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Effect of nutritional supplements treated *Quercus serrata* leaves on life cycle and economic traits of oak tasar silkworm (*Antheraea proylei*)

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Abstract
The experiment entitled "Effect of nutritional supplements treated *Quercus serrata* leaves on life cycle and economic traits of Oak tasar silkworm *(Antheraea proylei*)" was conducted during April 2024 to June 2024 at experiential site, Central Research and Training Institute, Chauntra, district Mandi, Himachal Pradesh with the objectives *viz.*, to study the effect of nutritional supplements treated *Q. serrata* leaves on life cycle, growth parameters and economic traits of oak tasar silkworm (*A. proylei)*. Three types of artificial diets; Artificial diet$_1$ (vitamin C - complex @ 1%), Artificial diet$_2$ (protein @ 1%), Artificial diet$_3$ (vitamin B - complex @ 1%) and fourth was the control (natural diet) were used for rearing of Oak tasar silkworm *(A. cnproylei*). Formulated diets were compared with natural diet and the observations on life cycle, growth parameters and economic traits were recorded. The study revealed that amongst the three formulated artificial diets, artificial diet$_2$ (Q. *serrata* leaves treated with protein @ 1%) was found to be promising for all the five larval stages of silkworm and selected for further studies. Life cycle parameter of instar like larval duration (46.44 days), pupal duration (12.75 days), moth emergence (94.11%) and growth parameters like larval length (87.84 mm), larval breadth (188.26 mm), larval weight (21.28 g), cocoon weight(6.99 g) as well as economic traits like silk filament length (1164.28m) were significantly maximum in T_2 treatment (*Q. serrata* leaves treated with protein @ 1%) followed by T_1treatment (vitamin C - complex @ 1%) and T_3 treatment (vitamin B - complex @ 1%). The lowest larval duration, pupal duration, larval length and breadth in fifth instar larvae ((39.16 days, 9.20 days, 84.22 mm and 18.56 mm, respectively) was recorded in untreated control. Lowest larval weight (17.08 g) and silk filament length (891.78 m) in fifth instar was also observed in larvae feeding on untreated *Q. serrata* leaves.

Keywords: Artificial diet, *Quercus serrata*, *Antheraea proylei*

Introduction
Sericulture encompasses the rearing of silkworms and the production of silk known for its unparalleled elegance, natural sheen and various desirable qualities like high absorbance, light weight and durability [7]. Silk often referred to as the "Queen of Textiles" is composed of two proteins: sericin and fibroin with fibroin being the structural core and sericin the surrounding adhesive material [14]. Most insects including lepidopteron are phytophagous in feeding and the degree of specificity to particular host plants varies considerably [8]. Oak tasar silkworm (*Antheraea proylei* Jolly) is a polyphytohagous insects thriving on a number of oak plants through species specificity varied from region to region and altitude to altitude because of availability of a specific species of food plant at a particular altitude range only [6,21,15]. The Indian Oak Tasar Silkworm (*Antheraea proylei* J.) is a beneficial insect with great economic importance in India for its silk production. India holds a significant position in the global production of Oak Tasar silk primarily through the commercial exploitation. *A. proylei* culture serves as a crucial economic activity particularly benefiting marginalized communities inhabiting the oak belt of the sub-Himalayan region spanning states such as Manipur, Nagaland, Assam, Mizoram, Meghalaya, Arunachal Pradesh in the North-East, Jammu & Kashmir, Himachal Pradesh and Uttarakhand in the North-West Himalayan region. Policy makers highlight sericulture as key avenue for socio-economic development in countries like India [7]. Singh emphasized that the growth and development of biological organisms including silkworms are greatly influenced by the type of food they consume.

He highlighted the significance of oak as a food source for silkworms as it plays a crucial role in their growth, development, cocoon yield and reproduction. Insects feeding on nutritionally enriched leaves directly impact the growth and development of silkworm larvae as well as the quality and quantity of silk production. Proteins are fundamental substances in the body that play a vital role in cellular structure and survival [10]. Their ability to combine with other active substances such as enzymes and hormones allows them to regulate metabolism and physiological functions in silkworm. Approximately 70% of the silk proteins synthesized by silkworms are derived directly from the protein present in mulberry leaves [3]. Adequate protein intake is essential for the development of ovaries and eggs in adult female silkworms as it is necessary for the secretion of juvenile hormones. In contrast, male silkworms typically do not require protein to mature their sperm upon reaching adulthood. Optimal protein nutritional requirements vary by age, sex, physiological status and stress [17,2]. The growth and development of silkworm primarily depend on the intake of vitamin B complex and vitamin C complex. Vitamins are organic compounds that predominantly serve as enzyme co-factors and catalysts. They regulate cellular metabolism and physiological [23].

