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Research Article

SYNERGETIC EFFECT OF THE NUTRITIONAL FORTIFICATION OF NANOPARTICLES OF ALANINE AND RIBOFLAVINON THE ECONOMIC PARAMETERS AND FECUNDITY OF MULBERRY SILKWORM, BOMBYX MORIL.

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ABSTRACT

Silkworm, *Bombyxmori* is a monophagous insect that feeds mainly on the leaves of the mulberry plant. Good quality of cocoons and silk mainly depends on the nutrition. Mulberry leaves were smeared with synergetic solutions of nanoparticles of alanine and riboflavin in different concentrations. The enriched leaves were air dried. The fifth instar larvae of mulberry silkworm *B. mori* L. were fed with enriched mulberry leaves thrice a day. Economic characters and fecundity were studied and recorded. Synergetic supplementation of nanoparticles showed a positive impact and significantly enhanced the cocoon weight by 94.366%, pupal weight by 64.285%, silk yield by 89.44% and fecundity by 48.44% when compared to control.

Keywords: Riboflavin, Alanine, Nanoparticles, Enrichment, Silk glands

1. INTRODUCTION

In silkworm, *Bombyxmori* efficient utilization of nutrients from the mulberry leaves influences the metamorphosis, growth and cocoon yield. Enrichment of the mulberry leaves by nutrient supplementation is one of the strategies by which growth of the larvae can be improved, cocoon and silk productivity can be increased and the quality can be enhanced and maintained. Nutritional supplements include minerals, vitamins, proteins, amino acids and sugars [1, 2].

The nutritional status of the mulberry leaves can be enriched by fortifying vitamins and other nutrients. Fortification of mulberry leaves with nutrients were found to increase thelarval growth and post cocoon characteristics [3, 4]

Fortification of different aminoacids has been reported to enhance the quantity and the quality of silk can be enhanced by fortifying amino acids [1, 2, 5-11]. It has been shown that 75% of mulberry leaf protein is directly converted into silk protein and 25% goes to the body tissues of the silkworm larvae [12]. β-alanine is a derivative of B5 vitamin (pantothenic acid) and coenzyme A [13]. Although quite a few studies have been conducted on amino acid supplementation, their results vary [2, 6, 7, 9, 10]. L-Alanine is the most predominant composition of silk. 34.36% of silk is composed of alanine [14]. 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 [15]. Riboflavin is important in promoting the release of energy from carbohydrates, fats and proteins *i.e.* "in the metabolic pathway for ATP production" [16]. Therefore alanine and riboflavin have been chosen as fortifying agents in this study.

There is an increasing optimism that nanotechnology in the advancement of functional feed which delivers the nutrients effectively. Nanotechnology affords a revolutionary way to enhance the growth and production in the field of nutrition. But the works on the supplementation of nanonutritive particles in silkworms are scanty. Considering the key role played by both alanine and riboflavin absorption in silkworm nutrition and development, attempts have been made here to assess the effects of synergetic nanoparticles of alanine and riboflavin supplementation on the growth and the economic traits of the silkworm, *B. mori*.

2. MATERIAL AND METHODS

2.1. Silkworm Rearing

The double hybrid mulberry silkworm breed (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 [17].
Alanine manufactured by Loba-Chemie Indo Austranal Co, Bombay. Riboflavin manufactured by Shreya Life Sciences Pvt. Ltd. Uttarakhand was used for this study. In this work, the size of the alanine and riboflavin particles was reduced to the size of 267nm and 240nm respectively by ball milling method using Pulverisette 7. Such nanoparticles of alanine and riboflavin were supplemented in synergetic form to the fifth instar larvae along with mulberry leaves in different concentrations. 150 newly emerged larvae were taken and divided into five groups so that each group contained 30 larvae. The control larvae were fed with untreated mulberry leaves. Sucrose larvae were fed with Mulberry leaves smeared with 2% Sucrose solution(2g/100ml).Synergetic set I larvae were fed with mulberry leaves smeared with a mixture of 5μg/ml of nano alanine + 20μg/ml of nano riboflavin solution and 2% sucrose solution. Synergetic set II larvae were fed with mulberry leaves smeared with a mixture of 10μg/ml of nano alanine + 40μg/ml of nano riboflavin solution and 2% sucrose solution. Synergetic set III larvae were fed with mulberry leaves smeared with a mixture of 15μg/ml of nano alanine + 60μg/ml of nano riboflavin solution and 2% sucrose solution.

