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Effect of vital dye supplimentation on nutritional indices of mulberry silkworm, *Bombyx mori* L.

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Abstract
There is a growing interest to introduce more new functionalities into silk while maintaining its advantageous intrinsic properties. Various methods with their merits are assessed to produce functional silk, specifically those with colour and luminescence. Vital dyes are stains can be applied on living cells without killing them. Silkworms fed on supplemented leaves dipped in different vital dye mixed mulberry leaves to all the larval instars four times in a day produce coloured larvae and cocoon. Hence it is necessary to study the overall nutritional quality index for nutritional rating. Lower concentrations of vital dyes proved to be considerably effective along with mulberry feed without any drastic effects on their digestive system, feeding efficacy, nutritional indices, their length and weight, colouring of larva and cocoons, survivability and cocooning, shell ratio, weight of shell and cocoon. These findings reported that economic traits was noticed and found to be variously coloured. Vital dyes which performed best results amongst the treatments and natural diet. This report showed that, mulberry treated with vital dyes significantly improved the silk production along with colouring of silkworm and their cocoons.

Keywords: Silkworm, mulberry leaves, vital dyes, nutritional indices, economic traits

Introduction

Sericulture is an art of rearing silkworm for the production of cocoons that is the raw material for the production of silk. It particularly suits rural-based farmers, entrepreneurs and artisans, as it requires low investment with potential for relatively higher returns. In India, Sericulture is mostly a village-based industry providing employment opportunities to a large section of the population. The present global scenario clearly indicates the enormous opportunities for the Indian silk industry. Mulberry silk is the most popular variety and contributes more than 80% of the Country’s silk production. Silk and silk products are very good foreign exchange earners. The commonly domesticated silkworm *Bombyx mori* feeds on the leaves of mulberry plant and converts leaf proteins into silk proteins, is one of the most valued of textile fibers. The success of sericulture industry reflects several factors such as morphometric parameters, economic traits, biological characters, etc. In order to obtain these desirable outputs, skillful utilization of hybrid varieties and use of improved variety of food supplementation are necessary. Feeding is the most important operation in silkworm rearing as it finally decides the success and profitability of sericulture (Krishnaswamy *et al* 1973) [6]. Nutritional quality serves to accelerate the growth, metamorphosis and forms the physiological foundation for sericulture. Therefore, nutrition is an important growth regulatory factor and a physiological need, which depends on the nature of nutritional resources, pattern of acquisition, allocation of incoming resources and the metabolic capacity for stored reserves in the insect which are all critical for fitness (Nirmal Kumar,S., 2012) [7]. Nutritional requirement in food consumption have direct impact on the overall genetic traits such as larval growth and cocoon weight, amount of silk production, pupation and reproductive traits (Ganesh prabu, P.,*et al* (2012) [4]. Thus the enrichment of mulberry leaves by nutrient supplementation is one of the strategies by which cocoon and silk productivity can be increased and the quality can be enhanced Silkworm requires a balanced nutrition for a robust growth. Recently there are many investigations which involves the enrichment of mulberry leaves by nutrient supplementation (Rouhollah, R 2010 [9]., Ganesh prabu., P *et al* 2012 [4], Samatha *et al* 2014) [10]. In recent years, many attempts have been made to improve the quality and quantity of silk through enhancing the leaves with nutrients, spraying antibiotics, vitamins, hormones and hormone analogues or plant extracts. There have been a series of trial and error investigations from the 20th century to generate coloured silk using dye added diet (Koyler J. M 1965) [5]; Edwards 1921) [2]. Some researchers observed that certain dyes pass through the midgut of insects Roeder (K.D.1953 [8], Zacharuk R.Y.1963) [13]. These investigations aimed to analyze the toxic effect of colours on the physiology of insects and to understand the retention time of mulberry leaves in the silkworm gut. These works did not conclusively indicate whether the dyes were successfully incorporated into silk and more importantly in silk fibroin due to the lack of appropriate and accurate characterization techniques at that time.

Materials and Methods

Mulberry leaves: The V-1 (Fig.1) mulberry plant leaves were washed thoroughly in tap water and shade dried to remove the water drops before feeding. From 3rd instar onwards, they were shifted to large trays and they were fed on mulberry leaves, *ad libitum*.

