

Effect of Nutritional Fortification of Nanoparticles of Riboflavin on the Growth and Development of Mulberry Silkworm, *Bombyx mori* L.

M. Kamala* and A. Karthikeyan

Dept. of Zoology, Government Arts College (Autonomous), Karur, Tamil Nadu (639 005), India



Open Access

Corresponding Author

M. Kamala

e-mail: kamala2519a@gmail.com

Keywords

Riboflavin, nanoparticles, enrichment, silk glands

How to cite this article?

Kamala and Karthikeyan, 2019. Effect of Nutritional Fortification of Nanoparticles of Riboflavin on the Growth and Development of Mulberry Silkworm, *Bombyx mori* L.. *Research Biotica* 1(1), 23-30.

Abstract

The freshly collected mulberry leaves were smeared with solutions of nanoparticles of riboflavin a vitamin (B2) in different concentrations. The enriched leaves were air dried. The fifth instar larvae of mulberry silkworm *Bombyx mori* L. were fed with enriched leaves thrice a day. Feeding parameters, Food conversion efficiency, growth and economic characters were studied and recorded. Supplementation of nanoparticles of vitamin B2 showed a positive impact and significantly enhanced the growth of the larvae (28.985%), silk gland weight (111.392%) and silk yield (194.44%) when compared to control. So vitamin B2 nanoparticles can be used as a fortification agent for improving the silk production.

1. Introduction

The mulberry silkworm, *Bombyx mori* L. is an economically important insect in the silk industry. Its economic significance is attributed to its silk secreting ability. The successful harvest of quality cocoons depends exclusively on the nutrition of the silkworm (Nagesh and Devaiah, 1996). Nutrition of silkworm is the sole factor which almost individually augments the quality and quantity of cocoon production (Laskar and Datta, 2000). Therefore among all the insects, the mulberry silkworm was considered the fittest organism to carry out the proposed study.

Silkworm is an oligophagus insect feeds mainly on mulberry leaves. 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. Enrichment of the 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 improve the economic value of cocoons (Krishnaswami *et al.*, 1971). Fortification of mulberry leaves with supplementary compounds like vitamins (Saha and Khan, 1996, Nirwani and Kaliwal, 1996, Nirwani *et al.*, 1998,

Etebari *et al.*, 2004, Rahmathulla *et al.*, 2007); hormones (Magadum *et al.*, 1992; Trivedy *et al.*, 1993; Saha and Khan, 1997), aminoacids (Khan and Faruki, 1990; Qadar *et al.*, 1994; Saha *et al.*, 1994; Saha and Khan, 1997) and minerals (Magadum *et al.*, 1992; Islam and Khan, 1993; Khan and Saha, 1995) to increase the larval growth and development. The influence of micronutrients on larval development and cocoon characters of silkworm were also studied (Vishwanath and Krishnamurthy, 1983).

Several studies have been conducted on food supplementation and developing artificial diet for rearing of silkworms. The perusal of literature indicated food supplementation of silkworm larvae have resulted in the improvement of commercial and biological aspects of sericulture whereas artificial diet had little success in commercial rearing (El-Karaksy and Idriss, 1990; Kabila *et al.*, 1994; Sarkar *et al.*, 1995; Yasmin *et al.*, 1995; Nirwani and Kaliwal, 1996 and 1998; Zaman *et al.*, 1996; Basit and Ashfagh, 1999; Goudar and Kaliwal, 1999; Etebari and Matindoost, 2005). These studies show that nutritional supplements have a significant impact on larval growth and cocoon production (Ito and Tanaka, 1962).

Vitamins are a group of organic compounds needed only in minute quantities in the diet that are essential for specific metabolic reactions within the cell and necessary for normal growth and maintenance of health. Dosages of vitamins are

Article History

RECEIVED on 18th October 2019

RECEIVED in revised form 27th December 2019

ACCEPTED in final form 28th December 2019

very determinative for normal growth of silkworm. Effects of multivitamins on the silkworm have been confirmed by Saha and Khan (1996), and Etebari and Matindoost (2004). However, it has been reported that dietary supplementation of vitamins and minerals produced a significant increment in the growth and development of *B. mori* (Saha and Khan, 1996). Supplementation of mulberry leaves with Vitamin B enhanced resistance in silkworm larvae against conditions of environmental stress which resulted in body weight increment as compared to control (Das and Medda, 1998; Rahmathulla *et al.*, 2002; Raman *et al.*, 2007).

Nanotechnology is emerging out as the greatest imperative tools and has positive impact in the advancement of functional feed which deliver the nutrients effectively. Nanotechnology a rapidly evolving field affords novel ways to enhance the growth and production in the field of nutrition. Applications of nanotechnology studies in nutrition of silkworm are limited. Considering the key role played by vitamin absorption in silkworm nutrition and development, attempts have been made here to assess the effects of supplementation of riboflavin nanoparticle on the growth and the economic traits of the silkworm, *B. mori* L.