2.2. 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 Cocoon parameters like cocoon weight, cocoon shell weight, cocoon shell ratio, and pupal weight were recorded by using standard methodology described [18].

2.3. Fecundity

After emergence from the cocoon, male and female moths in equal number were kept on trays and were allowed for copulation for 4hr after which they were decopulated gently. Female moths were then placed on sheets and covered with funnel for egg laying. After completion of egg laying, the total number of eggs per female were counted. [19].

2.4. Statistical analysis

Collected data were subjected to statistical analysis such as ‘t’ test to find out whether the difference between control and experimental groups was significant and ANOVA to find out whether variations among the different experimental groups were significant.

3. RESULTS AND DISCUSSION

3.1. Cocoon parameters

The cocoon weight and pupa weight of the control larvae were 0.71g and 0.56g respectively (Table 1). In sucrose supplemented larvae and all the experimental larvae the weight of cocoon and pupa was significantly greater than that of the control larvae. Both cocoon weight and pupa weight were found to be increasing with increasing concentration of the synergetic solutions. Shell weight was found to be significantly greater in the third set of experimental larvae (0.32g) than all other larvae (Fig 1). F value indicates that the increase in concentration of synergetic nanoparticles had significant effect on the cocoon weight (Fig. 2). Shell ratio depends upon the quantity of the silk produced from each cocoon and was found to be greater in synergetic set I 21.996% (Fig. 3) thanthe control and other sets.
The increase in the growth of the larvae due to the fortification agents can be ascribed as the reason for the enhancement of cocoon and shell weight as reported [20]. The present findings are in agreement with the finding of Kamala M [21] and Babu VP [22], who also reported higher pupal weight in silkworms reared on glycine fortified leaves. Increased pupal weight can be attributed to the increased fecundity also such pupae with more nutritious substances would be more valuable as a fish feed and poultry feed.
In silkworms, silk fibroin is derived mainly from four amino acids: alanine, serine, glycine and tyrosine which come from their dietary source of protein and amino acids [23]. Silkworms obtain 72-86% of their amino acids from mulberry leaves. More than 60% of the absorbed amino acids is used for silk production [5, 24] indicated that the addition of aspartic acid in concentration of 1 or 2% to mulberry leaves increased the economic characteristics of the silkworms. This observation falls in line with the findings of Nirwani [26] who observed that the economic parameters had significant effect on experimental sets than control. Similar results have also been recorded by Pai [27] also. In the opinion of Babu VP [22], the effect of Vitamin C-enriched mulberry leaves on rearing of *B. mori* and reported that daily application of 1.5% VC in 1st and 2nd instar of silkworm improved the economic traits.

This corroborates with the findings of Etebari *et al.* [2] who reported that feeding of silkworm on mulberry leaves enriched by with multi-vitamins from 4th instar increased female cocoon shell weight in 2.5%concentration, while female pupal weight increased in 1% concentration. Male and female shell ratio did not increase compared to controls. The present observation disagrees with Rouhollah [28] who reported that cocoon weight, pupal weight and shell weight had significant increase compared to control and cocoon shell percentage were also maximum than control. Despite increase in cocoon weight and shell weight in synergetic set III the shell ratio was found to be low, which could be ascribable to higher pupal weight. The present observation coincides with findings of Uma Maheshwari and coworkers [29] who reported that there was a significant increase in cocoon weight and shell weight whereas shell ratio was found to be low in the silkworm treated with kinetin.

Table 1: Synergetic effect of Nanoparticles of Alanine and Riboflavin on the Cocoon parameters of silkworm, *Bombyx mori L*

Groups			Synergetic set			
Parameters	Control	Sucrose	I Alanine 5µg/ml +Riboflavin 20µg/ml	II Alanine10µg/ml+ Riboflavin 40µg/ml	III Alanine 15µg/ml+ Riboflavin 60µg/ml	ANOVA
Cocoon Weight(g)	0.71	0.82± 0.044 [15.492%] t=-1.946*	0.97±0.027 [36.619%] t= -7.707*	1.11± 0.069 [56.338%] t=-9.220*	1.38± 0.046 [94.366%] t=-18.248*	F = 71.847*
Shell Weight(g)	0.15	0.16 [6.67%] t=-0.372**	0.25 [66.67%] t= -1.922*	0.24 [60%] t=-1.775*	0.32 [113.33%] t=-3.318*	F = 66.280*
Pupa Weight (g)	0.56	0.66 [17.857%] t=-1.614**	0.70 [25%] t=-2.327*	0.86 [53.571%] t=-4.135*	1.05 [64.285%] t=-5.972*	F= 15.00*
Shell Ratio%	21.126	19.512 [-1.639%] t=8.536*	25.773 [4.647%] t=-9.860*	21.621 [0.495%] t=-2.204*	23.188 [2.062%] t=-7.891*	F= 47.745*

