

Rearing of 5th instar larvae

Sex-wise assessment of larvae were done only in the 5th instar during which, identification of male and female larvae was easily possible in the basis of external features.

Environmental Conditions

Larvae become restless above 30^{o}C and inactive below 20^{o}C. The relative humidity requirement of early instars (I to III) is slightly higher (75-85%) than the late (IV and V) instars (60-70%). The optimum R.H. for moulting larvae was 40-50% as higher humidity renders the casting off of the old skin difficult (Jolly, *et al*., 1979).

Growth Parameters

Gain in body weight (G), duration of feeding(T), excreta(E), consumption index (CI), growth rate(GR), approximate digestibility (A.D.), efficiency of conversion of digested food (ECF), efficiency of conversion of ingested food(CI), fecundity, eggs retained, total egg production, coefficient of egg laying, hatching, cocoon weight, shell weight and silk ratio were calculated to test the difference among the sample means of both silkworm larvae.

Statistical analysis

Statistical methods as described by Pense and Sukhatma (1985) were followed for analysis of variance, regression and correlation of different nutritional and economic parameters.

Correlation analysis

$$r = \frac{cov\,(xy)}{v(x).v\,(y)}$$

Regression analysis

$$R = \frac{r_{12}^2 + r_{13}^2 - 2r_{12}\,r_{23}\,r_{13}}{1 - r_{12}^3}$$

where

V(x) = variance of x

V(y) = variance of y

x = Mean value of variable

y = Mean value of y variable

Co(x, y) = co-variance of x and y variable

r = correlation coefficient

R = Multiple correlation

where r_1, r_2, r_3 is the (mutual coefficient correlation)

$$b = \frac{\sum \log x.\log y - \sum \log x \sum \log y/_n)}{\sum \log x^2 - \sum \log x^2 /_n}$$

where

b = Regression coefficient, x and y are two variables

n = number of variables

$$b = \frac{1}{2}[\Sigma \log y - b\Sigma \log x]$$

$$a = \text{antilog}\frac{1}{2}[\Sigma \log y - b\Sigma \log x]$$

where

a = intercept of variables

Weight and length relationship formula

Isometric Growth $Y = a + b_x$

Allometric Growth $Y = aW^b$

$\log y = \log a + \text{blo } g^w$

where,

Y = growth parameters of silkworms

a = intercept

b = regression coefficient

W = weight of larvae.

RESULTS AND DISCUSSION

The comparative growth analysis of tasar and mulberry silkworms between weight, length and breadth from 1st to 5th instars have been depicted in Tables I_a – I_f and II_a –II_f respectively. The regression analysis between weight and breadth of larval instars 1st to 5th of *Antheraea mylitta* have been depicted through tables I_b to I_f respectively. The regression analysis of weight, breadth and length of mid value of *A. mylitta* was observed from 0.9540 to 9.8098 in 1st instar and 61.74 to 121.75 in 5th instar respectively, and the mid value of Y = 11.07 x-0.153 (R^2 = 1) to Y = 18.756 x -103.31 (R^2 = 1) to Y = 6.4908 x + 0.255 (R^2 = 0.9617) respectively. The regression analysis of weight and breadth of mid value of *A. mylitta* was observed from 0.46808 to 1.833 in 1st instar and 16.102. to 31.5 in 5th instar respectively and their mid value of Y = 1.7153 x +0.2893(R^2 = 1) to Y = 2.457 x -4.42 (R^2 = 0.9881)

Table Ia: Summation of Growth Analysis of *A.mylitta* in overall developmental period

Stage	Wt. Of Larvae (gm)	Breadth (mm)	Length (mm)
1st Instar	*0.50*	*1.22*	*6.00*
2nd Instar	3.9	3.06	16.80
3rd Instar	4.64	6.16	37.37
4th Instar	6.85	12.07	73..50
5th Instar	10.62	21.63	111.67

Multiple correlation 0.992

Table Ib: Statistical analysis of the summation of growth analysis of *A. mylitta* in 1st instar larvae

Stage	Wt. Of Larvae (gm)	Breadth (mm)	Length (mm)
1st Instar	0.50	1.22	6.00
St-Deviation	024	0.452	3
Correlation	1	0.998612829	1
Regg-X-line	0	-0.14033149	-0.1
Regg Y-line	0	0.27	1