Silkworm larvae: The eggs of CSR2 mulberry silkworm strains (Fig.2) were collected from the government grainage centre, at Manikandam, Tiruchirappalli. These were grown in rearing room under hygienic conditions.

Fig 1: V-1 Mulberry

Fig 2: Silkworm *Bombyx mori* -CSR2

Vital dyes

Neutral red, Rhodamine B, Acridine orange, Eosine and Light green are some of the common biological dyes used. All the experimental groups were observed to find the effect of dyes on the larval body, haemolymph, silk gland, cocoon and in the degummed cocoons. As colour manipulation was the focus of this investigation, all the groups thoroughly noticed after the consumption of dye added diet (Anumol Anto 2017) [1]. Along with the observation of colour change, visible effects such as normal growth, larval duration and mortality were also evaluated.

Analysis of nutritional traits: Nutritional indices were studied for fourth and fifth instar larvae of CSR2. Control and five dye added diet fed groups were used at standard conditions described by (Krishnaswamy *et al* (1973)[6].The temperature and humidity were maintained at 25^0C and 70% respectively. To measure the selected dietary parameters, a known quantity of mulberry leaves should be provided to the animals.

Ingesta = Dry weight of given leaf – Dry weight of leftover leaf

Digesta = Dry weight of the ingested food – dry weight of excreta

Approximate digestibility (AD%) = Dry weight of digesta/Dry weight of ingesta X 100

Reference Ratio (RR) = Dry weight of ingesta/Dry weight Digesta

Efficiency conversion of ingesta to larva (%) (ECI of larva) = Maximum dry of larvae/dry weight of ingesta X 100

Efficiency conversion of ingesta to cocoon (%) (ECI to cocoon) = Dry weight of cocoon/dry weight of ingesta X 100

Efficiency conversion of ingesta to shell (%) (ECI to shell) = Dry weight of shell/dry weight of ingesta X 100

Efficiency conversion of digesta to larva (%) (ECD) to larva = Maximum dry weight of larva/dry weight of digesta X 100

Efficiency conversion of digesta to cocoon (%) (ECD to cocoon) = Dry weight of cocoon/dry weight of digesta X 100

Efficiency conversion of digesta to shell (%) (ECD to shell) = *Dry weight of shell/dry weight of digesta* X 100

From the observed values, the data on the biomass of the larva and cocoon was obtained for the following 10 nutrigenetic traits and the standard gravimetric procedure. The data subjected to statistical analysis using Two way (SPSS Version 16) ANOVA.

Measurement of larval growth: The mean weight of the thirty randomly selected fourth and fifth instar larvae from each replication were recorded everyday using an electronic balance. Along with that the length of the same larvae were measured manually by marking the two proximities of larva and then the distance between the two points measured Fujia Chen *et al* (2012) [3].

Result

Silkworm nutritional indices are consumption of the feed and the conversion of the components for their growth and metabolism. *Bombyx mori* is a monophagous insect solely depends on the proteins of mulberry plant. Digested leaves are converted into fibroin and sericin in their silk gland for synthesis of silk. The experiments revealed the data for the nutritional efficacy of coloured mulberry with vital dyes.

Table 1: Recorded the data on Ingesta, Digesta, Approximate Digestibility and Reference Ratio for the fourth and fifth instar larvae, The intake of mulberry leaves of control group of worms seems to be less than coloured leaves consumed worms. The highest intake was observed in Acridine orange mixed group consumed larvae as 37.25 ± 0.06^a during 4 th instar and 99.75 ± 0.01^a in the 5 th instar which is followed by Neutral red, Rhodamine B, Light green and Eosin yellow treated groups. The digesta of the treated groups were calculated and found to be same in par with ingesta. The Average Digestibility was more in Acridine orange group was 32.31 ± 0.01^a during 4th instar and 97.92 ± 0.01^a in their 5th stage, followed by Rhodamine B, Neutral red, Light green and Eosin yellow with not much difference. The same trend was observed in Reference Ratio of all the group of worms during 4th and 5th instar stages of silkworm larva.

