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Growth And Dietary Efficiency of Artificial Nutrients Fortification of *Bombyx Mori* (L).

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Abstract:
Sericulture has been serving the humanity by providing natural animal silk for several centuries. The quality as well as the quantity of mulberry leaves along with environmental factors have a direct influence on the production of raw silk spun by *Bombyx mori* L. Larvae before pupation in the form of cocoons. Study on the nutritional aspect of the silkworm is an essential prerequisite for its proper commercial exploitation. Silkworm nutrition is the sole factor which has almost individually augmented the quality and quantity of the silkworm cocoon production and productivity. Despite the balancing of the silkworm nutrients by the mulberry leaf, the quantity available is not sufficient for the larval growth owing to variation in mulberry plant and in its management. This created a requirement for the dietary fortification with good quality mulberry leaves in optimum quantity for successful cocoon production. In this study, mulberry leaves were fortified with Honey, Glucose, B- complex, Lemon and Calciferol and the growth and dietary efficiency of the larvae fed with the fortified diet were evaluated. The results indicated improvement in terms of growth as well as cocoon parameters. Our studies support the concept of dietary fortification the Mulberry leaves fed to silkworm for netter production of silk.
Keywords: Honey, Glucose, B- complex, Lemon, Calciferol, Fortification.

1 Introduction:

The most common employed silkworm called the *Bombyx mori L.* Is considered as a domesticated worm which is also monophagous and an economically important insect. It is a member of the order Lepidoptera and family *Bombycidae*. The silkworm has been known to the humans for about thousands of years owing to the fabrication of natural silk along with its significant role in the rural agro-industry in the subtropical as well as the tropical regions of the world [1-2]. The naturally produced Silk is composed of two proteins called the sericin and the fibroin. These are proteins are produced by the silkworms in their foremost silk gland [3] and are then ejected by the spinnerets during the final stage of the larval development called as the 5th instar stage [4-5]. The primary food source of the domesticated moth are the fresh mulberry leaves that serve as reservoirs of carbohydrates, lipids, proteins, moisture and inorganic matter [6-8]. The development of the silkworm is often related to the cultivation of

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the mulberry tree. The most important factor is that the quality of the mulberry leaf affects the normal development and growth of the larvae [9]. The availability of mulberry leaf of high nutritional quality, under temperate climatic conditions, is restricted to the spring-summer season thereby serving as a limiting factor in the selection of *Bombyx mori L.*

The dietary efficiency of silkworm is considered as a crucial factor for the conversion of the feed in to the produced silk of commercial importance. The concept of fortification of the mulberry leaves is a sophisticated technique that increases the nutritional value of the food making it more useful for the improvement of the silkworm health, cocoon and silk quality [10]. The highly nutritious mulberry leaves make the silkworms more resistant to the ailments as well as enable them to enrich the silk productivity [11]. The fed on artificial diets can possess great potential in sericulture. Several research has been done on the dietary supplementation of the mulberry leaves fed to silkworms for the improvisations in the commercial characters of the silkworm including the vitamins, sugars, proteins, amino acids and minerals etc. that are responsible for the proper growth and development of the silkworm, *B. mori.* The vitamins such as ascorbic acid, thiamin, niacin, folic acid and multi-vitamins were studied by Etebari 2002 and Etebari et al., 2004 and it was reported that the vitamins of B-complex group and certain essential sugars, proteins, amino acids, minerals etc. ensured the proper growth and development of the silkworm, *B. mor.* [12-13]

Earlier studies have reported that nutrition of silkworm is a factor that influences the quantity and high-quality of silk [14]. The impact of distinctive dietary supplements on the silkworm have been extensively studied and it has been reported that better volume and high-quality of the silk production can be done through the fortification of mulberry leaves with additional nutrients [15-17].

Honey is considered as a profitable supplementary diet. An earlier study has so far reported that when mulberry leaves treated with 2% aqueous honey were offered to fifth instar larvae, there was a modulation of the larval growth and metabolism with reduced sericulture waste and increased silk production and quality [18-19]. Honey serves as a cost effective and easily accessible food additive that not just stimulates the silk protein synthesis in the silk gland but is also involved in mobilizing the protein reserves from the body and in improving the economic parameter of sericulture [18-19].