Materials and Methods

The present investigations were conducted during spring season (March-June) in 2024 to study the effect of nutritional supplements treated *Quercus serrata* leaves on life cycle and economic traits of oak tasar silkworm (*Antheraea proylei)*. The experiment was conducted at Central Tasar Research and Training Insititute, Chauntra, District Mandi (H.P.). The experiment was performed in a completely Randomized Design with four treatment and four replications. Each treatment consisted of 80 freshly hatched larvae of *A. proylei* divided into four replications (each replication contains 20 larvae per treatment).

Materials

The nutritional supplement was fed to tasar silkworm larvae (first, second, third, fourth and fifth instar). The equipments used during the rearing programme are rearing trays, rearing stand, chopping knife, brush, paraffin paper, bamboo stick, cleaning nets, collapsible plastic mountages and digital electronic balance.

Rearing Methods

The eggs of oak tasar silkworm were obtained from Central Tasar Research & Training Institute Chauntra, Distt. Mandi (H.P.). The eggs of tasar silkworm were kept in black box for uniform hatching. A thermo-hygorghaph was set up in the lab to track the ambient temperature and relative humidity. Rearing equipments were thoroughly disinfected with 2 percent formalin solution according to recommended schedules. Larval rearing was carried out under hygienic conditions throughout the period of rearing. The Environmental factors *viz.*, optimum temperature range (22-28 °C), relative humidity of 60-90 percent and the length of time of light (14-16 hours) and darkness (8-10 hours) were all maintained in the laboratory in accordance with the requirements for every larval instar as suggested by [13].

Plate 1: Rearing of oak tasar silkworm

After hatching of the eggs, first larval stage were brushed per treatments per replication (20 larvae) into separate rearing trays. In early instar tender *Q. serrata* leaves and in late instar matured leaves sprayed with dietary supplement were fed to worm at 6,10,16 and 21 hours of each day. The silkworm rearing technology described by Krishnaswami (1978) and slightly modified by [16] was followed in the present investigation. Cleaning nets were used to remove waste material from the tray following each moulting bed cleaning. The amount of food was raised in accordance with the oak tasar silkworm growth.

The mature or fully grown worms were released on mountages to spin cocoons after reaching maturity. On the fifth day after the worms were released according to the protocols, the cocoon were harvested. To record cocoon parameters, ten cocoons from each replication in one treatment were chosen at random. The weight of the cocoon was recorded using the first lot. The single filament length was measured in the 35 - second lot and the moth emergence as seed cocoons was observed in the third lot.

Treatment details

The present experiment was undertaken to the effect of nutritional supplement treated *Quercus serrata* leaves on life cycle and economic traits of oak tasar silkworm (*A. proylei)*. The experiment consists of following treatments (Table 1.):

Table 1: Details of treatment used during the present investigation

Tr. No.	Treatments detail	Source of material
T_1	*Quercusserrata* leaves treated with vitamin C - complex @ 1%	L-Ascorbic acid 99.9%, Pvt. Ltd., Palampur
T_2	*Quercusserrata* leaves treated with protein @ 1%	Soya flour local market Palampur
T_3	Quercus serrata leaves treated with vitamin B - complex @ 1%	Neurobion Forte Tablets, Pvt., Ltd., Palampur
T_4	Control (*Q. serrata* untreated leaves)	

Plate 2: Various dietary supplements (Vitamin C-complex, protein, Vitamin B-complex) used during investigation

Results and Discussion

The experimental results and discussion of present investigation entitled "Effect of nutritional supplements treated *Quercus serrata* leaves on life cycle and economic traits of *Antheraea proylei* (oak tasar silkworm)" are presented and discussed under following headings.

(a). Effect of nutritional supplements treated *Q. serrata* leaves on life cycle of oak tasar silkworm (*A. proylei).*

Data on larval duration (days), pupal duration (days) and moth emergence (%) of oak tasar silkworm are given in table 2.