*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.*

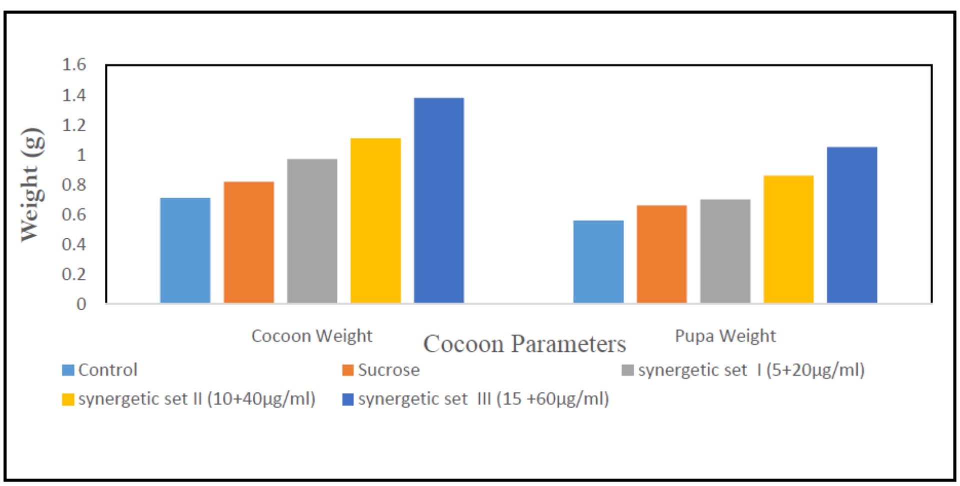

Fig. 1: Synergetic effect of Nanoparticles of Alanine and Riboflavin on the Cocoon weight and Pupa weight of the mulberry silkworm *Bombyx mori* L.

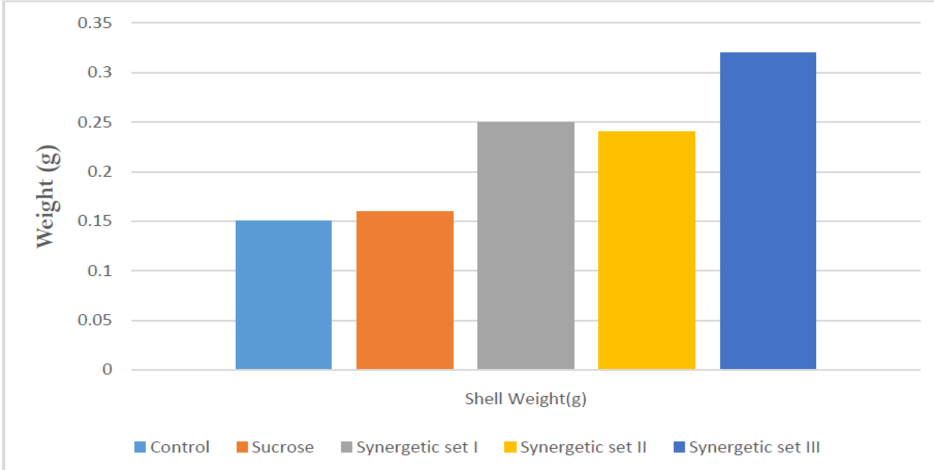

Fig. 2: Synergetic effect of Nanoparticles of Alanine and Riboflavin on the Shell weight of the mulberry silkworm *Bombyx mori* L.