2. Materials and Methods

2.1. Silkworm rearing

The commercial breed of the mulberry silkworm i.e., the double hybrid CSR6 × CSR26 × CSR2 × CSR27 collected from the silkworm rearing department, Rayanur, Karur district was used for this study. The silkworm rearing was followed by the standard protocol suggested by Regional Sericulture Research Station, Salem.

Riboflavin was purchased from Shreya Life Sciences Pvt. Ltd. Uttarakhand. In this work, the size of the riboflavin particles was reduced to the size of 240nm by ball milling method using Pulverisette 7. Such nanoparticles of riboflavin were supplemented to the silkworm larvae along with mulberry leaves in different concentrations. 450 newly emerged larvae were taken and divided into five groups as mentioned in the Table 1. Each group consisted of three trays each containing 30 larvae. The larvae were fed with the mulberry leaves as given in the Table 1.

2.2. Feed efficacy and cocoon parameters

The quantity of mulberry leaves offered to all groups was similar and the larvae were fed three times a day (9.00 AM, 1.00 PM and 5.00 PM). Unfed leaves larvae and faecal pellets were weighed daily and recorded. Based on the data recorded feed efficacy parameters like approximate digestibility, efficiency of conversion of digested food, efficiency of conversion of ingested food, rate of consumption, rate of assimilation, rate of production and rate of metabolism, food consumption Index, coefficient of food utilization and tissue somatic Index were calculated by using the formulae of Macfadyen and Petruszewicz (1970). The growth of the larvae

Table 1: Control and Experimental groups used in this study to determine the effects of nanoparticles of Riboflavin in silkworm larvae.

Groups of Larvae	Description – Silkworm larvae were fed with
Control	Mulberry leaves without any supplementation.
Sucrose (2g/100ml)	Mulberry leaves smeared with 2% Sucrose solution.
Riboflavin I (20µg/ml)	Mulberry leaves smeared with a mixture of 20µg/ml of nano-riboflavin solution and 2% sucrose solution.
Riboflavin II (40µg/ml)	Mulberry leaves smeared with a mixture of 40µg/ml of nano-riboflavin solution and 2% sucrose solution.
Riboflavin III (60µg/ml)	Mulberry leaves smeared with a mixture of 60µg/ml of nano-riboflavin solution and 2% sucrose solution.

was assessed by measuring the weight of the larvae. Cocoon parameters like cocoon weight, cocoon shell weight, cocoon shell ratio, and pupal weight were recorded by using standard methodology described by Etebari *et al.* (2002).

2.3. Statistical analysis

Collected data were subjected to statistical analysis such as student 't' test and single way ANOVA to find out the significant difference between control and experimental groups.

3. Results and Discussion

Feeding parameters of the larvae were recorded in the Table 2 and Figure 1. Feeding parameters like Consumption rate, Assimilation rate, Production rate were significantly greater in the riboflavin supplemented larvae than in sucrose supplemented larvae and control. Though significant difference in these factors was observed among the larvae supplemented with different concentration of nanoparticles of riboflavin, there was no regular increase or decrease in these parameters with the increasing concentration of the riboflavin. The CR, AR and PR were higher in the larvae supplemented with less concentrated nanoparticles of riboflavin than all the other control and experimental larvae. However MR and AD were higher in larvae supplemented with high concentration of riboflavin, whereas ECD, ECI and FCI were higher in larvae supplemented with moderate concentration of riboflavin. It was interesting to note that CFU and TSI were highest in larvae supplemented with high concentration of riboflavin. These data indicated that the

Table 2: Effect of nanoparticles of Riboflavin on the feed efficacy of the Mulberry Silkworm *Bombyx mori* L.

