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## Effect of drone brood and protinex fortification on feeding indices of silkworm, *Bombyx mori* L.

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**Abstract**

Nutrition plays key role in improving growth, development, health, food consumption and conversion of silkworm (*Bombyx mori* L.). Feeding indices of silkworm is a crucial factor in converting the feed ingested to produce commercially important silk. In the present investigation, the silkworm bivoltine double hybrid FC<sub>1</sub> × FC<sub>2</sub> [(CSR<sub>6</sub> × CSR<sub>26</sub>) × (CSR<sub>2</sub> × CSR<sub>27</sub>)] was reared on fortified mulberry leaves with drone brood, protinex and their combinations. Protinex (10%) caused the highest positive significant effects on ingesta (45.17g), digesta (31.57g), approximate digestibility (69.89%), relative growth rate (0.041), reference ratio (3.321), consumption index (1.154), respiration (0.640), metabolic rate (0.060), Efficiency of conversion of ingesta (34.00%) and digesta (48.65%) to larva when compared to other treatments and control. This study has been indicated that the protinex (10%) exhibit the presence of more growth stimulant activity and can be used to increase the silk yield in commercial silkworm rearing.

**Keywords:** Drone brood, protinex, fortification, silkworm

**1. Introduction**

Sericulture is an age old, agro based, women friendly cottage industry with combination of rural, agricultural and industrial based activities which includes host plant cultivation, silkworm rearing and post cocoon technology with high employment potential and economic benefits. The silkworm, *Bombyx mori* L. is an important economic insect and also a tool to convert mulberry leaf protein into commercially valuable silk protein. Silkworm larvae are monophagous in nature due to morin (Zhang *et al.*, 2018) [42] and obtain all nutrients from mulberry leaves to build its body, sustain and spin cocoons. Mulberry leaves provide proteins, vitamins and other nutrients from which silk proteins are synthesized. Quality and quantity of mulberry leaves along with environmental factors affect production of raw silk spun by larvae before pupation in the form of cocoons. Sengupta *et al.* (1972) [37] revealed that *Bombyx mori* L. requires specific essential sugars, amino acids, proteins and vitamins for its normal growth, survival and also for improvement in the growth of silk gland. Good quality cocoons can be obtained when silkworms fed on nutritionally supplemented leaves their results improved the silk production (Seki and Oshikane, 1959) [36]. In silkworms, silk fibroin is derived mainly from four amino acids: alanine, serine, glycine and tyrosine (Kirimura, 1962) [11] which come from their dietary source of protein and amino acids (Ito, 1983). Silkworms obtain 72-86 per cent of their amino acids from mulberry leaves and more than 60 per cent of the absorbed amino acids are used for silk production (Lu and Jiang, 1988) [17].

Under different environmental, feeding and nutritional conditions silkworm showed significant difference in its ability to ingest, digest, absorb and convert to body matter (Rahmathulla and Suresh, 2012) [26]. The capacity of silkworm to ingest mulberry leaf, digest, absorb, assimilate and convert it to silk fibre also differs from race to race (Jadhav *et al.*, 2016) [10]. Nutritional intake has direct impact on the overall genetic traits such as larval and cocoon weight, amount of silk production, pupation and reproductive traits. Nutritional ecology of insects is the prerequisite for a better knowledge on their ethnobiology and physiology which is often being neglected (Scriber and Slansky, 1981) [35]. Nutritional quality as well as environmental conditions has greater impact on regulation over the quantum of ingesta, digesta and approximate digestibility of food among silkworm (Ito, 1972). Silkworm growth is a composite result of various physiological activities of an organism by which matter accumulates in the body as a result of balance between assimilation & dissimulation (Ueda, 1982) [40] and efficiency of conversion of ingested mulberry leaves into silk.

The climatic condition of the North India is suited for bivoltine sericulture but the unit production and the quality of the silk produced is much lower than the sericulturally advanced countries like Japan and China. The cocoon productivity in North India is 34.17 kg/100 Dfls at commercial level and the average renditta is 9.50 kg, while it is 6.50 kg at National Level. As of now the production is about 40 kg/100 Dfls with renditta of 7.50 Kg.