Table 1: Nutrition consumption traits of CSR2- 4TH & 5 TH instar of *Bombyx mori* (Value are Mean ± Standard deviation of all the results are significant (p > 0.05)

Experiments	Ingesta (g)		Digesta (g)		AD (g)		RR (g)	
	IV instar	V instar	IV instar	V instar	IV instar	V instar	IV instar	V instar
Control	17.32± 0.02^{f}	90.99± 0.01^{d}	13.71± 0.01^{f}	94.92± 0.02^{c}	79.05± 0.05^{f}	92.89± 0.01^{c}	4.75± 0.05^{f}	14.05± 0.05^{c}
Neutral red	23.51± 0.01^{c}	74.99± 0.01^{f}	19.52± 0.02^{d}	68.99± 0.01^{f}	83.04± 0.04^{e}	91.99± 0.01^{d}	5.89± 0.01^{e}	12.49± 0.01^{d}
Eosin yellow	20.94± 0.05^{e}	89.99± 0.01^{e}	17.72± 0.02^{e}	82.15± 0.05^{e}	84.53± 0.03^{c}	91.31± 0.01^{e}	6.44± 0.04^{c}	11.51± 0.01^{e}
Rhodamine-B	23.43± 0.04^{d}	98.49± 0.01^{b}	20.63± 0.04^{b}	95.99± 0.01^{b}	88.05± 0.05^{a}	97.43± 0.04^{a}	8.35± 0.06^{a}	18.72± 0.01^{a}
Acridine orange	37.25± 0.06^{a}	99.75± 0.01^{a}	32.31± 0.01^{a}	97.92± 0.01^{a}	86.64± 0.04^{b}	94.42± 0.02^{b}	7.49± 0.01^{b}	17.99± 0.01^{b}
Light green	23.62± 0.02^{b}	92.69± 0.01^{c}	19.68± 0.01^{c}	91.91± 0.02^{d}	83.24± 0.04^{d}	90.90± 0.01^{f}	5.94± 0.05^{d}	10.99± 0.01^{f}

Table 2: showed the data of Efficiency conversion of Ingesta (ECI) of Larva, Cocoon and Shell. During 4th larval stage the ECI of larva was almost equal along with control group. When comes to cocoon and shell Eosine Yellow and Rhodamine B are resembling the control group followed by light green, neutral red and Acridine Orange. The results obtained during 5th instar was not in par with the control group of worms. Eosin yellow, Rhodamine B and Neutral red fed animals more efficiency of converting their ingested leaves than light green and Acridine Orange with Larva, cocoon and the shell with meager differences.

Table 2: Nutrition efficiency traits of CSR2- 4th & 5th instar of *Bombyx mori mori* (Value are Mean ± Standard deviation of all the results are significant (p > 0.05)

Experiments	ECI of larva (g)		ECI of cocoon (g)	ECI of shell (g)
	IV instar	V instar		
Control	4.43± 0.05^{a}	4.95±0.05^{b}	1.65±0.05^{e}	0.33±0.03^{d}
Neutral red	3.82±0.05^{d}	5.10±0.03^{a}	1.91±0.01^{c}	0.32±0.02^{e}
Eosin yellow	4.32±0.05^{b}	4.93±0.03^{c}	2.15±0.05^{a}	0.45±0.05^{a}
Rhodamine-B	3.88±0.05^{c}	4.90±0.01^{d}	2.01±0.01^{b}	0.43±0.03^{b}
Acridine orange	3.15±0.05^{f}	4.86±0.04^{f}	1.19±0.01^{f}	0.27±0.01^{f}
Light green	3.38±0.05^{e}	4.89±0.01^{e}	1.85±0.05^{d}	0.35±0.05^{c}

Table 3: was obtained from the results of the data of Efficiency conversion of Digesta (ECD) of the Larva, Cocoon and Shell. During 4th larval stage the ECD of larva the Eosine yellow worms showed 4.88±0.04 was almost equal along with control group. When comes to cocoon and shell Eosine Yellow and Rhodamine B are showing similar data to the control group followed by light green, neutral and Acridine Orange. The results obtained during 5th instar showed ECD of Larva for neutal red, rhodamine B and eosine yellow are more loser with control group of worms. ECD of Cocoon and Shell was also followed the the above trend of efficiency of converting their digested feed.