Table 2: Effect of nutritional supplements treated *Q. serrata* leaves on larval duration, pupal duration and moth emergence of oak tasar silkworm

Sr. No.	Treatments detail	Mean larval duration (days)					Total larval duration (days)	Mean pupal duration (days)	Moth emergence (%)
		Early instars			Late instars				
		First	Second	Third	Fourth	Fifth			
T_1	*Q. serrata* leaves treated with vitamin C-complex @1%	6.15	6.46	7.86	7.96	13.99	42.42	11.75	88.84
T_2	*Q. serrata* leaves treated with protein @1%	7.49	7.39	8.48	8.66	14.42	46.44	12.75	94.11
T_3	*Q. serrata* leaves treated with vitamin B-complex @1%	5.73	6.34	7.85	7.64	13.40	40.96	10.90	85.55
T_4	Control (natural diet)	5.50	5.68	7.34	7.48	13.16	39.16	9.20	83.88
	C.D. at 5%	0.80	0.83	0.52	0.65	0.57	3.37	0.92	1.96

Larval duration (days)

There were five larval instars of *Antheraea proylei* fed on the three nutritional supplements (Vitamin C-complex, Protein and Vitamin B-complex) and control (natural diet). The overall larval period of oak tasar silkworm varied from 39.16 to 46.44 days. In all the larval instars, the longest total larval duration (46.44 days) was seen in T_2 treatment (*Q. serrata* leaves treated with protein @1%) followed by T_1 treatment (*Q. serrata* leaves treated with vitamin C-complex @1%) (42.42 days), T_3 treatment (*Q. serrata* leaves treated with vitamin B-complex @1%) (40.96 days) and control (natural diet) having 39.16 days of total larval duration. These results are related with the findings of [4] who concluded that fortification of mulberry leaves with nutrient supplements *viz.*, royal jelly, dietary proteins, amino acids, vitamin B_3 and vitamin B_6 had affirmative effects on the growth and development of *Bombyx mori* silkworm larvae. [2] noticed that the oral supplementation of different concentrations of vitamin B and C to 3rd, 4th and 5th instar larvae of *B. mori* resulted significant increase in larval characters. Complete L-ascorbic acid (vitamin C) deprivation during larval cycle affected larval growth [5].

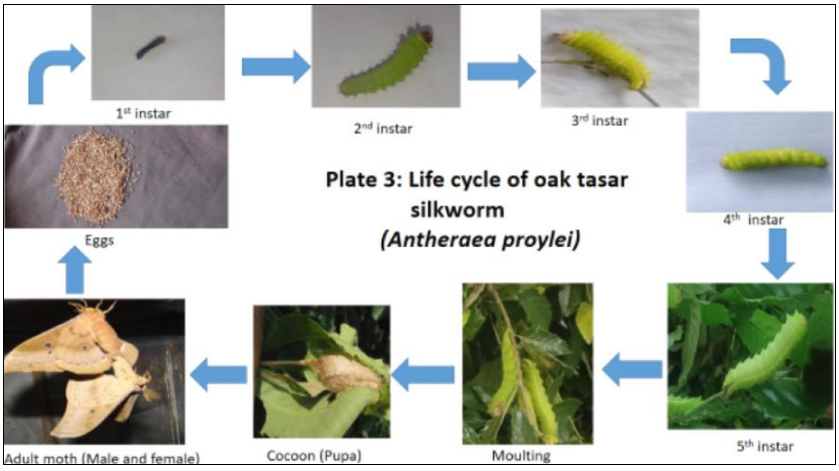

Plate 3: Life cycle of Silk worm (*Antheraea proylei*)

(Pupal duration (days)

The pupal duration of oak tasar silkworm varied from 9.20 to 12.75 days. Significantly highest mean pupal duration (12.75days) was recorded in treatment T_2 (*Q. serrata* leaves treated with protein @ 1%) followed by T_1 (*Q. serrata* leaves treated with vitamin C-complex @ 1%) (11.75days) which was at par with T_3 treatment (*Q. serrata* leaves treated with vitamin C-complex @ 1%) (10.90 days). However, the lowest pupal duration (9.20 days) was registered in the larvae fed with natural diet. These results are related with the findings of [2] who noticed that the oral supplementation of vitamin B and C to 3rd, 4th and 5th instar silkworm larvae resulted in significant increase in pupal characters. [14] showed that the pupal duration of *B. mori* was significantly decreased at 5 percent by incorporating amoxicillin in to natural diet. However [15] observed insignificant differences in the pupal stage duration when larvae of *B. mori* were fed on a semi-artificial diet containing soybean protein during the 5th instar