Vitamin B6 known as Pyridoxine is a water-soluble vitamin involved in the stimulation of the growth in silkworm. Vitamin B6 is an important in the protein metabolism of silkworm. Faruki (2005) in his study reported the effects of vitamin B6, he observed an increased reproductively potential in the *Bombyx mori L*. But the results showed that all concentrations of the vitamin had significantly reduced the fecundity and egg-viability of the silkworm [20].

The L-ascorbic acid (vitamin C) is considered indispensable for the growth and development of Bombyx mori. In fact, the ascorbic acid is present naturally in large amounts in the mulberry leaves and insects are incapable of synthesizing it. The drying of mulberry leaves causes degradation of the vitamin C and due to this the leaves cannot be relied as a source of ascorbic acid. Vitamin C is usually added to silkworm food generally varying from 1–2% of the dry weight of the artificial diet and this is considered as an optimum content of this vitamin [21].

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It is a well-known fact that the *Bombyx mori* biological parameters are determined by the nutritional value of the mulberry leaves. In this direction, we have studied the impact of silkworm diet enriched with Honey, Glucose, Lemon, B- complex and Calciferol.

2 Material Methods:

The model organisms selected for this study was the mulberry silkworm, *Bombyx mori L* which belongs to the order Lepidoptera, class Insecta and Family *Bombicidae*. The race chosen was a hybrid of ($CSR_2 \times CSR_4$) Mulberry silkworm which feeds mainly on the mulberry leaves. It is a holometabolous insect whose life cycle has four distinct stages namely the egg, larva, pupa and adult.

2.1 Rearing of silkworm:

Eggs of *B. mori* ($CSR_2 \times CSR_4$) were obtained from the grainage of the regional sericulture centre (V.M Chatram). The larvae were reared by the tray rearing method following the method described in krishanaswami *et al.,* (1970) [22]. In this method, the tray were lined with newspaper, required temperature and humidity were maintained and mulberry was used as feed for the worms. The fresh healthy leaves were collected during the cool hours of the day and stored in wet gunny bugs. The leaves were then chopped and fed to the early larval instars. The larvae were fed 5 times a day; the beds were cleaned every day and sufficient spacing was adapted during rearing. This typical insect has different stages in its life cycle and represents the most advanced form of metamorphosis. The complete life cycle through the serial progression with four distinct stages of development such as the Egg, larva, pupa, adult (Moth).

2.2 Experimental studies

Newly molted third instars larvae were fed with mulberry leaves treated with different treatment such as the Organic and inorganic fertilizer of growth. After 24 hours, unfed leaves and fecal pellets were removed and fed with fresh mulberry leaves with least disturbance to the larvae and the collected fecal pellets and uneaten leaves were dried separately at 50 - 60 °C for gravimetric calculation purposes. Care was taken to separate fine unfed food particles from the fecal pellets. Dead larvae were removed up to the third and fourth in stars every 24 hours. Trays were covered with wet muslin cloth. Each experiment was replicated 5 times with 10 larvae for each replication. The dried leaves were weighed in monopan (Dhona160D) and weight was recorded in order to find out the energy budget parameters. The larvae were periodically measured to find the growth parameter.

2.3 Growth parameter analysis

The survival rate, pupation rate, cocoon shell ratio, egg hatchability and relative growth rate were analyzed through given formula. The initial and final larval weight was noted to determine the survival rate (Formula 1.) of larvae with the impact of fly ash incorporated leaves.

$$\text{Survival rate (\%)} = \frac{\text{No. of larvae survived}}{\text{No. of larvae dead}} \times 100$$

Similar to larval survival, pupation rate was also calculated with the respective formula 2. At the end of fifth instar stage, the silk worm starts spinning in cocoon. The cocoons of various treatments were collected and incubated separately. Fully developed cocoons from

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various treatments were taken and the shell was cut down to find the shell weight and the pupa were weighed in monopan electric balance (Dhona, 160 D).

$$\text{Pupation rate (\%)} = \frac{\text{No. of larvae pupated}}{\text{No. of larvae introduced}} \times 100$$