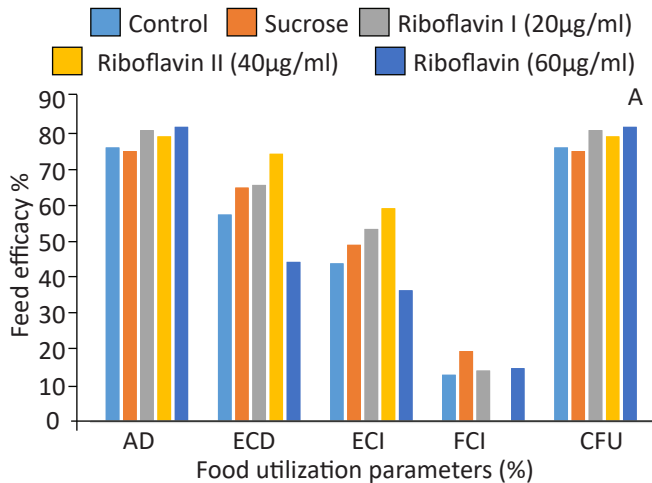
Parameters↓	Larvae Supplemented with					'F' value
	Control	Sucrose	Nanoparticles of Riboflavin I (20µg/ml)	Nanoparticles of Riboflavin II (40µg/ml)	Nanoparticles of Riboflavin III (60µg/ml)	
Consumption Rate (CR) mg/day	181.708 ±3.300	152.264±2.10 [-8.917%] t= 18.440*	200.283±0.980 [10.222 %] t= -13.212*	175.466±1.118 [-3.435 %] t= 4.014*	190.923±0.033 [9.215%] t= -6.829*	148.12*
Assimilation Rate(AR) mg/day	139.026 ±0.489	114.998±0.719 [-17.283%] t= 67.639*	163.089±0.566 [17.308%] t= -78.681*	139.725±0.752 [0.502%] t= -1.897*	157.201±0.609 [13.073%] t= 7.152*	211.52*
Production rate (PR) mg/day	80.381 ±0.853	75.151±0.610 [-6.506%] t= 12.215*	107.864±0.059 [34.190%] t= -78.593*	104.507±1.089 [30.014%] t= -42.662*	70.107±0.190 [-12.781%] t= 28.687*	638.41*
Metabolism Rate (MR) mg/day	102.296 ±0.774	46.804±0.929 [-54.246%] t=112.221*	64.119±0.614 [-37.320%] t= 94.515*	44.613±0.765 [-56.388%] t= 129.667*	87.093±0.551 [-14.861%] t= 39.056*	642.61*
Approximate digestibility (AD)%	76.510±9.952	75.525±15.65 [-1.287%] t= 0.415**	81.429±4.437 [6.429%] t= -1.681**	79.631±5.674 [4.079%] t= -0.976**	82.337±7.270 [7.615%] t= -1.378**	0.411**
Efficiency of conversion of digested food (ECD) %	57.817±47.741	65.349±35.296 [13.027%] t= 0.608**	66.138±28.661 [14.391%] t=-0.149**	74.794±27.753 [29.363%] t= -0.142**	44.597±15.563 [-22.865%] t= 0.796**	0.950**
Efficiency of conversion of ingested food (ECI)%	44.236±11.042	49.355±8.399 [11.572%] t= 0.424**	53.855±28.649 [21.744%] t= -0.505**	59.559±22.430 [34.639%] t= -0.503**	36.720±12.967 [-16.990%] t= 0.519**	0.958**
Food consumption Index (FCI)	13.125±0.482	19.705±0.297 [50.133%] t= 28.353*	14.284±0.739 [8.830%] t=-3.156*	16.353±0.757 [24.594%] t= -8.786*	14.962±0.698 [13.996%] t= -5.274*	12.639*
Coefficient of food utilization (CFU)	76.512±0.924	75.524±0.424 [-1.291%] t= 2.372*	81.429±0.607 [6.426%] t= -10.879*	79.631±0.294 [4.076%] t= -7.841*	82.339±0.420 [7.615%] t= -14.021*	54.163**
Tissue Somatic index (TSI)	60.769±0.488	84.72±0.545 [39.413%] t= 79.944*	96.786±0.566 [59.268%] t=-118.056*	120±0.908 [97.469%] t=-140.688*	144.189±1.043 [136.962%] t=-177.356*	535.50*

Note: Values with ± sign is Standard deviation; Values inside the square brackets indicate the % of change over the control; *Significant at the level of p<0.05, **Not significant at the level of p< 0.05.

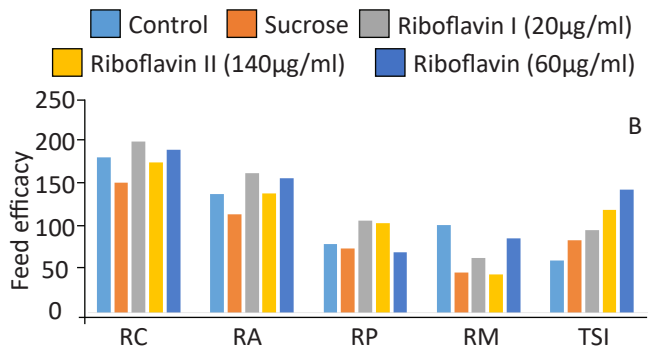
increase in concentration of nanoparticles of riboflavin had no effect on feeding parameters but nanoparticles have definitely influenced the feeding parameters significantly. Riboflavin is an important co-enzyme in energy metabolism and so it plays important roles in the enzymatic reactions. Riboflavin is a vitamin that is needed for growth and overall good health. It helps the body break down carbohydrates, proteins and fats to produce energy, and it allows oxygen to be used by the body (Victor, 2011). Our results are in line with the earlier works reporting Multivitamin and Mineral supplementation of mulberry leaves enhances food intake and conversion efficiency of silkworm (Prasad, 2004).

The increase in food consumption and utilization might be due to the supplementation of nanoparticles of riboflavin which increase the proportion of food nutrients that pass across the gut tissue rather than passing the food directly through the digestive system. Nanoparticles may pass into the cells more readily than the normal riboflavin. This would have accelerated their assimilation process.

