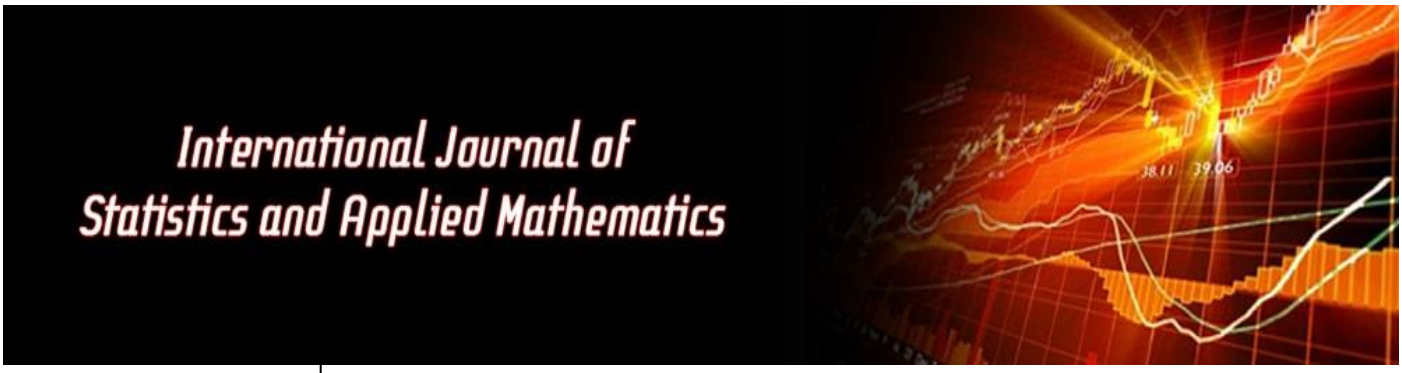

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Effect of mulberry leaves enrichment with nutritional supplements on economic parameters of FC_1 x FC_2 Silkworm, *Bombyx mori* L.

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Abstract
The nutritive value of mulberry leaves has a direct influence on various economic parameters of the silkworm including the quality of cocoon and silk derived from it. The mulberry leaves enriched with additional nutritional supplements have often proved to be beneficial in the production of better cocoons and silk yield. The present investigation was undertaken to know the effect of mulberry leaves enrichment with combinations of various nutritional supplements *viz.*, vitamin C, vitamin B-complex, soya flour, methionine and L-serine on economic characters in FC_1X FC_2 silkworm hybrid. The result of the study revealed that marked improvement in the economic parameters such as single cocoon weight, single shell weight, cocoon shell ratio, ERR by number, ERR by weight, filament length, filament weight and denier when the FC_1 X FC_2 silkworm larvae reared on vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5% over control batches.

Keywords: Silkworm, *Bombyx mori* L., nutritional supplements, economic parameters

Introduction
Sericulture is the practice of rearing silkworm for the production of silk. Silk is the most elegant textile in the world with unparalleled grandeur, natural sheen, inherent affinity for dyes, light weight, high absorbance, soft touch and high in longevity. Due to these unique features silk is known as the "Queen of Textiles" the world over (CSB; 2022). Silkworm, *Bombyx mori* L. is monophagus and feeds entirely on mulberry leaves only due to the presence of a chemical morin (Masthan *et al*., 2017) [21].

The life cycle of *Bombyx mori* L. includes four stages *viz.*, embryo, larva, pupa and adult moth and considered one of the most advanced forms of metamorphosis. The silk worm is the larval stage of the silk moth's life cycle. Moths lay eggs which develop into larvae (caterpillars) commonly called Silkworms. They eat for 20-30 days, consuming large amounts of mulberry leaves and undergo moulting through five changes of skin or instars. (Soumya *et al*., 2017) [19]. Two kinds of silk proteins, first being real silk fiber namely fibroin and second being adhesive, sticky substance i.e. sericin noted as major component of silk cocoon (Selin, *et al*., 2018) [18].