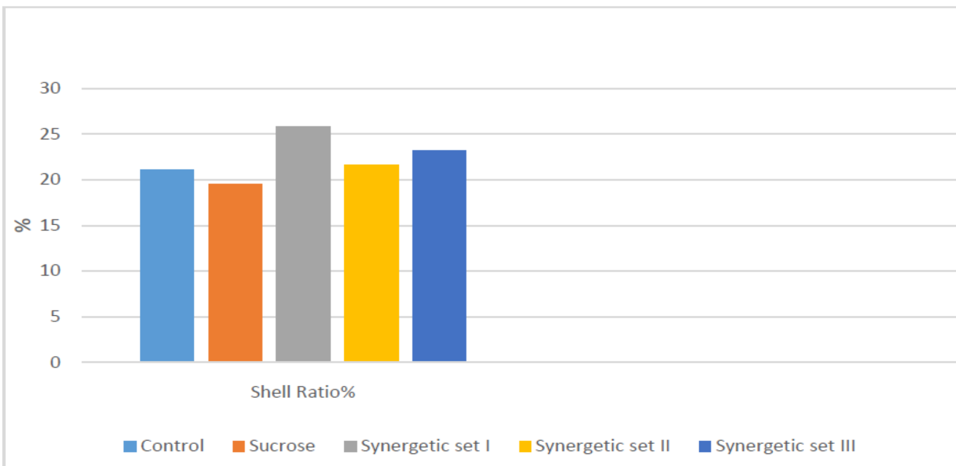

Fig. 3: Synergetic effect of Nanoparticles of Alanine and Riboflavin on the Shell Ratio of the mulberry silkworm Bombyx mori L.

3.2. Filament parameters

Filament length and weight were significantly greater in both sucrose supplemented larvae synergetic sets than that of the control (Table 2). Filament length was found to be increasing with increasing concentration, so filament length was higher in the third set than the others (Fig. 4). Silk filament length was found to be higher in synergetic set III (800 m) which is 92.77% higher than control, Maximum silk filament weight was found in silk produced by the synergetic set III (0.50g) which is 177.77% higher than control, The minimum filament weight was in control (0.18 g). Higher silk filament weight may be due to supplementation of alanine through the mulberry leaves at an optimum level which resulted in higher silk filament length in turn resulted in higher silk filament weight.

Denier is the unit of measuring the linear mass density of fibers, It 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 [17]. Denier was significantly greater in both sucrose supplemented larvae and larvae of synergetic sets than that of the control. Denier was found to be increasing with increasing concentration, so higher in the third synergetic set. Among the treatments, the filament of synergetic set III was found to possess higher denier (5.625) with 44.119% increase over control and least denier was recorded in 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 were reported by Chakrabarthy [30] found that application of arginine, histidine and their mixtures improved denier over the control. Vitamins and amino acids, and their derivatives participate in intracellular functions as diverse as nerve transmission, regulation of cell growth and the biosynthesis of various compounds in silkworm [31].

Table 2: Synergetic effect of Nanoparticles of Alanine and Riboflavin on the silk filament parameters of the silkworm, *Bombyxmori L*

Groups→ Parameters↓	Control	Sucrose	Synergetic set I Alanine5µg/ml + Riboflavin 20µg/ml	Synergetic set II Alanine10µg/ml+ Riboflavin 40µg/ml	Synergetic set III Alanine15µg/ml + Riboflavin 60µg/ml
Silk Filament Weight (mg)	0.18	0.25 [38.8%]	0.42 [133.33%]	0.45 [150%]	0.50 [177.77%]
Filament Length(m)	415	538 [29.638%]	758 [82.650%]	765 [84.337%]	800 [92.771%]
Denier	3.903	4.182 [7.14%]	4.986 [27.747%]	5.294 [35.639%]	5.625 [44.119%]

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

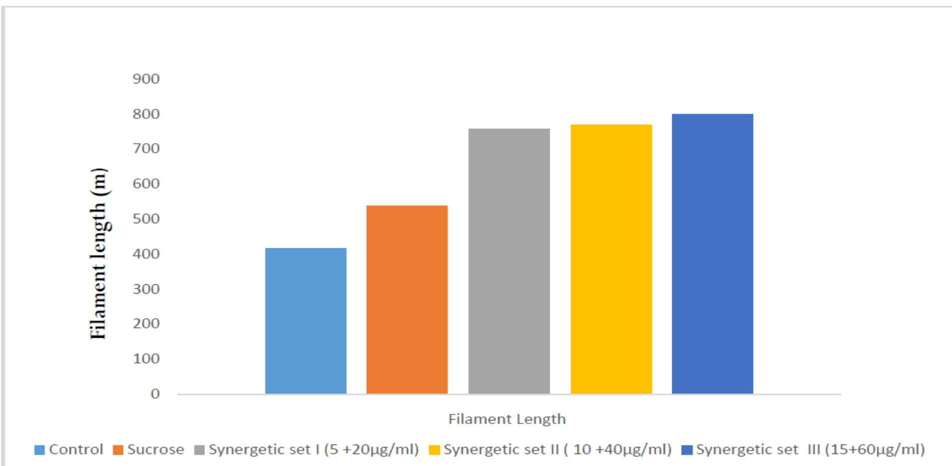

Fig. 4: Synergetic effect of Nanoparticles of Alanine and Riboflavin on the filament length of the mulberry silkworm *Bombyx mori* L.

