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Nutritional parameters of CSR2 and PM X CSR2 strains of *Bombyx mori* fed with Neutral red as a food supplement

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ABSTRACT

Recently, there are various methods to incorporate additional properties into silkworm silk, during its production in the silk gland itself. These advanced properties involve additional mechanical strength, colour, ultraviolet resistance, etc. and it is utilized for a wide range of applications. The property which focused in this study is the colour, an important quality attribute of silk. Effect of neutral red, a vital dye used as dietary supplement and its nutritional effects on *Bombyx mori (B. mori)* along with the colour manipulating efficiency on the larvae were observed. The colour change noticed from larval to cocoon stage confirms its efficiency to get into the silk gland of the animal crossing through the integument and haemolymph. The results of the observed nutritional parameters confirms that the selected dye in the selected concentration is suitable to produce colour cocoons without disturbing the nutritional traits such as ingesta, digesta, approximate digestibility percentage, growth rate, efficiency conversion rate, etc. of the larva. The findings elucidates that more vital dyes may use for the production of colour silk without causing any harm to the silkworm physiology. As the dye has no harmful effect in the dietary parameters, which plays a major role in the growth of the larva and production of silk, it is assumed that it may not affect the economical and biochemical parameters also. However, it has to be confirmed by further analyses.

Keywords**:** *Bombyx mori*, neutral red, nutritional traits, efficiency conversion

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1. INTRODUCTION

Silkworm silk is a fibrous protein reeled from silkworm cocoons. It is one of the most popular textile fibers used for various applications due to its incredible physical, chemical and mechanical properties. Nowadays there are attempts to improve the properties of silk which increases its popularity and demand all the world over. Recent research by (Lingyue et al., 2015) [1].

Elucidates the synthesis of more ultraviolet resistant silk by adding titanium dioxide nanoparticles as dietary supplements to the silkworm *B. mori*. Similar investigations to improve or manipulate the colour of cocoons by adding selected dyes in selected concentrations are reported by several researchers [2-5].

As colour is an attracting quality of silk, dyeing of silk is unavoidable in the textile industry. However, this dyeing process is a threat to the environment as it affect the water bodies, aquatic population and in turn the human beings as it releases dye waste abundantly into the environment especially to the water bodies [6].

It also requires a huge quantity of water for the processing and releases volumes of toxic water as byproduct [7]. As it considered as one of the most polluting industries, there is a need to develop eco friendly methods to reduce dye waste pollution. On this background, the above mentioned researches aims the reduction of dye waste pollution by introducing a new technology for the production of colour silk in the silk gland itself before spinning.

Among a few investigations regarding colour silk production, some reported the production of cocoons with remarkable colour change, which persisted in the silk thread even after degumming [4]. Thus, it offers a natural silkworm system which produces coloured cocoon in a variety of colours which can be obtained by the selection of suitable dyes in appropriate concentrations. By further researches and improvements of this technology, pollution due to dyeing industry may be reduced to a remarkable extend.

However, it is necessary to analyze the effect of these dyes on the silkworm characters other than colour. Trivedy et al (2016) [4] recorded the physical characters of CSR2 X CSR4 Strain of *B. mori* fed on rhodamine B. Anumol *et al* (2017) [5] reported the changes of the larval and cocoon parameters of *B. mori* fed on various vital dyes. There are not much reports regarding the physical and physiological traits of *B. mori* fed with dye added diet. Nutrition plays an important role in improving the growth and development of the silkworm *B. mori* like other organisms [8].

Besides that, nutrition affects nearly all biological processes including the rates of biological and physiological reactions [9, 10] and also it affects the quality and quantity of cocoons [11, 12]. Silkworm requires a balanced nutrition for a robust growth. Nutrition 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 [13]. As nutrition plays an important role in the profitability of sericulture, it is important to confirm the influence of the selected dyes on the nutritional traits of the larvae [23-26].