Tissue somatic index (TSI) signify the percentage of tissue in entire body and it was found to be significantly greater in the riboflavin supplemented larvae and less in sucrose supplemented larvae than that of the control. Tissue somatic



AD- Approximate Digestibility; ECD- Efficiency of Conversion of Digested Food; ECI- Efficiency of Conversion of Ingested food; FCI- Food Consumption Index; CFU- Coefficient of Food Utilization



RC: Rate of consumption; RA: Rate of assimilation; RP: Rate of production; RM: Rate of metabolism; TSI: Tissue somatic index

Food utilization parameters (mg/day)

Figure 1: A & B Effect of the Nanoparticles of Riboflavin on food utilization parameters of the mulberry silkworm *Bombyx mori* L.

index was found to be increasing with increasing concentration, so highest TSI was noticed in larvae supplemented with higher concentrations of (60µg/ml) riboflavin.

Vitamins play significant role in the physiology and metabolism of organisms like silkworm and enhance their performance where direct supplementation of vitamin on larvae affect larval metabolism (Mora *et al.*, 2008; Castillo *et al.*, 2016). Feeding trials conducted by several workers proved that the level of nutrients in different varieties of mulberry have significant influence on growth and development of silkworm and cocoon production (Krishnaswami *et al.*, 1970; Bari *et al.*, 1985; Machii and Katagiri, 1990; Ponraj Ganesh Prabu *et al.*, 2011; Ganesh Prabu *et al.*, 2012). Moisture content in the leaves enhances the feed efficacy of the larvae which increase the growth rate (Ueda and Suzuki, 1967).

3.1. Larval weight and Silk gland weight of the V instar larva

The larvae of the control sets and experimental sets were found to be increasing in weight daily. While comparing with the control larvae, significant increase in weight of the larvae was observed in all experimental sets. Single larva weighed about 0.5 to 0.9 g during first day of V instar, weight of the larva was found to be increasing daily and at the last day of V instar the weight was about 1.33g in control and 2g in 60µg/ml riboflavin supplemented larvae. Fortification of riboflavin resulted in increased larval weight in all 3 groups, Riboflavin I (0.724%), Riboflavin II (13.768%), Riboflavin III (18.11%). Statistically the results were significant. Silk gland weights were found to be higher in all vitamin treated sets than the control. Highest silk gland weight with 111.392% increase over control was recorded in Riboflavin III (60µg/ml) (Table 3 and Figure 2).

The average weight of the larva fed with normal mulberry leaves (control) was found to be 1.38g. though the larval weight was found to be greater in sucrose supplemented larvae the increase was not significant statistically. The weight of the vitamin supplemented larva was significantly

Table 3: Effect of nanoparticles of Riboflavin on the Larval parameters of silkworm, *Bombyx mori* L.

Groups→ Parameters↓	Control	Sucrose	Larvae supplemented with		
			Nanoparticles of Riboflavin I (20µg/ml)	Nanoparticles of Riboflavin II (40µg/ml)	Nanoparticles of Riboflavin III (60µg/ml)
Larval weight (g)	1.38±0.092	1.457±0.097 [5.579%] t=-1.229**	1.37±0.136 [-0.724%] t= 0.216*	1.57±0.140 [13.768%] t= -2.511*	1.63±0.160 [18.11%] t=-0.603*
Silk Gland weight (g)	0.79±0.092	1.19±0.116 [50.632%] t= -6.197*	1.25±0.134 [58.227%] t=-6.382*	1.62±0.161 [105.063%] t= -10.103*	1.67±0.162 [111.392%] t=-10.557*

Note: Values with ± sign is Standard deviation; Values inside the square brackets indicate the % of change over the control; *Significant at the level of p < 0.05, **Not significant at the level of p < 0.05

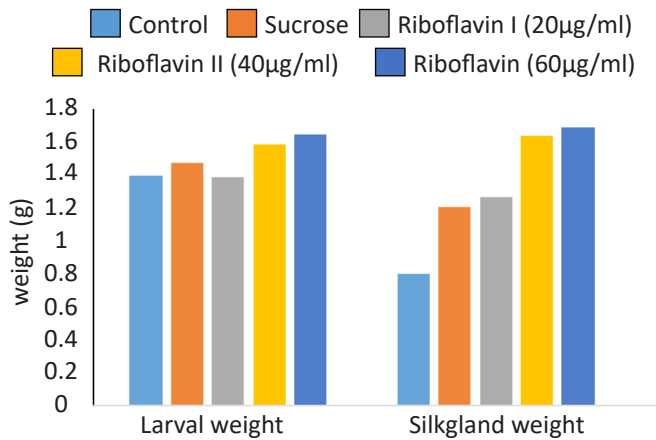


Figure 2: Effect of the nanoparticles of Riboflavin on the Larval growth and silk gland weight of the Mulberry silkworm *Bombyx mori* L.

greater than that of the control and sucrose supplemented larvae. Highest larval weight was recorded in the larvae supplemented with (60µg/ml) riboflavin. Larval weight was found to be increasing with increasing concentration, significant difference was noticed in larval weight among the larvae in the experimental sets over control. This indicates the increase in concentration of nanoparticles of riboflavin had a significant effect on the larval weight.