Fortification of mulberry leaves is one of the strategies by which cocoon and silk productivity can be increased and the quality can be enhanced and maintained by fortifying nutrient supplement. Feeding of nutritionally enriched leaves showed better growth and development of silkworms as well as improved the economic value of cocoons (Krishnaswami *et al.*, 1978) [12]. Fortification of mulberry leaves with supplementary compounds like vitamins (Saha and Khan, 1996; Nirwani and Kaliwal, 1996; Nirwani and Kaliwal, 1998; Etabari *et al.*, 2004; Rahmathulla *et al.*, 2007) [25, 21, 22, 32], hormones (Magadum and Hooli, 1992; Saha and Khan, 1997) [30, 33], amino acids (Khan and Faruki, 1990; Qadar *et al.*, 1994; Saha *et al.*, 1994; Saha and Khan, 1997) [31, 33] and minerals (Magadum and Hooli, 1992; Khan and Saha, 1995) [18, 30] was done by different workers to increase the larval growth and development of silkworm. Further, it is evident that the digestion and assimilation process also interrupts silk production. The late age feeding is the maximum lively feeding stage of silkworm. The larva accumulates an enormously vast range of fuel reserves in several tissues and is endowed with unique biochemical variations to keep dietary sources and make them accessible. When some new dietary elements are added to silkworm feed during the late age rearing it can alter the nutritional efficiency and food consumption parameters. The present study has been aimed to find out the feed efficacy of drone brood, protinex and their combinations treated mulberry leaves with regard to food utilization by larvae and ultimate impact on the cocoon parameters of silkworm in Jammu & Kashmir Union Territory climatic conditions.

## 2. Methodology

The present investigation was conducted at Sericulture Research Laboratory of Division of Sericulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu during spring season of 2021-22. For the experiment, the test insect, bivoltine double hybrid FC<sub>1</sub> × FC<sub>2</sub> [(CSR<sub>6</sub> × CSR<sub>26</sub>) × (CSR<sub>2</sub> × CSR<sub>27</sub>)] silkworm, *Bombyx mori* L. was used. The silkworm eggs were procured from the Sericulture Development Department of Jammu and Kashmir Union Territory and then incubated at ambient temperature of 25 ± 1° C with relative humidity of 80 ± 5 per cent until hatching. The newly hatched worms were then transferred to rearing beds and reared as per the rearing method suggested by Krishnaswami, 1978 [12].

Drone brood and protinex powder were used for the present investigation as the feed supplementation proteins. Drone brood was collected from the Apiary maintained by Division of Entomology, SKUAST-Jammu. However, protinex powder was procured from the nearby market. Known quantity of drone brood (g) weighed on an electronic balance was mixed with known quantity of distilled water (ml) on weight by volume basis (1:1) using a glass rod. The material was then squeezed through double layered muslin cloth and the filtrate was maintained as stock solution, considered as 100 per cent and kept in refrigerator at -4°C for further use. Protinex

powder was procured from the nearby market, known quantity of protinex powder (g) weighed on electronic balance was mixed with known quantity of distilled water (ml) on weight by volume basis (1: 4) using a glass rod in order to make protinex solution and kept in refrigerator at -4°C for further use.

The experiment was conducted during fifth instar. The experiment was laid down in a completely randomized design with three replications each consisted of 100 silkworm larvae. Weighted and equal quantity of fortified mulberry leaves was fed to all treatments in fifth instar except control where fresh mulberry leaves were fed. Simultaneously, an additional batch of silkworm larvae was maintained to determine the dry weight on subsequent daily increments in larval weight. Left over leaves and excreta was collected after every fortified feed, separated manually and dried in a hot air oven at 65°C for 5 hours.

### 2.1 Estimation of Feeding Indices Traits

During the silkworm nutritional study, data was collected on the biomass of larva for nutrigenic traits on ingesta, digesta, approximate digestibility, reference ratio, consumption index, relative growth rate, respiration & metabolic rate, efficiency of conversion of ingesta and digesta for larva (Waldbauer, 1968; Scriber and Feeny, 1979; Kogan and Parra, 1981; Slansky and Scriber, 1985) [41, 34-36, 13], the equation with brief description of the nutrigenetic traits are given below.