Multiple correlation 0.9920

Table 1c: Statistical analysis of the summation of growth analysis of *A.mylitta* in 2nd instar larvae

Stage	Wt. Of Larvae (gm)	Breadth (mm)	Length (mm)
2nd Instar	3.9	3.06	16.80
St-Deviation	0.344	0.684	7.51
Correlation	1	0.992578	0.9922303
Regg-X-line	0	-0.11142	-0.087261
Regg Y-line	0	0.222843	1.032995

Multiple correlation 1.002

Table Id: Statistical analysis of the summation of growth analysis of *A. mylitta* in 3rd instar larvae

Stage	Wt. Of Larvae (gm)	Breadth (mm)	Length (mm)
3rd Instar	0.950	20.667	16.80
St-Deviation	0.7375	1.35	7.51
Correlation	1	0.992578	0.9922303
Regg-X-line	0	-0.11142	-0.087261
Regg Y-line	0	0.222843	1.032995

Multiple correlation 1.00

Table Ie: Statical analysis of he summation of growth analysis of *A.mylitta* in 4th instar larvae

Stage	Wt. Of Larvae (gm)	Breadth (mm)	Length (mm)
4th Instar	6.85	12.07	73.50
St-Deviation	0.77	2.752	33.325
Correlation	1	0.994635	0.9943743
Regg-X-line	0	-2.291661	-1.765455
Regg Y-line	0	5.87398	24.40966

Multiple correlation 1.0048

Table If: Statistical analysis of the summation of growth analysis of *A.mylitta* in 5th instar larvae

Stage	Wt. Of Larvae (gm)	Breadth (mm)	Length (mm)
5th Instar	10.62	21.63	111.67
St-Deviation	0.9	5.504167	50.525
Correlation	1	0.993715	0.9935551
Regg-X-line	0	-1.781868	-6.65239
Regg Y-line	0	4.03584	4389837

Multiple correlation 1.003

respectively. The value of Y was varied considerably (2.05x + 0.255 (R^2 = 0.9617) to Y = 4.811 x -26.241(R^2 = 1).

The regression analysis of weight, breadth and length of mid value of *B. mori* was recorded from 0.9642 to 4.845 in 1st instar and 29.037 to 67.992 in 5th instar respectively and the mid value of Y = 25.874x -0.3295 (R^2 = 1) to Y = 7.0032 x +46.148 (R^2 = 0.268) respectively The regression analysis of weight, length and breadth of mid value of *B.mori* was found from 0.0976 to 0.448 in 1st instar and 5.36 to 5.58 in 5th instar respectively, and the mid value of Y = 2.3358x – 0.0192 (R^2 = 1) to Y = 1.1942 x + 2.215 (R^2 = 0.4737) respectively. The value of Y varied also considerably 2.6 x -0.05 (R^2 = 0.0965) to Y = 0.0068 x + 5.35 (R^2 = 1).

The data obtained revealed that the rate of nutritional conversion showed a gradual increase in body growth reaching maximum weight (10.62 gms), breadth (21.63 mm) and length(111.67 mm) in *Antheraea mylitta* (2.731 gms) and maximum growth in weight, (5.3 mm), breadth (5.50 mm) length in *Bombyx mori* respectively. The average weight of 1st instar was (0.125 gms), length (2.875 mm) and diameter breadth (0.275 mm) respectively.

From the obtained results it showed that, the gain of the weight of 1st instar was significantly increased with 2nd, 3rd, 4th and 5th instars of larvae. The mulberry silkworm larva attained the maximum length and breadth in late 5th larval instar and values were 65.50 mm and 5.388 mm respectively. The length of 2nd, 3rd and 4th instars of mulberry larvae were ranging as 9.750 mm, 20.67 mm, 44.00 mm, 65.50 mm and their diameters were measured as 0.9750 mm, 1.650 mm, 3.300 mm, and 5.388 mm respectively.

The regression analysis of weight, length and breadth of mid value of *A. mylitta* recorded from 0.4608 to 1.833 in 1st instar and 16.102 to 31.5 in 5th instar respectively.