Nutrition plays an important role in improving the growth and development of silkworm *Bombyx mori* L. Silk production is largely dependent on larval growth and nutritive value of mulberry leaves (Masthan *et al*., 2017) [21]. As the quality of mulberry leaf has an intimate relation to the healthy growth of the larvae and quality of their cocoon, the chemical composition of the leaves has a great scope in determining the food value (Anil Kumar and Sunil Kumar 2018) [2]. Fortification of mulberry leaves by using extra nutrients and feeding to the silkworms is a useful modern technique to increase the economic value of cocoon. (Rahmathulla *et al*., 2007) [14]. Several dietary compounds such as sugars proteins, amino acids, minerals, vitamins, salts, etc. whose supplementation can considerably enhance the growth and development of silkworm and hence improve the quality of cocoons (Anil Kumar and Jayaraju 2021) [3].

Vitamins are a group of unrelated organic compounds needed only in little quantities in the diet that are essential for specific metabolic reactions within the cell and necessary for normal growth and maintenance of health. It prevents associated deficiency diseases. Dosages of vitamins are very definitive for normal growth of silkworm (Ahsan *et al*., 2013) [1]. In silkworms, silk fibroin is mainly obtained from four amino acids *viz*., alanine, serine, glycine and tyrosine. Silkworm derive 72-86% of their amino acids from mulberry leaves and more than 60% of the absorbed amino acids are used for silk production (Radjabi, 2010) [12]. It is known that the rich sources of food proteins improve the economic features and promote the growth of silkworm such as soy protein (Rahman, 2018) [13].

Material and Methods

Present experimental analysis was conducted in laboratory Silkworm Seed Production Center, Gadhinglaj, Kolhapur, Maharashtra, to study the effect of mulberry leaves enrichment with nutritional supplements on economic parameters of silkworm *Bombyx mori* L. In the present experiment nine treatments and three replications were used. The experiment was carried out by using Completely Randomized Design.The silkworm race FC_1XFC_2 Bivoltine Double Hybrid, was obtained from the Silkworm Seed Production Center, Gadhinglaj, Kolhapur district, Maharashtra State. Two per cent formalin solution used as disinfectant, 0.3 per cent bleaching powder, Vijetha powder and lime powder was also used. The nutritional supplements used were vitamin C, vitamin B-complex, soya flour, L-serine and methionine. The fresh mulberry leaves of variety V_1 obtained from previous year established mulberry garden at Silkworm Seed Production Centre, Gadhinglaj, Kolhapur district (Maharashtra State).

Rearing method of silkworm breeds: The improved method of silkworm rearing used in this experiment. The disease free layings (Dfl's) of mulberry silkworm races were procured from Silkworm Seed Production Center, Gadhinglaj, Kolhapur, Maharashtra. The Dfl's were incubated at 25 ºC and 75 per cent relative humidity. In the rearing process, egg sheets were spread out in trays with single layer. Trays were covered by using paraffin paper to maintain the necessary humidity for incubation. When obtained the blue egg stage, the egg sheets were put inside black box and covered with black piece of fabric and left undisturbed for 48 hours to promote homogeneous embryo growth referred as "Black boxing". The eggs were exposed to bright day light for few minutes in the morning hour to ensure evenly hatching. Newly hatched silkworm larvae were brushed with the help of sterilized feather of bird and immediately fed with chopped pieces (0.5-1 sq. cm) of mulberry leaves of variety V_1. After passing each moult bed was disinfected with vijetha powder for control of infection caused by fungi, bacteria, protozoa and viruses. After reaching their full growth, the matured silkworms were released on chandrika. Silkworm spin the cocoon in between 48-72 hr. Fifth day after the discharge of the silkworm for mounting on chandrika, the cocoons were harvested. (Krishnaswami, 1979) [9].