2. MATERIALS AND METHODS

2. 1. Experimental animal – *Bombyx mori*

The silkworm, *B. mori* (Lepidoptera) PM × CSR2 and CSR2 strains were used in this study. Eggs of PM X CSR2 obtained from the Grainage centre, Sericulture Department in the Regional Deputy Director's office, Tiruchirappalli, Tamil Nadu. CSR2 larvae obtained at the first instar stage from the Government rearing centre, Manikandam, Trichy.

2. 2. Silkworm feed

Morus alba is the common mulberry plant and the sole preference of the silkworm larvae. This perennial plant is well suited for the Indian climate. All the essential elements such as protein, carbohydrate, inorganic salts, essential vitamins and minerals, required for the physiological functions of the silkworm are present in it. The V1 mulberry leaves to feed the control animal were collected from the college garden. The control animals fed regularly with mulberry leaves throughout the period. The experimental group fed with the prepared modified diet.

2. 3. Preparation of dye added diet

The dye selected in this study is neutral red (Nice Chemicals), a vital dye. After some trials, a concentration (0.02 wt %) which has no harmful effect on silkworm life cycle was selected. The dye solution sprayed uniformly on the mulberry leaves and fed the silkworms from the fifth day of fifth instar.

2. 4. Nutritional parameters of *B. mori*

In order to investigate the effect of the selected dyes on the nutritional and dietary efficiency of *B. mori*, 11 parameters were analyzed. After the fourth moult, three replications of each group of the two strains reared in separate trays at standard conditions described by Krishnaswami et al (1973) [14].

The temperature and humidity were_maintained_at 25 °C and 70% respectively. To measure the selected dietary parameters, a known quantity of mulberry leaves should be provided to the animals. For that, mulberry leaves were accurately weighed in an electronic balance and fed three times a day to both experimental and control groups of silkworms of the two strains. For the determination of the dry weight of the ingesta, the same quantity of mulberry leaves kept as dummies. For each treatment, additional batches of larvae were maintained to observe the subsequent changes in the larval weight as per the reports by Maynard and Loosli, 1962 [15]. From the observed values, the various nutritional parameters are calculated using the following equations described by Waldbauer, 1968 [16].

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 of excreta

Relative Growth Rate (RGR) = Weight gain of the larva during feeding period/mean fresh weight of larva during fifth instar X larval duration of fifth instar in days.

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

3. RESULTS AND DISCUSSION

3. 1. Colour manipulation in experimental group silkworms

Fig 1. V instar larvae of CSR2; a) control, b) neutral red and PM X CSR2; c) control, d) neutral red [5]