The cocoon weight, pupal weight and shell weight were taken separately and the cocoon Shell ratio was calculated by the formula 3 (Sakaguchi, 1978) [23].

$$\text{Cocoon shell ratio (\%)} = \frac{\text{Shell weight}}{\text{Cocoon weight}} \times 100$$

Fecundity assay was determined by the silk moths emerging from the treated larvae which were reared with normal adults of the opposite sex from the laboratory cultures. They were observed for the number of progenies produced when pairs were restricted in sleeve containers by providing detained castor leaves for oviposition. Calculation was done using the given formula 4.

$$Fecundity\ (\%) = \frac{No.\ of\ eggs\ laid\ by\ treated\ adults}{No.\ of\ eggs\ laid\ by\ control\ adults} \times 100$$

In addition, egg hatchability assay (Formula 5.) were determined by the eggs laid by the adults that emerged from different treatments of organic and inorganic fed treated larvae and compared their hatchability rate with egg hatchability ratio of adults.

$$\text{Egg hatchabilty (\%)} = \frac{\text{No. of eggs hatched in treatment}}{\text{No. of eggs hatched in control}} \times 100$$

2.4 Relative growth rate

The relative growth rate (RGR) of mulberry silkworm was calculated at 24, 48, 72, 96 and 120 h by the following formula 6 given by Isikiber and Copland (2002) [24].

$$\text{RGR} = \frac{\text{Final weight of insect} - \text{Initial weight of insect}}{(\text{Final weight of insect} + \text{Initial weight of insect})/2} \times \text{Days}$$

2.5 Gravimetry

The fourth and fifth instar larvae utilization for oral treatment of organic and inorganic plant leaves were done by weighing their fecal pellets separately. All weighing were analyzed using a monopan electronic balance with an accuracy of 0.001 mg. The accuracy of balance was checked for every 20 weighing. The following formulas were used to analyze the different parameters.

$$\text{Growth (mg)} = \text{Final weight} - \text{Initial weight}$$

$$Growth\ rate\ (mg) = \frac{Growth\ (mg) \times 100}{\text{Initial wet weight} \times \text{Duration}}$$

$$\text{Food consume rate (\%)} = \frac{Dray\ food\ counsume\ (mg)}{\text{Wet weight gain (mg)} - \text{Animal weight (mg)}}$$

$$Conversion\ rate (\%) = \frac{Dryfood\ counsume\ (mg)}{\text{wet animal weight}} \times 100$$

$$Specific\ growth\ rate\ (\%) = \frac{Inl2 - Inl1}{\text{Experimental duration (day)}} \times 100$$

Where,

In-Natural log; I_2-Final live weight; I_1-Initial live weight

In majority of insects, the quantity of uric acid (U) excreted in the feces is negligible and amounts to 0.2 to 0.5 percent of the total quantity of feces egested. The food consumption

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of the fifth instars larvae was calculated by adding daily consumption of the larva. The mean fecal weight/ larva were also calculated similarly. The above quantitative estimation of C was made in terms of joules. Food energy converted into body substance (P=Growth) was estimated as the difference between the energy content of the test larvae at the beginning and at the end of fifth instar. Food assimilated (A) was calculated by subtracting the mean fecal energy from that of food energy consumed.

$$\text{Consumption} = \text{P} + \text{R} + \text{F} + \text{U}$$

Where,

C - Food energy consumed; P – Growth; R - Energy released as heat dud to metabolism

F+U – Loss of energy through feces and nitrogenous excretory products.

$$\text{Assimilation (mg)} = \text{c} - (\text{f} + \text{u})$$

Rates of consumption, Assimilation and production were calculated by dividing the respective amounts of energy by the product of mid body weight (g) of the worms and the duration (days) required for the completion of the fifth instar the rates were expressed in terms of mg live larva/day. Efficiencies of conversion of ingested matter to body substance (ECI) and digested matter to body substances (ECD) were calculated relating P to C and A separately and expressed as percentage.

$$Conversion\ rate(\%) = \frac{Dry\ animal\ weight\ (mg)}{\text{Wet animal weight (mg)}} \times 100$$

2.6 Pupal stages

At the end of the fifth instar on sixth or seventh day the silk worm starts spinning in cocoon. The pupal or chrysalis period may last for 8-14 days formed within the cocoon. Ideal conditions of temperature (23-25 ± 1 °C) and humidity (34-83%) were maintained to form pupa. The sixth- or seventh-days larvae crawls over the Netrika and spins the cocoon. The cocoons of various concentrations were collected and incubated separately.