Silk gland weight was greater (111.392%) in nanoparticles of riboflavin (60µg/ml) supplemented larvae (1.67g) and minimum in control (0.79 g). Silk gland weight was significantly greater in both sucrose and riboflavin supplemented larvae than that of the control. Silk gland weight was found to be increasing with increasing concentration, so higher concentrations of (60µg/ml) riboflavin increased the silk gland weight.

Our findings were also in line with earlier researchers who reported growth indices were affected by doses of vitamin

B and C supplemented up to certain limits by producing significant increment in larval body weight (Ahsan *et al.*, 2013; Evangelista *et al.*, 1997).

Vitamins are organic compounds needed only in minute 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 determinative for normal growth of silkworm. Supplementation of mulberry leaves with Vitamin B enhanced resistance in silkworm larvae against conditions of environmental stress which resulted in body weight increment as compared to control (Das and Medda, 1998; Rahmathulla *et al.*, 2002; Raman *et al.*, 2007). Vitamin having significant role in cell metabolism associated with glucose, may improve physiology of silkworm contributing in enhanced performance for biological and economic traits (Ewer, 2005).

3.2. Cocoon parameters

After complete spinning of larvae cocoons were formed, those cocoons were analysed, the results revealed that the weight of the control cocoon was 0.71g and cocoon weight was found to be maximum in the larvae fed with mulberry leaves supplemented with 20µg/ml riboflavin (1.20g) This indicate the increase in concentration of nanoparticles of riboflavin had no effect on the cocoon weight (Table 4 and Figure 3).

Shell weight was found to be about 6% greater in sucrose supplemented larvae than that of the control, whereas it was greater in riboflavin supplemented larvae. This indicate the increase in concentration of nanoparticles of riboflavin had significant effect on the shell weight.

Pupa weight was significantly greater in both sucrose and riboflavin supplemented larvae than that of the control. There was no regular increase or decrease in the weight of the pupa with the increasing concentration of the riboflavin.

Table 4: Effect of nanoparticles of Riboflavin on the Cocoon parameters of silkworm, *Bombyx mori* L.

Groups→ Parameters↓	Control	Sucrose	Larvae supplemented with		
			Nanoparticles of Riboflavin I (20µg/ml)	Nanoparticles of Riboflavin II (40µg/ml)	Nanoparticles of Riboflavin III (60µg/ml)
Cocoon Weight (g)	0.71	0.82 [15.492%] t=-1.946*	1.20 [69.014%] t=-7.175*	1.20 [69.014%] t=-7.215*	1.19 [67.60%] t=-10.082*
Shell Weight (g)	0.15	0.16 [6.66%] t=-0.372**	0.24 [60%] t=2.058*	0.23 [53.33%] t=-1.775*	0.27 [80%] t=-3.318*
Pupa Weight (g)	0.56	0.66 [17.857%] t=-1.614**	0.94 [67.85%] t=-4.700*	0.97 [73.214%] t=-4.158*	0.92 [64.285%] t=-4.872*
Shell Ratio%	21.126	19.512 [-7.639%] t=8.536*	20 [-5.329%] t=3.341*	19.166 [-9.277%] t=40.536*	22.689 [7.398%] t=-5.919*

Note: Values inside the square brackets indicate the % of change over the control; *Significant at the level of p < 0.05, **Not significant at the level of p < 0.05

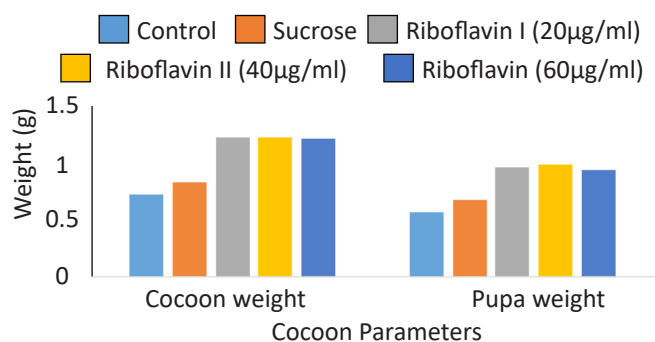


Figure 3: Effect of nanoparticles of Riboflavin on the Cocoon weight and pupa weight of the mulberry silkworm *Bombyx mori* L.

The weight of the pupa formed from the larvae with 40µg/ml riboflavin was found to be greater than control. The maximum pupal weight was found in 40µg/ml riboflavin supplementation (0.97g) as compared to control (0.56g). These findings are of great significance from the view point of rearing silkworms for production of seed cocoons as fecundity of silk moth is dependent on pupal weight. This necessitates the application of feed additives to silkworm at an optimum level. The present findings are in agreement with the finding of Sridhar and Radha (1986). So such pupae with more nutritious substances would be more valuable as a fish feed and poultry feeds

Shell ratio depends upon the quantity of the silk produced from each cocoon and was found to be lesser in sucrose supplemented and riboflavin supplemented larvae and was found to be greater in 60µg/ml riboflavin supplemented larvae.