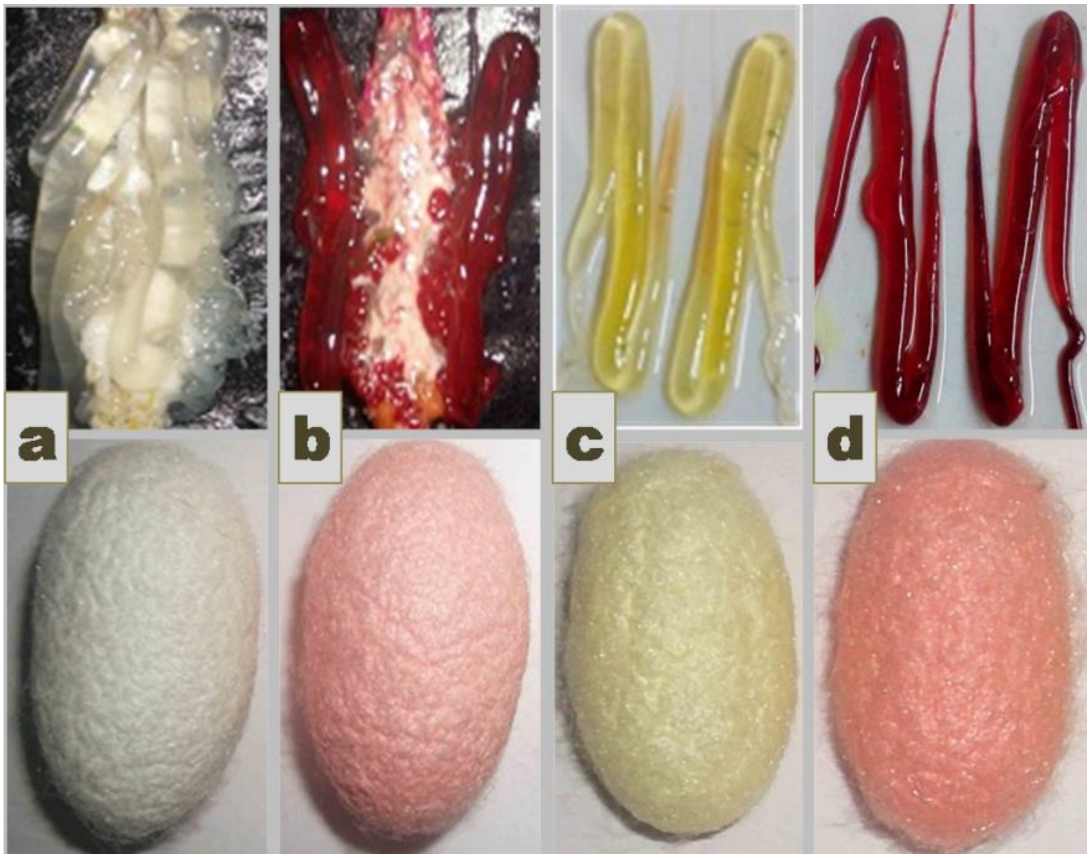

Fig 2. Silk gland and cocoon of CSR2; a) control, b) neutral red and PM X CSR2; c) control, d) neutral red [5]

In the present investigation, the colour of the larva, silk gland and cocoon were changed by feeding neutral red added diet to the fifth instar larvae. Our previous study reported the synthesis of the colour cocoons through a green way by feeding neutral red added diet [5]. Both CSR2 and PM X CSR2 strains of *B. mori* used for the experiment and the colour changes observed in the larvae, silk gland and cocoons of both the strains (Fig. 1 and 2). This green process without any chemical pollution offers the development of an insect system that produce coloured silk directly from the mulberry silkworm *B. mori* through the in vivo uptake of vital dyes. In order to analyze the nutritional effects of this dye on *B. mori*, the experiment repeated at the same standard conditions and the similar colour change noticed in the animal groups fed with dye added food. The body colour of the larvae changed into dark pink from the day of treatment. The reason for it is the dye absorption from the gut into the silk gland through haemolymph. Further studies with different concentration of various dyes may lead to the production of silk with a variety of colours without much pollution of the environment.

3. 2. Nutritional consumption and efficiency traits

Under standard nutritional estimation condition, data were recorded for food intake (ingesta), digesta, approximate digestibility, reference ratio, relative growth rate, efficiency conversion of ingesta and digesta for larva, cocoon and shell.

Table 1. Nutrition Consumption Traits of CSR2.

Groups	Ingesta	Digesta	AD %	RR	RGR
CN	3.920±0.095	1.584±0.011	40.409±0.728	1.679±0.022	0.110±0.001
NR	3.897±0.125	1.579±0.008	40.548±1.094	1.682±0.031	0.110±0.001

AD = Approximate Digestibility, RR = Reference Ratio, RGR = Relative Growth Rate. Values given are the mean ± standard deviation of three values from three experimental set up.

Table 2. Nutrition Consumption Traits of PM X CSR2.

Groups	Ingesta	Digesta	AD %	RR	RGR
CN	3.843±0.068	1.530±0.008	39.815±0.904	1.660±0.028	0.102±0.001
NR	3.843±0.068	1.530±0.008	39.826±0.867	1.662±0.024	0.102±0.000

AD = Approximate Digestibility, RR = Reference Ratio, RGR = Relative Growth Rate. Values given are the mean ± standard deviation of three values from three experimental set up.

Table 3. Nutritional Efficiency Conversion Traits of CSR2.

Groups	ECI larva (%)	ECD larva (%)	ECI cocoon (%)	ECD cocoon (%)	ECI shell (%)	ECD shell (%)
CN	20.42±0.65	50.52±0.75	15.11±0.33	37.38±0.35	7.26±0.12	17.98±0.27
NR	20.53±0.16	50.66±1.01	15.22±0.30	37.55±0.29	7.27±0.09	17.94±0.35

ECI = Efficiency Conversion of Ingesta, ECD= Efficiency Conversion of Digesta.
Values given are the mean ± standard deviation of three values from three experimental set up.