Moth emergence (%)

The moth emergence of tasar silkworm ranged from 83.88 to 94.11%. Treatment T_2 (*Q. serrata* leaves treated with protein @1%) showed significantly maximum moth emergence (94.11%) followed by Treatment T_1 (*Q. serrata* leaves treated with vitamin C-complex @1%) (88.84%) and T_3 (*Q. serrata* leaves treated with vitamin B-complex @1%) (85.55%). However, *Q. serrata* leaves treated with natural diet showed minimum moth emergence (83.88%). Proteins play a fundamental and physiological role in the growth and development of silkworms. These results are in conformity with the findings of [15] who reported that larvae fed on a combination of macro and micro nutrients recorded 77.41% moth emergence in *B. mori*.

Plate 4: Moth emergence

(b). Effect of nutritional supplements treated *Q. serrata* leaves on the growth parameter of oak tasar silkworm (*A. proylei*)

Data on larval length, larval breadth and larval weight of oak tasar silkworm are given in table 3.

Plate 5: Larval length

Table 3: Effect of nutritional supplements treated *Q. serrata* leaves on larval length, breadth and weight of oak tasar silkworm (*A. proylei*)

Sr. No.	Treatments detail	Mean larval length (mm)					Mean larval breadth (mm)					Mean larval weight (g)				
		Early instar			Late instar		Early instar			Late instar		Early instar			Late instar	
		First	Second	Third	Fourth	Fifth	First	Second	Third	Fourth	Fifth	First	Second	Third	Fourth	Fifth
T_1	*Quercus serrata* leaves treated with vitamin C-complex @1%	7.90	16.11	64.57	69.36	86.34	1.18	2.59	4.81	10.33	18.70	0.006	0.085	3.09	10.39	19.10
T_2	*Quercus serrata* leaves treated with protein @1%	8.53	17.72	65.52	70.66	87.84	1.66	2.66	4.92	10.49	18.82	0.014	0.099	4.10	11.44	21.28
T_3	*Quercus serrata* leaves treated with vitamin B-complex	7.85	16.84	63.21	68.98	85.12	1.12	2.50	4.71	10.25	18.67	0.005	0.091	3.08	9.34	18.09
T_4	Control (natural diet)	7.74	15.81	55.81	68.85	84.22	1.06	2.44	4.60	10.15	18.56	0.004	0.063	2.25	8.43	17.08
	C.D. at 5%	0.08	0.109	0.014	0.032	0.018	0.06	0.11	0.07	0.07	0.10	0.001	0.018	0.292	0.062	0.022

Larval Length, Breadth and Weight

Length

In all the larval instars (first, second, third, fourth and fifth), highest growth in terms of length was observed in T_2 treatment (*Q. serrata* leaves treated with protein @1%) (8.53, 17.72, 65.52, 70.66 and 87.84 mm). This clearly indicated that *A. proylei* silkworm grows better with T_2 treatment (*Q. serrata* leaves treated with protein @1%) followed by T_1 treatment (*Q. serrata* leaves treated with vitamin C-complex @1%) (7.90, 16.11, 64.57, 69.36 and 86.34 mm) which was at par with T_3 treatment (*Q. serrata* leaves treated with vitamin B-complex @1%) (7.85, 16.84, 63.21, 68.98 and 85.12 mm). The length was observed lowest when fed on T_4 treatment (control) (7.74, 15.81, 55.81, 68.85 and 84.22 mm).

Breadth

Highest larval breadth in all the five larval instars was also observed in T_2 treatment (*Q. serrata* leaves treated with protein @1%) (1.66, 2.66, 4.92, 10.49 and 18.82 mm) followed by T_1 treatment (*Q. serrata* leaves treated with vitamin C-complex @1%) (1.18, 2.59, 4.81, 10.33 and 18.70 mm) and T_3 treatment (*Q. serrata* leaves treated with vitamin B-complex @1%) (1.12, 2.50, 4.71, 10.25 and 18.67 mm). The larval breadth was minimum in control (1.06, 2.44, 4.60, 10.15 and 18.56 mm).