Saha and Khan (1996) found that vitamins and minerals significantly increased the growth of pupae compared to the untreated controls. It was observed that vitamin supplementation slightly reduced the larval periods in *B. mori* at lower concentrations as compared to the controls. Nirwani *et al.* (1998) observed that dietary supplementation of riboflavin significantly decreased the larval period in all the treated groups. Similar results have also been recorded by Pai *et al.* (1988) also.

3.3. Filament parameters

Filament length and weight were significantly greater in both

sucrose and riboflavin supplemented larvae than that of the control. Filament length was found to be increasing with increasing concentration, so higher concentrations of (60µg/ml) riboflavin increased the filament length. Maximum silk filament weight was found in silk produced by the larvae supplemented with 60µg/ml riboflavin concentration. The highest silk filament weight was found in larvae supplemented with 60µg/ml riboflavin (0.53g) which is 194.44% higher than control, The minimum filament weight was in control (0.18 g). Higher silk filament weight was increased due to vitamin supplement through the mulberry leaves at an optimum level which resulted in higher silk filament length in turn resulted in higher silk filament weight (Table 5 and Figure 4).

Denier is the unit of measure the linear mass density of fibers, is the mass in grams per 9000 meters of the fiber and is used to estimate the number of cocoons required to reel the silk. Denier was significantly greater in both sucrose supplemented larvae and riboflavin supplemented larvae than that of the control. Denier was found to be increasing with increasing concentration, so higher concentrations of (60µg/ml) riboflavin. The maximum denier was recorded (5.910) and minimum was in (3.903). Among the treatments, mulberry leaves treated with (60µg/ml) riboflavin concentration was having higher denier (5.910) with 51.421% increase than control and least was recorded in absolute control (3.903). The significant increase in the denier may be due to fineness of the silk filament. As silk filament length and silk filament weight increased the denier also increased over the control. The similar findings are found in case of Chakrabarty and Kaliwal (2012) who experimented application of supplements to the silkworms and found improved denier at a rate of 150µg/ml over the control.

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. Several studies have been conducted on food supplementation and developing artificial diet for rearing of silkworms. Food supplementation has resulted in the improvement of commercial and biological aspects of sericulture. Vitamins and aminoacids, and their derivatives participate in intracellular functions as diverse as nerve

Table 5: Effect of Nanoparticles of Riboflavin on the Filament parameters of silkworm, *Bombyx mori* L.

Groups→ Parameters↓	Control	Sucrose	Larvae supplemented with		
			Nanoparticles of Riboflavin I (20µg/ml)	Nanoparticles of Riboflavin II (40µg/ml)	Nanoparticles of Riboflavin III (60µg/ml)
Silk Filament Weight (mg)	0.18	0.25 [38.8%]	0.50 [177.77%]	0.50 [177.77%]	0.53 [194.44%]
Filament Length(m)	415	538 [29.638%]	803 [93.493%]	803 [93.493%]	807 [94.457%]
Denier	3.903	4.182 [7.14%]	5.603 [43.556%]	5.603 [43.556%]	5.910 [51.421%]

Note: Values inside the square brackets indicate the % of change over the control

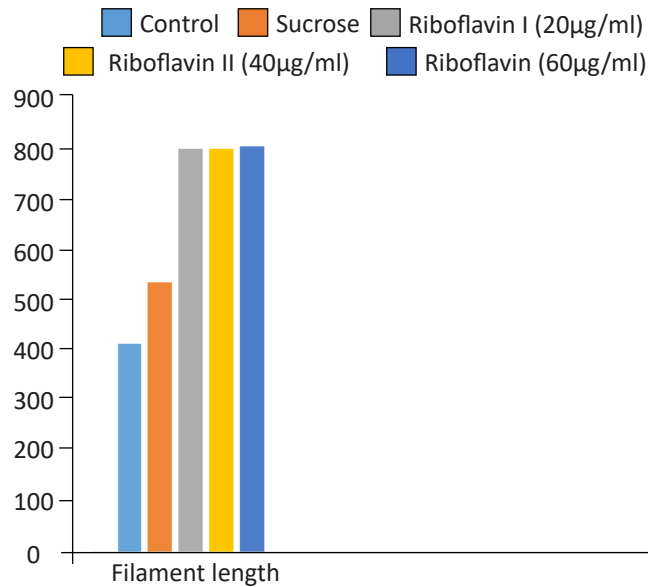


Figure 4: Effect of nanoparticles of Riboflavin on the filament length of the mulberry silkworm *Bombyx mori* L.

transmission, regulation of cell growth and the biosynthesis of various compounds in silkworm (Rodwell, 1993).