3.3. Fecundity

It is the total number of eggs laid by a single mother moth and was calculated by counting the total number of eggs laid by a female moth. Average of three layings in each replicate was recorded for analysis purpose.Fecundity was significantly greater in synergetic set III (766 Nos. 48.44%) than that of control (516 Nos.) (Table 3). Fecundity was found to be increasing in increasing concentration (Fig. 5). The results obtained are in agreement with the finding of Rouhollah [28] who reported thatalanine treatment could significantly increase egg number. Larval feeding has effects on genital development, and fecundity of adults (Chapman 1998).This observation is in accordance with Legay [32] who reported that the effects of amino acids on fecundity of insects can be related to corporaallata discharge due to vitellogenesis. And this phenomenon will lead to lay more eggs. This observation falls in line with Krishnappa [33] who found that fecundity and fertility were well pronounced due to amino acid supplementation. He also reported that amino acids reduce the total larval duration and mortality of larvae and pupae. Similarly, Khan AR [34] observed that proline and leucine enhanced the reproductive potentiality (i.e., fecundity). The present observation disagrees with Khan MD [35] who observed that alanine and glutamine significantly decreased the larval and pupal periods.

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. The nanoparticles of both alanine and riboflavin can be readily or easily absorbed from the intestine and can reach the haemolymph easily and hence they are effectively used for the synthesis of silk protein. They can inhibit the bacterial attachment to intestinal cells. Nanoparticles offer a very larger surface for absorption because of their ultrafine structure and hence function very effectively than the larger particles of the same nutritional substances.

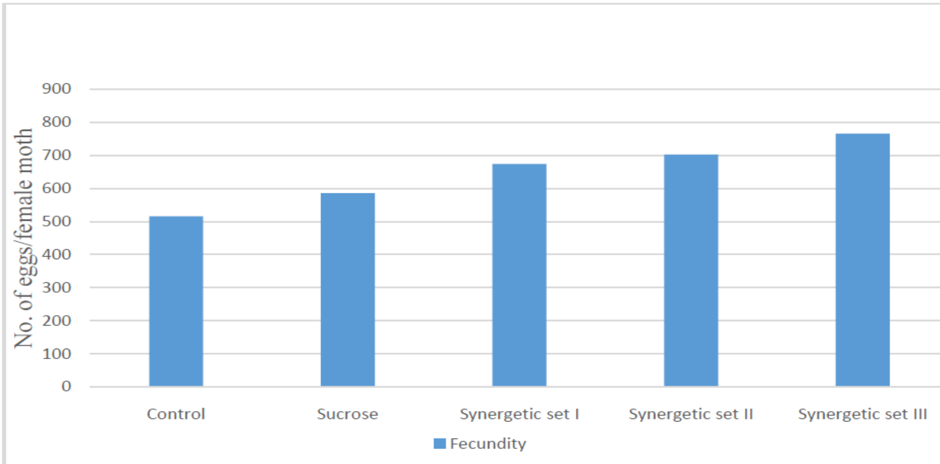

Fig. 5: Synergetic effect of Nanoparticles of Alanine and Riboflavin on the Fecundity of the mulberry silkworm *Bombyx mori* L

Table 3: Synergetic effect of Nanoparticles of Alanine and Riboflavin on the Fecundity of silkworm, *Bombyxmori L.*

GROUPS	Fecundity
CONTROL	516±19.106
SUCROSE	586±8.854 [13.565%] t = - 6.591*
Synergetic set I (Alanine5µg/ml+ Riboflavin 20µg/ml)	674±16.338 [30.620%] t = - 12.585*
Synergetic set II (Alanine10µg/ml+ Riboflavin 40µg/ml)	702±41.456 [36.046%] t = - 8.105*
Synergetic set III (Alanine15µg/ml+ Riboflavin 60µg/ml)	766±18.810 [48.44%] t = - 18.499*
ANOVA	F= 68.803*

*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.*

4. CONCLUSION

Fortification of synergetic nanoparticles of alanine and riboflavin resulted a significant increase in quality and quantity of silk and fecundity. Therefore synergetic nanoparticles of alanine and riboflavin shall be recommended as a fortifying agent to the sericulture farmers in improving the silk yield. They can be commercially manufactured on a large scale.

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