Table 4. Nutritional Efficiency Conversion Traits of PM X CSR2.

Groups	ECI larva (%)	ECD larva (%)	ECI cocoon (%)	ECD cocoon (%)	ECI shell (%)	ECD shell (%)
CN	18.13±0.47	45.54±0.17	12.79±0.16	32.12±0.43	4.80±0.05	12.05±0.33
NR	18.22±0.11	45.75±0.80	12.83±0.06	32.20±0.56	4.76±0.07	11.96±0.45

ECI = Efficiency Conversion of Ingesta, ECD = Efficiency Conversion of Digesta.
Values given are the mean ± standard deviation of three values from three experimental set up.

All these observed nutritional consumption and efficiency traits are the major factors for evaluating the effect of food on the animal. It influences the productivity and profitability of sericulture. The main objective of this study was to find out the efficiency conversion of ingesta to larval biomass, cocoon and larvae when fed with dye added mulberry leaves. The nutritional efficiency of conversion contributes directly to the major portion of the cost benefit ratio of silkworm rearing, and is considered an important physiological criterion for evaluating nutritional superiority. The nutritional parameters measured is presented in Tables 1 and 2. There was no considerable variation between the nutrigenetic traits of control and experimental groups in both the strains of *B. mori*.

In the case of CSR2 strains, control and experimental groups showed only slight variations in the nutritional consumption traits. Approximate digestibility is around 40 % in the control and treated groups. The differences in ingesta (3.920 and 3.897 for control and neutral red groups respectively), digesta (1.584 and 1.579 for control and neutral red groups respectively) and reference ratio (1.679 and 1.682 for control and neutral red groups respectively) between the groups are not remarkable while relative growth rate is exactly same (0.102) in both the groups. For both the control and experimental groups of PM X CSR2 strain the ingesta, digesta and relative growth rate were 3.843, 1.530 and 0.102 respectively. Approximate digestibility was 39.826 for the control and 39.815 for neutral red fed groups. Reference ratio also has almost similar values for both the groups (1.660 and 1.662 respectively). Thus it can be concluded that there is no significant differences between the nutrition conversion traits of control and experimental groups in both CSR2 and PM X CSR2 strains.

Nutritional efficiency conversion traits analysis also provided the results with same trend where control and neutral red added diet fed groups had no remarkable difference in both the silkworm varieties. There was an increase in the efficiency conversion of ingesta and digesta percentage in the experimental groups of the both strains than that of their control groups. The efficiency of conversion of ingesta and digesta of larva are 20.42 and 50.52 for control while it is 20.53 and 50.66 for experimental group of CSR2 strains. Efficiency conversion of cocoon also showed the same trend with 15.11 and 37.38 for control and, 15.22 and 37.55 for experimental group respectively. Similar results observed for efficiency conversion of ingesta and digesta of larva and cocoons in the PM X CSR2 strains of *B. mori.*

However, a slight decrease observed in efficiency conversion of ingesta to shell of the experimental group in PM X CSR2 strain and the same trend in efficiency conversion of digesta to shell in both the strains were observed. However, it also is not a remarkable difference.

4. CONCLUSION

The efficiency of conversion of ingesta to cocoon and shell, which are otherwise referred to as leaf-cocoon and leaf-shell ratios, are the ultimate indices to evaluate nutritional efficiency in terms of the cocoon or shell [17-22]. On considering the nutritional efficiency conversion traits, it can be identified that the the addition of dye into the diet, caused no noticeable changes in CSR2 and PM X CSR2 strains of silkworms. These analyses of the phenotypic expression of 11 selected nutrigenetic traits confirmed that the dye added diet cause no disturbance on the nutritional parameters of both the selected traits of *B. mori*. As there is no disturbance on these important nutritional parameters, it may considered that the selected dye would have been no adverse effect on the other physiological and biochemical characters. However, it has to be confirmed by further studies.

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