The increase in quantity of silk yield, decrease in some feeding parameters in larvae supplemented with nanoparticles of riboflavin over control larvae can be correlated with the greater efficiencies of conversion of digested food and ingested food. This confirms the fact that riboflavin increases the efficiency of conversion and metabolic rate.

Since the absorbance of riboflavin is more when the size of riboflavin is less, it is concluded that riboflavin nanoparticle supplementation increased the growth of the larvae, quantity and quality of the silk. Therefore riboflavin nanoparticle shall be recommended as a fortifying agent to the sericulture farmers after analyzing the whole molecular mechanism of the role of vitamins in improving the silk yield. Organic nanoparticles increase the nutritional value, they can be processed and commercially manufactured on a large scale. Such capsules enclosing nanoparticles increase the bioavailability and deliver the nutrients without affecting the taste.

Bose *et al.* (1995) have reported that succulent mulberry leaves with less fiber and higher mineral contents presumably stimulated the metabolic activities in silkworm, resulting in qualitative improvement of cocoon and silk.

4. Conclusion

Nutrition is known to play an important role on growth, development and overall performance of the cocoon components. Better quality of vitamin treated mulberry leaf variety, therefore, leads to the elevated value of growth and yield attributing parameters in the present investigation. From the present observations, it was also evident that

consistently better rearing performance was obtained from feeding nanovitamin treated mulberry leaves. All the parameters governing yield and quality of cocoon were influenced significantly by the dietary supplementation of larvae with nanoparticles of vitamins. This might be attributed due to better absorption and utilization of major nutritive substances and influence of nanoparticles of vitamins on growth and metabolism in silkworm larvae. The silkworm responded significantly to leaf treated with nanovitamin than to untreated mulberry leaves resulting in the production of cocoon and silk of superior quality.

5. Acknowledgement

The authors express their sincere thanks to the Principal, Head and Staffs of PG and Research Department of Zoology of Government Arts College (Autonomous), Karur, Tamil Nadu for providing the laboratory facilities to carry out this research work.

6. References

- Ahsan, K.M., Khan, A.R., Ferdous, T., 2013. Growth and development of the mulberry silkworm, *Bombyx mori* L. On vitamin b and c supplemented diet. Bangladesh J. Zool 41(2), 199–206.
- Balasundaram, D., Selvisabanyakam, V., Mathivanan, 2008. Studies on comparative feed efficacy of mulberry leaves MR2 and MR2 treated with vitamin C on *Bombyx mori* L. (Lepidoptera: Bombycidae) in relation to larval parameters. J. Curr. Sci. 12(2), 31–35.
- Basit, M.A., Ashfaq, M., 1999. Collaborative effect of optimum dosage of N, K, Ca, P and Mn on the larval development and silk yield of *Bombyx mori* L. Pak. J. Biol. Sc. 2, 1002–1005.
- Castillo, Y., Suzuki, J., Watanabe, K., Shimizu, T., Watarai, M., 2016. Effect of Vitamin A on *Listeria monocytogenes* Infection in a Silkworm Model. PloS one. 11(9), e0163747.
- El-karasy, I.R., Idriss, M., 1990. Ascorbic acid enhances the silk yield of the mulberry silkworm, *Bombyx mori* L. J. Appl. Entomol. 109, 81–86.
- Etebari, K., 2002. Effect of enrichment mulberry leaves (*Morus alba*) with some vitamins and nitrogenous compounds on some economic traits and physiological characters of silkworm *Bombyx mori* L. MSc Thesis, Isfahan University of Technology, Isfahan, Iran.
- Etebari, K., Matindoost, L., 2005. Application of multi-vitamins as nutrients on biological and economical characteristics of silkworm *Bombyx mori* L. J. Asia-pacific Entomol. 8, 1–6.
- Ganesh, P., Selvisabhanayakam, P., Balasundaram, D., Pradhap, M., Vivekananthan, T., Mathivanan, V., 2012. Effect of food supplementation with silver nanoparticles (AgNps) on feed efficacy of silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). Int. J. Res. Biol. Sci. 2, 60–67.