**2.1.1 Ingesta (g):** Total intake of the dry weight (g) of mulberry leaves by silkworm larvae during the 5<sup>th</sup> stage upto the spinning or ripening stage: (Dry weight of leaf fed – Dry weight of left over leaf).

**2.1.2 Digesta (g):** Total assimilated dry food from the intake or ingesta of dry weight of mulberry leaves by silkworm larvae during the 5<sup>th</sup> stage until the spinning or ripening stage: (Dry weight of leaf ingested – Dry weight of litter).

**2.1.3 Approximate digestibility (%):** Directly indicates the assimilation efficiency of mulberry leaves and depends on the passage rate of food through gut in silkworm: (AD = Dry weight of digesta / Dry weight of food ingested × 100).

**2.1.4 Reference ratio:** It is an indirect expression for assimilation and absorption of food. Expresses the ingesta required per unit excreta produced: (RR = Dry weight of food ingested / Dry weight of excreta).

**2.1.5 Consumption index:** Relates to the rate of food intake to the mean weight of the larvae during the feeding period: (CI = Ingesta / 5<sup>th</sup> stage mean fresh larval weight (g) × 5<sup>th</sup> stage larval duration in days).

**2.1.6 Relative growth rate:** It refers to the larval gain biomass and indicates the efficiency of conversion of nutrition into larval biomass: (RGR = Weight gain of the larva during feeding period / 5<sup>th</sup> stage mean fresh larval weight (g) × 5<sup>th</sup> stage larval duration in days).

**2.1.7 Respiration:** It is a catabolic reaction in which total oxidation of the digested or assimilated food for releasing energy required for the entire biological activities by breakdown of macromolecules into simpler molecules: (Dry weight of food digested – Maximum dry weight of larvae).

**2.1.8 Metabolic rate:** It is measure of total biochemical reactions involving both catabolic and anabolic reactions of an organism, associated with the degradation of macromolecules into smaller unit and vice-versa. (MR = Respiration / 5<sup>th</sup> stage mean fresh larval weight (g) × 5<sup>th</sup> stage larval duration in days).

**2.1.9 Efficiency of conversion of ingesta to larva (%):** It is associated with the efficiency conversion of ingested nutrition into biomass or body matter at different stages and expressed in percentage. ECI to larva was the efficiency of conversion of ingested food into larva: (ECI larvae = Maximum dry weight of larva / Dry weight of Ingesta × 100).

**2.1.10 Efficiency of conversion of digesta to larva (%):** It is the expression of efficiency of conversion of digesta into larval biomass: (ECD larvae = Maximum dry weight of larva / Dry weight of digesta × 100).

**2.2 Statistical analysis:** All the observations obtained for various parameters were tabulated and subjected to statistical analysis by using the SPSS version 16.

### 3. Results

**3.1 Nutrition consumption traits of silkworm fed with fortified mulberry leaves with different proteins:** Mulberry leaves fortified with drone brood (6%), protinex (10%), drone brood (3%) + protinex (3%) and drone brood (5%) + protinex (5%) showed profound influence on the nutritional consumption traits. In this study, the highest nutritional consumption parameters viz., ingesta (45.17g), digesta (31.57g), approximate digestibility (69.89%), relative growth rate (0.041), reference ratio (3.321), respiration (0.640), metabolic rate (0.060) and consumption index (1.154) were recorded in protinex (10%) treated batch when compared to other treatments and control. The effect of fortified mulberry leaves fed to silkworms on nutritional consumption characteristics are presented in Table 1 and 2.

**Table 1:** Effect of fortified mulberry leaves with different proteins on nutrition consumption characteristics of silkworm larvae.