Treatment Details

Table 1: Treatment details

Tr. No	Treatment details
T_1	Vitamin C 0.25% + soya flour 0.25%
T_2	Vitamin B-complex 0.25%+ soya flour 0.25%
T_3	Vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5%
T_4	Vitamin B-complex 0.25%+ soya flour 0.25% + vitamin C 0.5%
T_5	Vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%
T_6	Vitamin B-complex 0.25%+ soya flour 0.25% + vitamin C 0.5% + methionine 0.5%
T_7	Vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%+ L-serine 0.5%
T_8	Vitamin B-complex 0.25%+ soya flour 0.25% + vitamin C 0.5% + methionine 0.5%+ L-serine 0.5%
T_9	Untreated control

The details of treatment used during the present investigation are given in Table 3.1 Fresh mulberry leaves were soaked in each concentration for 15 min and then were dried in air for 20 min. The supplementary leaves were fed to the mulberry silkworm larvae till pupation. The procedure outlined by Etebari *et al*. (2007) [7] was used for preparation of test solution.

Method of recording observation

Economic parameters such as single cocoon weight, single shell weight, cocoon shell ratio, ERR by number, ERR by weight, filament length, filament weight and denier were recorded (Formulae given).

$$\text{Weight of single cocoon} = \frac{\text{Weight of 10 male cocoons (g) + weight of 10 female cocoons (g)}}{\text{Number of cocoon taken (20)}}$$

$$\text{Single shell weight} = \frac{\text{Shell weight of 10 male cocoon + 10 female cocoon shell weight (g)}}{\text{Total number of cocoon taken (20)}}$$

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

$$\text{ERR by number (\%)} = \frac{\text{No. of cocoon harvested}}{\text{Total no. of larvae retained after 4}^{\text{th}}\text{ moult}} \times 100$$

$$\text{ERR by weight (kg)} = \frac{\text{Weight of good cocoons harvested in kg}}{\text{Total no. of larvae retained after 4}^{\text{th}}\text{ moult}} \times 10{,}000$$

Filament length(m) = No. of rotations x circumference of wheel (m)

$$\text{Denier} = \frac{\text{Filament weight (g)}}{\text{Filament length (m)}} \times 9000$$

Statistical analysis

The experimental data collected on various economic traits were statistically analyzed by standard 'analysis of variance' as per method and the critical difference (CD) was calculated wherever the 'F' test was found significant. The data are presented with the level of significance at 5 per cent. The data pertaining to economic traits *viz*., cocoon weight, shell weight, cocoon shell percentage, ERR by number, ERR by weight,

average filament length, weight and denier was recorded and computed.

Results and Discussions

Single cocoon weight (g): The present findings (Table 2) indicate that the highest mean single cocoon weight was recorded in T_5 (1.83 g) followed by T_3 (1.77 g), T_1 (1.76 g) and the lowest mean single cocoon weight was observed in T_9, the control group (1.41 g). These results are in accordance with Anil Kumar and Sunil Kumar (2018) [2] who found that mulberry leaves enriched with 0.5% methionine enhanced cocoon weight of silkworm, *Bombyx mori* L. These results are similar to findings like Balasundram *et al.* (2013) [5] examined, feeding vitamin C-treated MR_2 mulberry leaves to *B. mori* larvae in their last instar resulted increase cocoon weight of group fed with 0.2% vitamin C, with the silkworm larvae (V instar) being more than those in the control and other vitamin C-treated groups (0.1, 0.4, and 0.8 percent). According to Sawant *et al.* (2016) [17], supplementing silkworms (*Bombyx mori* L.) with 0.2% ascorbic acid improved their economic metrics, such as single cocoon weight. Quraiza *et al.* (2016) [11] demonstrated fortification of the mulberry leaves by nutrient supplementation, it resulted that 7 % plant protein (soya flour) showed significant increase in parameters such as cocoon weight over control. Ravi Kumar and Anil Kumar (2016) reported that folic acid supplementation of mulberry leaves had a favourable influence on cocoon weight. Deepa *et al.* (2020) [6] observed that when silkworm larvae fed with mulberry leaves supplemented with amino acids during late instars resulted in maximum single cocoon weight. The latest results indicate that a combination of 0.25% vitamin C, 0.25% soya flour, 0.5% vitamin B-complex, and 0.5% methionine functions as a successful growth promoter, resulting in a significant increase in single cocoon weight.