3. Results And Discussions:

Overall, the highest initial growth was seen in glucose with 2.72±0.95 mg at a concentration of 5, the highest final growth was seen in Honey with 8.46±2.17mg at a concentration of 2.5. The highest relative growth rate was seen in B-Complex with 275.48±47.12 at a concentration 2.5. The highest food consumed was shown in B- complex at a concentration of 2.5 with 826.17±41.35 mg. The highest food consummation was 6.62±3.07% at a 7.5 concentration of B-complex. The highest assimilation was 68.12±49.76% at a 12.5 concentration of B-complex. The highest co-efficiency of utilization was 84.722±2.280% at 2.5 concertation of calciferol. The growth obtained in the study has been tabulated in table 1.

Overall, the highest cocoon sheet ratio was 16.329±0.714 at 2.5 concentration of lemon. The highest pupal weight was 95.10±2.16 recorded at 2.5 concentration of B- complex. The highest moth was 94.62±1.68 at concentration 5 of calciferol. The highest egg laying moth was 402.13±40.66 at 5 concentration of calciferol. The highest total egg laying was 982.00±244.52 at 2.5 concentration of honey. The highest hatchability was 92.64±11.66 at 2.5 concentration of lemon. The cocoon parameters obtained in the study has been tabulated in table 2.

The nutritional intake of the silkworm has a direct impact on the overall genetic traits such as the larval and cocoon weight, amount of silk production, pupation and reproductive traits [25]. The efficient conversion of the ingested mulberry leaves into silk is considered as a better economic index in the cocoon production [26]. In a study by Magadum et al. (1996), the results reported that the total digesta increases with an increased ingesta [27].

In our study, the highest initial growth was seen in glucose with 2.72±0.95 mg at a concentration of 5 but this is in disagreement to the study by Ito.,1960. In their study, Glucose was a very weak stimulant for the silkworm and sucrose had a positive impact. The larva in their study was stimulated to feed by sucrose even when no leaf powder was added to the diet [21]. The highest final growth was seen in Honey with 8.46±2.17mg at a concentration of 2.5. This is in agreement to the study by Bhatti et al,.2019. In their study 2% aqueous honey has positive impact on the larval growth and cocoon yield of *B. mori* [18]. Our study is also in agreement to several other studies [28-30].

The highest pupal weight was 95.10±2.16 recorded at 2.5 concentration of B- complex. The highest relative growth rate was seen in B-Complex with 275.48±47.12 at a concentration 2.5. The highest food consumed was shown in B- complex at a concentration of 2.5 with 826.17±41.35 mg. The highest food consummation was 6.62±3.07% at a 7.5 concentration of B-complex. The highest assimilation was 68.12±49.76% at a 12.5 concentration of B-complex. This is agreement to the study by Ibrahim et al., 2022. The results of this study indicated that larvae treated with Melatonin and Vitamin B6 gave the highest parameters and improvement in larval weight, cocoon weight, cocoon shell and pupal weight [31].

The highest co-efficiency of utilization was 84.722±2.280% at 2.5 concertation of calciferol. The highest moth was 94.62±1.68 at concentration 5 of calciferol. The highest egg laying moth was 402.13±40.66 at 5 concentration of calciferol. This is in agreement to the study by Akram et al., 2017. In their study, the impact of different levels of the Vitamin D3 supplementation was evaluated by rearing silkworm larvae under controlled conditions during last three larval instars and the results pertaining to Cocoon Weight, Cocoon Shell Weight and Cocoon Shell Percentage also showed positive trends when reared on supplemented food [32].

Lemon juice is considered as a natural repository of vitamins and minerals with the potential to stimulate fibroin synthesis in the silkworm and also enhances growth rate and metabolism in silkworm. In our study the highest cocoon sheet ratio was 16.329±0.714 at 2.5 concentration of lemon and the highest hatchability was 92.64±11.66 at 2.5 concentration of lemon. Our findings are in agreement to the several studies [13,33].