The regression analysis of weight, breadth and length of mid value of *B. mori* was raised from 0.9642 to 4.845 in 1st instar and 29.037 to 67.992 in 5th instar respectively. The value of regression analysis of weight, breadth and length of *A. mylitta* varied from 2 mm to 10 mm in 1st and 99 mm to 120 mm in 5th instar respectively. The regression analysis of weight, breadth and length of *B. mori* varied from 1 mm 5 mm in 1st and 58 mm to 70 mm in 5th instar and weight and breadth had 0.1mm to 0.5 mm in 1st and 4.5 mm to 6.0 mm in 5th instar respectively.

It was investigated that the reduction in leaf consumption by late instar (5th instar) larvae of tasar silkworm were recorded due to the maturation of silk gland and the spinning of silk thread. The gradual increase in body growth was recorded maximum (10.62 gms) in 5th instar larvae. The length and breadth was recorded as 111.67 mm and 21.63 mm in final instar larvae respectively.

Similar results were obtained in *B. mori* that the growth was increased during 1st instar. The initial weight gain in larva was calculated to be 0.05 gm with respect to length showing increasing trend 1.0 mm to 5.0 mm. The breadth was also varied 0.1 mm to 0.5 mm with respect to logarithmic regression 0.0976 to 0.448 mm respectively. The maximum growth in breadth occurred at 3rd instar because more consumption and digestibility of food supplements indicating congenial physiological state for silk biosynthesis in later developmental instars.

The breadth of 4th instar varied from 2.4 mm to 4.2 mm in comparison to logarithmic value 2.323 mm to 4.08 mm respectively. In this state there was maximum increase in weight and length that occurs with respect to all instars due to more consumption of food materials in 5th stage which indicate maximum silk yield in late 5th instar. The larvae stopped feeding at late 5th instar stage. As a result the metabolism and growth

Table IIa: Statistical analysis of the summation of growth analysis of *Bombyx mori* in overall developmental period.

Stage	Wt. Of Larvae (gm)	Length (mm)	Breadth (mm)
1st Instar	0.125	2.875	0.275
2nd Instar	0.450	9.750	0.925
3rd Instar	0.950	20.667	1.650
4th Instar	1.850	44.0	3.300
5th Instar	2.731	65..50	5.388

Multiple correlation 1.000

Table IIb: Statistical analysis of the summation of growth analysis of *Bombyx mori* in 1st instar larvae

Stage	Wt. Of Larvae (gm)	Length(mm)	Breadth (mm)
1st Instar	0.125	2.875	0.275
St-Deviation	0.05	1.5	0.1
Correlation	1	0.99591	0.982708
Regg-X-line	0	-0.019388	-0.022857
Regg-Y-line	0	0.5	0.0

Multiple correlation 0.73295

Table IIc: Statistical analysis of the summation of growth analysis of *Bombyx mori* in 2nd instar larvae

Stage	Wt. Of Larvae (gm)	Length (mm)	Breadth (mm)
2nd Instar	0.450	9.750	0.925
St-Deviation	0.1	4.65	0.35
Correlation	1	0.989778	0.989778
Regg-X-line	0	-0.11186	-0.08305
Regg-Y-line	0	2.1	0.16

Multiple correlation 0.84914

Table IId: Statistical analysis of the summation of growth analysis of *Bombyx mori* in 3rd instar larvae

Stage	Wt. Of Larvae (gm)	Length (mm)	Breadth (mm)
3rd Instar	0.950	20.667	1.65
St-Deviation	0.15	9.858333	0.35
Correlation	1	0.9937	0.988505
Regg-X-line	0	-0.364551	-0.04
Regg-Y-line	0	12.4476	0.102857

Multiple correlation 1.00

Table IIe: Statistical analysis of the summation of growth analysis of *Bombyx mori* in 4th instar larvae

Stage	Wt. Of Larvae (gm)	Length (mm)	Breadth (mm)
4th Instar	1.850	44.0	3.300
St-Deviation	0.22857	21.075	0.75
Correlation	1	0.988315	0.990474
Regg-X-line	0	-0.339552	-0.416071
Regg-Y-line	0	8.63918	0.8768

Multiple correlation 1.00

analysis had been shown at decreasing and retardation trend with respect to all instars of silkworm larvae. The larvae utilized the food materials already assimilated in the 4th instar stage.