Fortification of mulberry leaves with	Ingesta (g)	Digesta (g)	Approximate Digestibility (%)	Relative growth rate
Drone brood (6%)	44.44±0.02 <sup>d</sup>	29.30±0.03 <sup>c</sup>	65.93±0.03 <sup>c</sup>	0.033±0.000 <sup>b</sup>
Protinex (10%)	45.17±0.01 <sup>e</sup>	31.57±0.01 <sup>d</sup>	69.89±0.02 <sup>d</sup>	0.041±0.005 <sup>c</sup>
Drone brood (3%) + Protinex (3%)	43.10±0.01 <sup>c</sup>	26.56±0.01 <sup>b</sup>	61.62±0.01 <sup>b</sup>	0.033±0.000 <sup>b</sup>
Drone brood (5%) + Protinex (5%)	42.94±0.02 <sup>b</sup>	26.51±0.02 <sup>b</sup>	61.73±0.02 <sup>b</sup>	0.034±0.001 <sup>b</sup>
Control	41.17±0.01 <sup>a</sup>	23.05±0.04 <sup>a</sup>	55.98±0.10 <sup>a</sup>	0.013±0.001 <sup>a</sup>

**Note:** Each value is a mean ± standard error (SE) of three replications. Mean ±SE followed by same letter in column are non significant by Tukey HSD test.

**Table 2:** Effect of fortified mulberry leaves with different proteins on silkworm reference ratio, consumption index, respiration and metabolic rate

Fortification of mulberry leaves with	Reference ratio	Consumption index	Respiration (g)	Metabolic rate
Drone brood (6%)	2.935±0.003 <sup>c</sup>	1.101±0.002 <sup>c</sup>	0.520±0.005 <sup>c</sup>	0.038±0.000 <sup>c</sup>
Protinex (10%)	3.321±0.004 <sup>d</sup>	1.154±0.001 <sup>d</sup>	0.640±0.005 <sup>d</sup>	0.060±0.000 <sup>d</sup>
Drone brood (3%) + Protinex (3%)	2.605±0.003 <sup>b</sup>	1.086±0.002 <sup>b</sup>	0.420±0.005 <sup>b</sup>	0.021±0.000 <sup>b</sup>
Drone brood (5%) + Protinex (5%)	2.613±0.001 <sup>b</sup>	1.072±0.009 <sup>b</sup>	0.413±0.008 <sup>b</sup>	0.021±0.000 <sup>b</sup>
Control	2.272±0.005 <sup>a</sup>	0.989±0.007 <sup>a</sup>	0.310±0.005 <sup>a</sup>	0.007±0.000 <sup>a</sup>

**Note:** Each value is a mean ± standard error (SE) of three replications. Mean ±SE followed by same letter in column are non-significant by Tukey HSD test.

**3.2 Nutritional conversion efficiency characteristics of silkworm fed with fortified mulberry leaves with different proteins:** The efficiency of mulberry leaves ingested converted into silkworm larval biomass varied significantly among the treatments. The highest efficiency conversion of ingesta (ECI) to larva was recorded in protinex 10 per cent (34.00%) administered batches of silkworm and the least in

control (26.50%). With regard to efficiency conversion of digesta (ECD) to larva, significant increase was observed among the treatments and more efficient conversion of digested food into larval biomass in protinex 10 per cent (48.65%) administered batches of silkworm and less efficient in control (40.33%) were noticed (Table 3).

**Table 3:** Effect of fortified mulberry leaves with different proteins on silkworm, *B. mori* nutritional conversion efficiency characteristics

Fortification of mulberry leaves with	Efficiency conversion of ingesta to larva (%)	Efficiency conversion of digesta to larva (%)
Drone brood (6%)	29.41±0.02 <sup>c</sup>	47.52±0.03 <sup>d</sup>
Protinex (10%)	34.00±0.01 <sup>d</sup>	48.65±0.02 <sup>e</sup>
Drone brood (3%) + Protinex (3%)	28.19±0.03 <sup>b</sup>	44.60±0.03 <sup>b</sup>
Drone brood (5%) + Protinex (5%)	29.34±0.01 <sup>c</sup>	45.74±0.06 <sup>c</sup>
Control	26.50±0.01 <sup>a</sup>	40.33±0.11 <sup>a</sup>

**Note:** Each value is a mean ± standard error (SE) of three replications. Mean ±SE followed by same letter in column are non-significant by Tukey HSD test.