Table 2: Effect of mulberry leaves enrichement with various nutritional supplements on economic parameters of silkworm

Tr. No	Treatments	Single cocoon weight (g)	Single shell weight (g)	Shell ratio (%)	ERR by No. (%)	ERR by weight (kg)	Filament length (m)	Filament weight (kg)	Denier
T_1	Vitamin C 0.25% + soya flour 0.25%	1.76	0.43	24.43 (29.62)	94.00 (75.95)	14.14	927.00	0.27	2.71
T_2	Vitamin B-complex 0.25%+ soya flour 0.25%	1.47	0.28	19.09 (25.90)	92.67 (74.53)	13.21	798.00	0.18	2.07
T_3	Vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5%	1.77	0.43	24.29 (29.53)	96.00 (78.72)	14.27	928.77	0.28	2.72
T_4	Vitamin B-complex 0.25%+ soya flour 0.25% + vitamin C 0.5%	1.58	0.31	19.62 (26.29)	92.00 (73.65)	13.38	800.00	0.19	2.13
T_5	Vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%	1.83	0.45	24.59 (29.73)	96.67 (79.85)	14.50	932.17	0.29	2.79
T_6	Vitamin B-complex 0.25%+ soya flour 0.25% + vitamin C 0.5% + methionine 0.5%	1.71	0.38	22.22 (28.12)	92.67 (74.53)	13.90	893.70	0.24	2.41
T_7	Vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5% + L-serine 0.5%	1.69	0.36	21.30 (27.48)	90.67 (72.37)	13.68	888.43	0.23	2.32
T_8	Vitamin B-Complex 0.25%+ soya flour 0.25% + vitamin C 0.5% + methionine 0.5% + L-serine 0.5%	1.64	0.33	20.12 (26.65)	90.00 (71.62)	13.44	876.33	0.21	2.15
T_9	Untreated control	1.41	0.25	17.42 (24.67)	88.00 (69.77)	12.81	780.00	0.17	1.96
S.Em ±		0.036	0.008	0.183	1.65	0.1	9.33	0.004	0.018
C.D at 5%		0.11	0.03	0.54	4.91	0.3	27.72	0.01	0.06

Single shell weight (g): Table 2 shows that higher single shell weight was recorded in T_5 (0.45g) followed by T_3 (0.43g), T_1 (0.43g) and lower in control (0.25g). These findings were in agreement with Anil Kumar and Sunil Kumar (2018) who found that mulberry leaves enriched with 0.5% methionine enhanced shell weight of silkworm, *Bombyx mori* L. According to Sawant *et al.* (2016) [17], supplementing silkworms (*Bombyx mori* L.) with 0.2% ascorbic acid improved their economic metrics, such as single shell weight. Quraiza *et al.* (2016) [11] demonstrated fortification of the mulberry leaves by nutrient supplementation, it resulted that 7 % plant protein (soya flour) showed significant increase in parameters such as shell weight over control. Ravi Kumar and Anil Kumar (2016) [16] reported that folic acid supplementation of mulberry leaves had a favourable influence on shell weight. Deepa *et al.* (2020) [6] observed that when silkworm larvae fed with mulberry leaves supplemented with amino acids during late instars resulted in maximum single shell weight.