In 5th instar the body growth of tasar silkworms was found 10.62 gm in weight, 111.67 mm in length and 21.63 mm in breadth and in mulberry silkworms these values were calculated as 2.73 gm, 65.5 mm and 5.38 mm respectively. The allometric growth of tasar silkworms was found 61.74 mm to 121 mm in length and 16.10 mm to 31.5 mm breadth respectively. As progressively in mulberry silkworms the allomeric length and breadth values were varied from 29.037 mm to 67.66 mm and 5.36 mm to 5.95 mm respectively.

Study of weight-length-breadth relationship is a significant tool in sericulture industry for knowing the potential yield and better silk performances both qualitatively and quantitatively for maximum silk production within stipulated time period. In the present study it was observed that in tasar silkworms in 4th instar larvae maximum accumulated growth achieved while in mulberry silkworms in 3rd instar larvae such trend was obtained. In these larval instars the silkworms assimilated maximum food in their bodies. It was due to maximum consumption of food and leaf conversion efficiency into protoplasm of these larvae. The data obtained indicated that in late 5th instar larvae the growth declined due to set in of the silk spinning process.

A silkworm multiplies its body weight about 10,000 times and weight of silk gland 14000 times during their life cycle. The accumulation of biomass and attainment of maximum body size is a pre-requisite for successful metamorphosis in insects Paul, 1992 and Sarkar *et al*, 2009. Larval weight gained improved significantly by the application of soft tender mulberry leaves and arjun leaves. The growth is affected by the efficiency of conversion of ingested food which is an overall measure of the ability of larvae to utilize the ingested food (Rajanna and Puttaraju, 2000). Kumari (2013) has studied the growth pattern in weight-length-breadth in mulberry and tasar silkworms and calculated their energy budgets. Kumari and Roy (2011a,b) studied the identification of nutritionally efficient silkworms as well as impact of environmental factors on the growth and sustainable development of these silkworms.

The calculated finding stated that the relation between length, breadth and body growth gives higher degree of positive significant correlation. The multiple correlations also give the positive correlation to each other. The coefficient of regression of weight, length and breadth in linear method was not constant. In this study the allometric relationship of length, weight and breadth across all developmental stages would reflect the convention scaling coefficient. The increased larval growth in 4th instar tasar and 3rd instar in mulberry silkworms had shown that there was high food consumption and metabolic activities in silkworms in these stages and decreased larval growth in 5th instar due to non-feeding in period. Hence, less consumption affect the metabolism and physiology that would significantly reduced larval growth and development as evident in the reduction efficiency of conversion of ingested food to larval biomass. The growth of silkworm is dependent on the quality and quantum of feed they have been taken and consideration of the balance between anabolic and catabolic reactions fuelled by the nutritive substance absorbed after digestion of food (Sinha *et al*., 2000, Meenal and Ninagi, 1995; Harsh Kumar and Thakur, 1999).

Environmental factors specially temperature (24.2 + 2 to 28 + 2°C), humidity (74 + 2 - 88 + 2%), photoperiod (100 – 242 hours/month^{-1}) and day length (11.00 - 12.50 hours) are essential physical regime. For healthy growth of silkworm photoperiod played a role to determine optimum time regime

Table IIf: Statistical analysis of the summation of growth analysis of *Bombyx mori* in 5th instar larvae

Stage	Wt. Of Larvae (gm)	Length (mm)	Breadth (mm)
5th Instar	2.731	65.50	5.388
St-Deviation	0.25625	29.79688	1.365625
Correlation	1	0.07333	0.688261
Regg-X-line	0	2.87275	0.519154
Regg-Y-line	0	66.35698	2.215462

Multiple correlation 0.9968

during a day cycle for moth emergence, coupling, eggs laying and hatching specially in tasar silkworms (Nagat and Nagaswa, 2006; Shiv Kumar *et al.,* 1997; Ueda *et al.*, 1969 and 1971). Joshi (1995) had provided a growth index for Eri silkworms, *Philosamia ricini* Hutt. Similarly in the present study regression analysis of weight-length-breadth relationships showing positive growth pattern with direct correlation existing therein indicating favourable physiological machineries for silk yield.

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