Table 3: Nutrition Efficiency Traits of CSR2- 4th & 5th instar of *Bombyx mori mori* (Value are Mean ± Standard deviation of all the results are significant (p > 0.05)

Experiments	ECD of larva (g)		ECD of Cocoon (g)	ECD of shell (g)
	IV instar	V instar		
Control	4.63±0.04^{c}	5.71±0.01^{d}	1.80±0.01^{d}	0.35±0.05^{d}
Neutral red	4.62±0.02^{d}	5.81±0.01^{a}	2.05±0.05^{b}	0.34±0.04^{e}
Eosin yellow	4.88±0.04^{a}	5.75±0.05^{c}	2.06±0.05^{a}	0.51±0.01^{a}
Rhodamine-B	4.53±0.03^{e}	5.64±0.02^{e}	2.04±0.04^{c}	0.43±0.04^{b}
Acridine orange	4.65±0.05^{b}	5.80±0.02^{b}	1.21±0.01^{e}	0.25±0.05^{f}
Light green	4.10±0.01^{f}	5.62±0.04^{f}	2.04±0.01^{c}	0.41±0.02^{c}

Table 4: Length and Weight of 4th & 5th larval stages of *Bombyx mori mori* (Value are Mean ± Standard deviation of all the results are significant (p > 0.05)

Experiments	Length of larva (cm)		Weight of larva (gm)	
	IV instar	V instar	IV instar	V instar
Control	4.22±0.14^{b}	7.19±0.04^{a}	0.77±0.01^{f}	4.48±0.14^{b}
Neutral red	4.02±0.12^{d}	7.18±0.03^{b}	0.90±0.10^{d}	4.01±0.01^{d}
Eosin yellow	4.00±0.12^{e}	7.16±0.07^{c}	0.91±0.10^{c}	4.01±0.01^{d}
Rhodamine-B	4.08±0.04^{c}	7.15±0.03^{d}	0.92±.0.10^{b}	4.44±0.01^{c}
Acridine orange	4.30±0.13^{a}	7.14±0.03^{e}	1.19±0.01^{a}	4.84±0.01^{a}
Light green	3.96±0.04^{f}	7.18±0.03^{b}	0.81±0.01^{e}	3.75±0.01^{e}

Physical parameters of larva such as the length and weight reflect the general health of the larva are presented in Table 4. The regular data obtained from control and all the vital dye treated silkworm was presented in Table 4. Regarding the length of the worms there in a increasing trend was noticed in neutral red, light green and Rhodamine B treated groups followed by Acridine orange and Eosin yellow with not much difference with control during 4th and 5th instar stages. The weight of the silkworm treated with acridine orange was more than other vital dye treated groups during 4th and 5th instar stage of silkworm larvae *bombyx mori.*

Fig 3: CSR2 mulberry silkworm *Bombyx mori* fed with vital dye fortified leaves A – Control, B–Neutral Red, C–Rhodamine-B, D–Eosin Yellow, E–Acridine Orange, F –Light Green

Discussion

It is evident that the larval growth and the cocoon quality are influenced up to an extent by the nutritional efficiency of silkworm and intern influenced by the quality of food. Therefore, from the data on nutritional parameters analyzed itself, it was expected that the larval physical traits or characters would not be affected by the dyes selected for this study. To confirm the expected results, the nutritional indices, length and weight of fifth instar larva were significantly same in control and vital dye treated groups of silkworm. It was observed that Neutral red, Rhodamine B, Acridine orange, Light green and Eosin yellow visually modified the cocoon colour noticeably (Tansil *et al*., 2011 [11], Trivedy *et al*., 2016) [12]. This proves it is a promising technology for producing various vital colour silk in the near future. It provides a cost effective method for dyeing silk, which avoids hazardous chemicals. The process has any unfavorable effects on the silkworm, as there is no significant difference in the observed traits of normal and experimental group silkworms. Using various vital dyes in appropriate concentrations, differently colour silk could be synthesized in the near future.

Conclusion

The final indices to assess nutritional efficiency in terms of the cocoon or shell are the efficiency of conversion of ingesta to cocoon and shell, also known as leaf-cocoon and leaf-shell ratios. Given the nutritional efficiency conversion features, it is clear that neither the CSR2 strains of silkworms significantly changed as a result of the dye being added to their diet. These investigations of the phenotypic expression of 10 chosen nutrigenetic features demonstrated that neither the dye added diet nor the chosen attributes of *B.mori* were affected by the nutritional factors. The selected dye may be assumed to have had no negative effects on the other physiological and biochemical characteristics given that there is no perturbation on these crucial nutritional indicators. But additional research is required to corroborate it.

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