Shell ratio (%): The present findings (Table 2) indicated that T_5 exhibited the highest mean cocoon shell ratio at 24.59 % followed by T_1 (24.43 %), T_3 (24.29 %) and the lowest cocoon shell ratio was observed in T_9, the control group, at 17.42%. These results were similar with findings of Anil Kumar and Sunil Kumar (2018) [2] who found that mulberry leaves enriched with 0.5% methionine enhanced shell ratio of silkworm, *Bombyx mori* L. According to Sawant *et al.* (2016) [17], supplementing silkworms (*Bombyx mori* L.) with 0.2% ascorbic acid improved their economic metrics, such as shell ratio. Quraiza *et al.* (2016) [11] demonstrated fortification of the mulberry leaves by nutrient supplementation, it resulted that 7 % plant protein (soya flour) showed significant increase in parameters such as shell ratio over control. Ravi Kumar and Anil Kumar (2016) [16] reported that folic acid supplementation of mulberry leaves had a favourable influence on shell ratio. Deepa *et al.* (2020) [6] observed that when silkworm larvae fed with mulberry leaves supplemented with amino acids during late instars resulted in maximum shell ratio.

Effective rearing rate (ERR) by number (%): The Effective Rearing Rate (ERR) serves as a measure of the survivability of silkworms throughout the silkworm crop. ERR by number was recorded significantly maximum in T_5 (96.67 %) followed by T_3 (96.00 %), T_1 (94.00 %) and the lower in T_9, the control group (88.00 %). These results showed similarity with Anil Kumar and Jayaraju (2021) [3] who found that when the FC_1 x FC_2 silkworm larvae were raised on phenylalanine supplementation at 1.5% compared to control batches, there was a noticeable improvement in the economic metrics, such as ERR. In terms of economic characteristics, all the breeds fared better with phenylalanine, methionine, and valine coming in order of preference. According to Sawant *et al.* (2016) [17], supplementing silkworms (*Bombyx mori* L.) with 0.2% ascorbic acid improved their economic metrics, such as effective rate of rearing by number. Rahul *et al*. (2017) [15] found positive effect of mulberry leaves fortified with yeast extract (0.5 and 1 % conc.) on effective rate of rearing. Deepa *et al*. (2020) [6] observed that when silkworm larvae fed with mulberry leaves supplemented with amino acids during late instars resulted in maximum effective rate of rearing.

Effective rearing rate (ERR) by weight (kg): ERR by weight was recorded significantly higher in T_5 (14.50 kg) followed by T_3 (14.27 kg), T_1 (14.14 kg) and the lowest Effective rearing rate by weight (kg) was recorded in T_9, the control group (12.81 kg). These results were in agreement with the earlier workers Rahul *et al*. (2017) [15] found positive effect of mulberry leaves fortified with yeast extract (0.5 and 1 % conc.) on effective rate of rearing. Deepa *et al*. (2020) [6] observed that when silkworm larvae fed with mulberry leaves supplemented with amino acids during late instars resulted in maximum cocoon weight (92.83g/50 cocoons). Rahmathulla *et al.* (2007) [14] examined the effects of oral folic acid feeding on *B. mori* bivoltine hybrid (CSR_2 x CSR_4) larvae in their fifth instar. They found that eating mulberry leaves sprayed with folic acid solution significantly increased the silkworm's ERR by weight. According to Sawant *et al.* (2016) [17], supplementing silkworms (*Bombyx mori* L.) with 0.2% ascorbic acid improved their economic metrics, such as effective rate of rearing by weight. The current findings demonstrate that a blend comprising 0.25% vitamin C, 0.25% soya flour, 0.5% vitamin B-complex, and 0.5% methionine serves as an effective growth promoter, leading to a substantial enhancement in cocoon yield per weight.