- Goudar, K.S., Kaliwal, B.B., 1999. Effect of cortisone on the economic parameters of the silkworm, *Bombyxmori L.* *Sericologia* 39: 555–561.
- Ito, T., 1978. Silkworm Nutrition, 121–157.
- Ito, T., Tanaka, M., 1962. Nutrition of the silkworm *Bombyxmori*, VI. Effects of concentration of sugar and protein added in artificial diets. *Bull. Seric. Exp. Stn.* 18, 1–34.
- Kabila, V., Subburathinam, K.M., Chetty, J.S., 1994. Growth and economic characters of silkworm, *Bombyx mori L.* on feed enriched with neutralized aspartic acid. *Indian J. Seric.* 33, 80–81.
- Khan, A.R., Faruki, S.I., 1990. Growth and development of *Bombyxmori L.* on feed supplemented with Para-Amino Benzoic Acid. *Univ. J. Zool. Rajshahi Univ.* 9, 47–53.
- Khan, M.D., Saha, B.N., 1995. Growth and development of the mulberry silkworm, *Bombyx mori L.*, on feed supplemented with alanine and glutamine. *Sericologia* 35, 657–663.
- Krishnaswami, S., 1978. New Technology of silkworm rearing. Central Silk Board, India.
- Magudam, S.B., Hooli, M.A., 1992. Effect of application of juvenile hormone analogue in IV instar followed by thyroxine in the pure mysore breed of *Bombyx mori (L.) sericologia* 32(3), 385–390.
- Mora, J.R., Iwata, M., von Andrian, U.H., 2008. Vitamin effects on the immune system: vitamins A and D take centre stage. *Nat. Rev. Immunol.* 8, 685–698.
- Nirwani, R.B., Kaliwal, B.B., 1996. Effect of folic acid on economic traits and the change of some metabolic substances of bivoltine silkworm, *Bombyx mori L.* *Korean J. Seric. Sci.* 38, 118–123.
- Nirwani, R.B., Kaliwal, B.B., 1998. Effect of thiamine on commercial traits and biochemical contents of the fat body and haemolymph in the silkworm *Bombyxmori L.* *Sericologia* 38, 639–646.
- Prasad, P.R., 2004. Effect of fortification of Ascorbic Acid through mulberry leaf on cocoon traits of Pure Mysore race of silkworm, *Bombyx mori L.* *Indian J. Entomol.* 66, 37–39.
- Qader, M.A., Haque, R., Absar, N., 1994. Amino acid contents in posterior silk gland of *Bombyx mori* (Lepidoptera: Bombycidae) influenced the quality of mulberry leaves. *Bull. Sericult. Res.* 5, 63–68
- Rahmathulla, V.K., Das, P., Ramesh, M., Rajan, R.K., 2007. Growth rate pattern and economic traits of silkworm, *Bombyx mori L.* under the influence of folic acid administration. *J. Appl. Sci. Environ. Manage.* 11(4), 81–84.
- Rahmathulla, V.K., Suresh, H.M., Mathur, V.B., Geethadevi, 2002. Feed conversion efficiency of Elite bivoltine CSR hybrids silkworm, *Bombyxmori L.* reared under different environmental conditions. *Sericologia.* 42, 197–203.
- Rajabi, K.R., Ebadi, R., Mirhoseini, S.Z., Seidavi, A.R., Zolfaghari, M., Etebari, M., 2007. A review on nutritive effect of mulberry leaves enrichment with vitamins on economic traits and biological parameters of silkworm *Bombyx mori L.* *ISJ.* 4, 86–91.
- Raman, C., Manohar, S.L., Xavier, N., Krishnan, M., 2007. Expression of silk gene in response to p- soytose (hydrolyzed soy bean protein) supplementation in the fifth instar male larvae of *Bombyx mori.* *J. Cell Biol. Mol. Sci.* 6(2), 163–174.
- Rodwell, V.W., 1993. Amino acids (Harper's Biochemistry. 23rd edition, R.K.Murray, D.K.Granner, P.A. Mayes and V.W. Rodwell eds.), Appleton & Lange, Connecticut, USA. 23–32.
- Saha, A.K., Rahman, M.S., Saha, B.N., Uddin, M., 1994. Effect of proline and leucine on the growth and development of silkworm *Bombyx mori L.* *Univ. j. zool. Rajshahi Univ.* 13, 75–79.
- Saha, B.N., Khan, A.R., 1997. The nutritive effects of Sinafort (R) on *Bombyxmori L.* *Entomon.* 22(1), 29–34.
- Sarkar, A., Haque, M., Rab, M., Absar, N., 1995. Effect of feeding mulberry (*Morus sp.*) leaves supplemented with different nutrients to silkworm, *Bombyxmori L.* *Curr. Sci.* 69: 185- 188. SAS institute. 1997. SAS/STAT User Guide for personal computers, Cary, NC: SAS institute.
- Tazima, Y., 2001. Improvement of Biological Functions in the Silkworm (Translated from Japanese). Science Publishers, Inc., Enfield, NH, USA.
- Victor, S.R., 2011. The Role of Food, Agriculture, Forestry and Fisheries in Human Nutrition – Volume IV. EOLSS Publications. p. 121. ISBN 9781848261952
- Vishwanath, A.P., Krishnamurthy, K., 1983. Effect of foliar spray on the larval development and cocoon characters of silkworm (*Bombyx mori L.*). *Indian .J. Seric.* 11/12, 1-6
- Yasmin, T., Absar, N., Sarkar, A.A., 1995. Effect of foliar spray of micronutrients and urea on the nutritional quality of mulberry (*Morus sp.*) leaves. *Indian J. Seric.* 34, 149–152.
- Zaman, K., Ashfaq, M., Akram, W., 1996. Effect of feeding Mg and N treated mulberry leaves on larval development of silkworm, *Bombyx mori L.* and silk yield. *Pakistan Entomologist* 18, 78–79.