4. Conclusions:

Nutrition plays a very crucial role in the growth and development of Silkworm. As sericulture poses a huge demand for increased quantity of good quality mulberry leaves, the application of dietary fortification can help in coping up with the demand for high quantities of mulberry leaves. Our study showed significant improvements in the growth and cocoon parameters by showing the highest initial growth with glucose, the highest final growth with Honey, highest relative growth rate, highest food consumed as well as assimilation and highest pupal weight with B-Complex, the highest co-efficiency of utilization with calciferol, the highest cocoon sheet ratio and hatchability with lemon. Our results support the concept of dietary fortification for sericulture.

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Samples	Concentration	Growth (mg)		RGR(relative growth rate)	Food consumed (mg)	Food consummation (%)	Assimilation	Co-efficiency of utilization (%)
		Initial	Final					
Honey	2.5	1.64±0.96	8.46±2.17	207.30±21.11	846.20±57.12	5.36±2.71	52.18±20.17	72.317±2.11
	5	1.48±0.92	7.08±1.92	208.64±26.16	756.12±49.17	5.78±2.96	49.76±29.60	69.814±1.690
	7.5	1.36±0.84	6.42±2.62	176.36±19.42	690.46±40.27	4.77±1.96	52.48±30.17	67.664±2.431
	10	1.76±0.77	6.72±3.08	19.6±29.20	756.25±39.12	5.46±1.72	48.25±29.17	70.664±2.431
	12.5	1.65±0.69	5.67±2.46	176.47±20.72	650.17±42.20	4.20±1.98	52.17±30.17	67.134±2.704
Lemon	2.5	1.25±0.28	8.12±2.46	196.22±19.40	756.20±40.17	5.10±2.46	55.46±29.17	65.592±3.584
	5	0.76±0.88	6.17±2.13	172.35±20.19	652.77±40.17	5.88±2.92	48.17±25.26	69.830±1.584
	7.5	1.62±0.17	9.18±2.46	205.46±21.32	690.29±35.72	5.17±2.36	52.25±30.25	70.932±2.305
	10	1.88±0.93	8.76±2.17	209.15±20.73	710.17±40.96	4.96±2.10	56.32±46.17	67.128±2.704
	12.5	1.69±0.78	6.88±2.46	196.22±17.25	690.26±35.48	4.72±2.96	55.32±20.17	71.162±2.105
Glucose	2.5	1.62±0.93	5.46±2.76	170.17±20.25	612.76±39.70	5.10±2.46	48.17±35.12	68.644±2.341
	5	2.72±0.95	3.21±2.96	198.46±17.69	590.47±40.22	5.22±2.22	55.22±20.17	70.934±2.304
	7.5	1.56±0.29	6.52±2.78	201.17±46.32	670.22±40.96	5.17±2.46	48.35±20.17	68.742±2.350
	10	1.77±0.88	7.09±2.56	179.23±35.74	721.17±40.98	5.62±2.17	48.17±30.12	67.128±2.704
	12.5	1.96±0.25	4.72±2.02	169.32±31.68	657.48±39.27	4.98±2.66	52.17±25.18	71.142±2.105
calciferol	2.5	1.46±0.36	6.47±2.16	205.46±42.13	460.32±40.17	5.76±2.46	32.25±20.19	84.722±2.280
	5	1.52±0.76	5.72±2.78	222.35±40.76	692.73±50.17	5.21±2.86	48.17±20.19	80.841±2.680
	7.5	1.72±0.48	6.94±2.17	258.52±30.98	690.77±49.56	5.50±2.64	35.17±30.12	82.644±2.331