Weight

All the larval instar (first, second, third, fourth and fifth) showed highest growth in terms of weight in T_2 treatment (*Q. serrata* leaves treated with protein @1%) (0.014, 0.099, 4.10, 11.44 and 21.28 g) followed by T_1 treatment (*Q. serrata* leaves treated with vitamin C-complex @1%) (0.006, 0.085, 3.09, 10.39 and 19.10 g) and T_3 treatment (*Q. serrata* leaves treated with vitamin B-complex @1%) (0.005, 0.091, 3.08, 9.34 and 18.09 g). The weight was observed lowest when fed on untreated *Q. serrata* leaves (0.004, 0.063, 2.25, 8.43 and 17.08 g).

Protein play a fundamental and physiological role in growth and development of silkworm larvae. These results are in concurrence with the findings of [1] who proved that larvae of *B. mori* fed with mulberry leaves enriched with anway nutrilite protein (10%) revealed significant enhancement in larval characters like larval weight. [19] documented that mulberry silkworm larvae fed on mulberry (MR_2) leaves sprayed with 25% concentration of AgNps enhanced the larval length, width and weight as compared to control. Larvae fed on mulberry leaves treated with 0.2% N+ 0.05% vitamin C affect body weight and body length of silkworm larvae statistically [9].

Plate 6: Larval weight

Effect of nutritional supplements treated *Q. serrata* leaves on economic traits of oak tasar silkworm (*A. proylei*).

Data on cocoon weight and silk filament length of oak tasar silkworm are given in table 4.

Table 4: Effect of nutritional supplements treated *Q. serrata* leaves on cocoon weight and silk filament length of oak tasar silkworm

Sr. No.	Treatments detail	Mean cocoon weight (g)	Mean silk filament length (m)
T_1	*Q. serrata* leaves treated with vitamin C-complex @1%	6.34	1036.14
T_2	*Q. serrata* leaves treated with protein @1%	6.99	1164.28
T_3	*Q. serrata* leaves treated with vitamin B-complex @1%	5.97	1025.36
T_4	Control (natural diet)	5.88	891.78
	C.D. at 5%	0.02	35.67

Cocoon weight (g)

Significantly maximum cocoon weight (6.99g) was recorded in T_2 treatment (*Q. serrata* leaves treated with protein @ 1%) followed by T_1 (*Q. serrata* leaves treated with vitamin C-complex) (6.34g) and T_3 treatment (*Q. serrata* leaves treated with vitamin B-complex) (5.97g). However, the minimum cocoon weight (5.88g) was obtained in control (untreated diet). These our results are matching with the findings of [20] who exhibited that mulberry leaves treated with *Z. mays* flour protein (10 per cent) fed larvae recorded maximum cocoon weight (2.32±0.07 g) of *B. mori*. However [11] revealed that maximum cocoon weight of tasar silkworm was obtained in leaves fortified with soya solution spray treatment followed by soya dust formulation.

Plate 7: Cocoon weight

Silk filament length

Significantly maximum silk filament length (1164.28 m) was recorded in T_2 treatment (*Q. serrata* leaves treated with protein @1%) followed by T_1 treatment (*Q. serrata* leaves treated with vitamin C-complex @1%) (1036.14 m) which was at par with T_3 treatment (*Q. serrata* leaves treated with vitamin B-complex @1%) (1025.36m). However, the minimum silk filament length (891.78 m) was obtained in control (untreated diet). These results are related with the findings of [12] demonstrated that mulberry leaves treated with aqueous solution of seed powder of cowpeas (*vigna unguiculata)* showed maximum silk filament length (0.323%) as compared to untreated control. [18] revealed that nutritional supplementation of *A. hybridus* plant extract (2.0%) to 3rd, 4th and 5th instar larvae of *B. mori* has improved silk filament length positively.

Plate 8: Silk filament of oak tasar silkworm (Antheraea Proyeli)

Conclusion

Proteins are bio-molecules that plays a fundamental and physiological role in growth and development of silkworms and synthesis of silk proteins in silk gland during larval development. The overall result concluded that the fortification of *Q. serrata* leaves with nutrient supplements protein @ 1%, vitamin C-complexand vitamin B-complex habited positive influence on the growth and development of *A. proylei* larvae as well as on cocoon and silk production. This might be due to increase in nutritional efficiency in larval stages which significantly influenced the resulting pupae and adult stages. The maximum larval weight, pupal weight, silk filament length, moth emergence and effective rate of rearing of silkworm larvae fed with *Q. serrata* leaves treated with protein@ 1% in comparison to other dietary supplements confirmed that protein is an ideal nutrient for the sericulture industry.

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