### 4. Discussion

The physiology of growth is manifested by the accumulation of organic matter resulting from the balance between anabolic and catabolic reactions fuelled by the nutritive substances digested in any animal. In silkworm, the food consumption has direct relevance to the weight of larva, cocoon, pupa and

shell. However, these parameters of consumption and productivity will vary depending on the season, breeds, feed and instars. Accumulation of nutrients in insect is greatly influenced by the nutritional richness of the host plant or diet fed and this storage nutrition function as the reservoirs for the supply both at the time of larval moult and during



metamorphosis. Variation in the quantity or quality of nutrition can have profound effect on insect development (Slansky and Scriber, 1985; Horie and Watanabe, 1983a; Horie and Watanabe, 1983b; Chapman, 1998) [36, 7-8, 35]. The rate of food consumption and leaf quality influenced significantly larval growth, weight and probability of survival (Murugan and George, 1992) [20]. Analysis of the nutritional indices like the rates of ingestion, digestion, assimilation and conversion in the growing larvae would be useful in understanding the differences in the digestive and assimilation abilities of the silkworm. Further, it was established that silkworm derives over 70 per cent of the protein from the mulberry leaves and in 5<sup>th</sup> instar up to 96 per cent of ingested protein is used for silk protein synthesis and variation in the quantity or quality of nutrition have profound effect on insect development (Fukuda *et al.*, 1963) [5]. In sericulture, nutritional requirement and its conversion efficiency contribute directly or indirectly on the cost benefit ratio of silkworm rearing. In silkworm 97 per cent of the total food intake during the last two instars and the feed utilization study confined to 5<sup>th</sup> instar larvae as 80-85 per cent of the total leaves consumed in this instar as silkworm very active metabolically at this stage (Rahmathulla *et al.*, 2005) [24].

Food ingesta is very important physiological and economic trait as far as sericulture is concerned. The food intake is regulated by the physical nature and presence of phagostimulants in food. Higher food intake in the treated category reflects the high silk production ability, as feeding influences the synthesis of total DNA, RNA and protein synthesis. The higher value of approximate digestibility indicates the greater suitability of food plants. High value of reference ratio indicates high rate of digestion and absorption of food. Growth rate directly influences the speed of development of larvae which in turn depends on the quality of leaf and physiological stages of the larvae. Mahmoud, (1989) [19] has concluded that leaves dipped in 0.2 per cent N solution produced the larvae with maximum weight as compared to the other doses. Rehman, (1997) [29] has concluded that optimum doses of minerals in various combinations, when used enhanced silk production and silkworm growth to a greater extent than control. In the present investigation, among the groups, the protinex (10%) group has the highest ingesta, digesta, approximate digestibility, reference ratio, relative growth rate, respiration, metabolic rate, consumption index, efficiency of conversion of ingesta and digesta to larva values. This may be due to the nutritional supplementation (Rani *et al.*, 2011; Balasundaram *et al.*, 2013) [2, 27]. There is a direct relation between food consumption and weight of larvae, cocoon, pupae and shell (Shivakumar, 1995) [38]. Remadevi *et al.* (1992) [30] reported that the variance in consumption and productivity parameters is dependent on the type of nutrition. In general, the present results are in agreement with the observations of earlier workers (Balasundaram *et al.*, 2008; Rath, 2010; Lakshmi Bai and Ramani Bai, 2011) [1, 16].

## 5. Conclusion

India is the second largest producer and biggest consumer of silk in the world. Silkworm (*Bombyx mori* L.) is the voracious feeder, the source of producing the silk. Fortification of mulberry leaves with supplementary nutrients such as drone brood and protinex and their combinations can give the good quality and quantity of the cocoons. In conclusion, the feed efficacy parameters like ingesta, digesta, approximate digestibility, relative growth rate, reference ratio, respiration,

metabolic rate, efficiency of conversion of ingesta and digesta to larva was comparatively enhanced by protinex (10%) fortified mulberry leaves than the control and other nutritional supplementary groups.

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