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	10	1.64±0.72	4.02±2.79	215.65±40.35	721.66±42.39	5.17±2.84	48.19±29.12	76.162±2.704
	12.5	1.32±0.96	2.65±1.66	217.30±46.17	686.25±39.17	5.20±2.72	30.95±28.12	78.83±2.5
B-complex	2.5	1.79±0.84	7.86±3.25	275.48±47.12	826.17±41.35	5.78±2.46	60.22±48.19	78.925±2.304
	5	1.64±0.54	6.92±2.70	262.35±31.22	777.35±40.79	5.66±2.17	59.76±42.56	72.835±2.570
	7.5	1.80±0.43	6.88±3.12	219.72±40.68	810.25±40.17	6.62±3.07	61.20±52.12	76.915±2.342
	10	1.84±0.42	6.46±3.09	220.32±40.99	790.66±39.17	5.89±2.98	59.17±50.25	75.693±2.374
	12.5	1.86±	6.12±3.46	215.75±40.62	678.36±41.17	5.36±2.88	68.12±49.76	74.162±2.104

Table 1: Growth of different nutrients fed in silkworm growth parameters studies.

	concentration treatment	Cocoon sheet ratio	Pupal weight	moth	Egg laying moth	Total egg laying	hatchability
Honey	0	15.046±2.253	86.12±1.06	87.22±0.128	149.20±33.10	746.11±421.16	77.00±12.82
	2.5	14.063±2.714	88.32±2.15	88.46±0.76	160.75±20.46	982.00±244.52	86.38±15.22
	5	15.763±2.246	67.18±1.62	91.05±0.26	199.22±54.16	486.12±274.22	69.50±16.40
	7.5	16.317±0.650	83.21±1.68	66.22±0.48	232.48±29.42	652.56±429.48	69.22±15.22
	10	14.312±0.329	78.16±4.18	88.48±0.36	220.33±20.94	278.42±262.12	72.56±12.26
Glucose	0	16.320±0.740	96.22±4.22	85.28±4.66	253.62±41.22	577.93±161.22	87.67±14.01
	2.5	14.318±0.319	78.20±6.12	79.62±2.72	310.16±28.32	594.53±121.46	86.48±14.02
	5	15.046±2.223	70.12±1.62	92.32±2.48	272.41±30.18	480.38±122.66	90.75±13.45
	7.5	15.043±1.526	89.18±1.42	74.26±0.98	286.12±52.22	927.36±120.14	88.37±11.92
	10	15.026±0.507	93.16±1.62	78.22±0.69	268.72±11.48	263.15±132.12	88.95±11.06

Lemon	0	15.046±0.526	90.75±3.62	86.52±0.26	210.42±52.42	656.52±120.16	87.78±0.41
	2.5	16.329±0.714	93.22±4.62	85.63±0.512	310.62±24.18	774.12±146.25	92.64±11.66
	5	15.076±2.826	75.20±4.12	85.48±0.18	270.62±46.18	588.16±152.46	81.96±19.45
	7.5	14.063±2.819	67.28±4.18	79.32±0.64	256.22±50.30	457.44±126.32	88.70±17.17
	10	15.046±0.607	60.22±1.15	85.42±5.34	285.42±11.46	661.49±22.68	86.60±11.18
B-Complex	0	15.046±2.223	93.16±1.28	88.62±5.120	239.11±32.74	526±122.46	80.14±15.32
	2.5	15.043±1.526	95.10±2.16	84.16±1.62	258.14±20.72	781.62±145.55	79.70±14.22
	5	14.042±0.240	93.22±4.61	92.48±1.66	284.12±12.32	873.33±232.17	72.48±22.06
	7.5	16.320±0.743	60.18±2.17	85.32±1.25	246.42±41.39	621±133.12	75.22±13.35
	10	14.053±2.226	78.22±1.69	8.36±42.18	263.17±19.48	397±142.18	74.66±14.28
Calciferol	0	14.063±2.817	70.12±1.38	74.16±0.62	346.67±19.72	395.14±212.30	83.62±19.22
	2.5	15.763±0.518	76.16±1.22	62.08±2.48	386.17±10.45	826.12±155.22	88.72±16.42
	5	14.056±0.260	86.18±1.62	94.62±1.68	402.13±40.66	480.38±127.12	85.28±13.22
	7.5	15.729±2.273	77.14±2.32	69.22±0.48	372.68±41.17	626.48±132.56	89.66±22.14
	10	15.073±2.192	72.18±1.35	92.10±1.62	352.26±23.62	776.22±120.36	92.61±15.22

Table 2: Cocoon parameters analysis in different nutrients fed silk worm.

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