Filament length (m): Table 2 indicated that T_5 exhibited the highest filament length at 932.17 m followed by T_3, with a filament length of 928.77 m and T_1 with 927.00 m, the lowest filament length was observed in T_9, the control group, at 780.00 m. These results were in accordance with Anil Kumar and Sunil Kumar (2018) [2] who found that mulberry leaves enriched with 0.5% methionine enhanced filament length of silkworm, *Bombyx mori* L. According to Sawant *et al*. (2016) [17], supplementing silkworms (*Bombyx mori* L.) with 0.2% ascorbic acid improved their economic metrics, such as filament length. Quraiza *et al.* (2016) [11] demonstrated fortification of the mulberry leaves by nutrient supplementation, it resulted that 7 % plant protein (soya flour) showed significant increase in parameters such as filament length over control. Ravi Kumar and Anil Kumar (2016) [16] reported that folic acid supplementation of mulberry leaves had a favourable influence on filament length. Deepa *et al*. (2020) [6] observed that when silkworm larvae fed with mulberry leaves supplemented with amino acids during late instars resulted in maximum filament length.

Filament weight (g): It is seen from table 2 that the treatment T_5, which consisted of vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5%, exhibited the highest filament weight at 0.29 g. Followed by T_3, including vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5%, with a filament weight of 0.28 g and T_1, including vitamin C 0.25% + soya flour 0.25%, with a filament weight of 0.27 g, the lowest filament weight was observed in T_9, the control group, at 0.17 g. The range of filament weight varied from 0.17 g to 0.29 g. These outcomes are connected to those of Khyade and Gosavi (2016) [8], who showed that mulberry leaves treated with an aqueous solution of cowpea (*V. unguiculata*) seed powder greatly increased the weight of silk filaments (21.317 %). Anil Kumar and Sunil Kumar (2018) [2] found that mulberry leaves enriched with 0.5% methionine enhanced filament weight of silkworm, *Bombyx mori* L. According to Sawant *et al.* (2016) [17], supplementing silkworms (*Bombyx mori* L.) with 0.2% ascorbic acid improved their economic metrics, such as filament weight. Ravi Kumar and Anil Kumar (2016) [16] reported that folic acid supplementation of mulberry leaves had a favourable influence on filament weight of silkworm, *Bombyx mori* L.

Denier: According to the data presented in Table 2, it is evident that treatment T_5, comprising 0.25% vitamin C, 0.25% soya flour, 0.5% vitamin B-Complex, and 0.5% methionine, demonstrated the highest denier at 2.97. In contrast, the control group, T_9, exhibited the lowest denier at 1.96. The range of mean denier varied from 1.96 to 2.97. These outcomes are connected to those of Khyade and Gosavi (2016) [8], who showed that mulberry leaves treated with an aqueous solution of cowpea (*V. unguiculata*) seed powder greatly increased the denier. Thulasi and Sivaprasad (2013) [20] demonstrated that mulberry leaves, when treated with ascorbic acid and lemon juice, exhibited a positive impact on denier. Anil Kumar and Sunil Kumar (2018) [2] found that mulberry leaves enriched with 0.5% methionine enhanced denier of silkworm, *Bombyx mori* L. According to Sawant *et al*. (2016) [17], supplementing silkworms (*Bombyx mori* L.) with 0.2% ascorbic acid improved their economic metrics, such as denier. Quraiza *et al.* (2016) [11] demonstrated fortification of the mulberry leaves by nutrient supplementation, it resulted that 7 % plant protein (soya flour) showed significant increase in parameters such as denier over control. Ravi Kumar and Anil Kumar (2016) [16] reported that folic acid supplementation of mulberry leaves had a favourable influence on denier. Deepa *et al*. (2020) [6] observed that when silkworm larvae fed with mulberry leaves supplemented with amino acids during late instars resulted finer denier.

Conclusion

The present investigation of research topic concluded that all economic parameters based on overall performance it was noticed that vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% + methionine 0.5% and vitamin C 0.25% + soya flour 0.25% + vitamin B-complex 0.5% performed best among all combinations of nutritional supplements for single cocoon weight, single shell weight, cocoon shell ratio, effective rate of rearing by number, effective rate of rearing by weight, filament length, flament weight and denier.

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