Effect of Food Supplementation with Silver Nanoparticles (AgNps) on Feed Efficacy of Silkworm, *Bombyx mori* (L.) (Lepidoptera: Bombycidae)

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Abstract
Silkworm *Bombyx mori* is an important economic insect and also a tool to convert leaf protein into silk protein. This study was carried out to determine the feed efficacy of silkworm *Bombyx mori* (V instar larvae) fed by MR1 mulberry (*Morus sinensis*) leaves and different concentrations of silver nanoparticles treated MR2 mulberry (*Morus sinensis*) leaves in relation to physiological parameters like food consumption (FC), food utilization (FU), approximate digestibility (AD), consumption index (CI) and coefficient of food utilization (CFU). The green synthesis of silver nanoparticles by using silver precursor (Silver Nitrate – AgNO₃) with *Morus sinensis* leaf extract as a reducing and stabilizing agent. The different concentrations (25%, 50%, 75% and 100%) of prepared silver nanoparticles were treated to the larval period. In the present study, it has been observed that the feed efficacy of larvae (5th instar), enhanced by 25% Silver nanoparticles treated group than control and other treated groups (50%, 75% and 100%). This study has been indicated that the Silver nanoparticles exhibit the presence of certain growth stimulant activity and can be used to increase the silk yield in commercial silkworm rearing with reference to Sericulture.

Keywords: *Bombyx mori*, *Morus sinensis*, Silver Nanoparticles, Mulberry, Feed Efficacy.

Introduction
The silkworm, *Bombyx mori*, is a monophagous lepidopteran insect which has been domesticated for more than five thousand years. The physiology of this species has been studied extensively due to the economic importance of silk production over the centuries. Further studies on food preferences and optimal nutrient levels for maximum larval growth and silk production eventually led to the development of an artificial diet. Today, *Bombyx mori* can be raised entirely on artificial diet from the first to the last larval instars. Mulberry leaves suitable as food for silkworms must contain several chemical constituents such as water (80%), proteins (27%) and carbohydrates (11%), other extracts, mineral matters, vitamins etc. and at the same time, they must have the favourable physical features such as suitable tenderness, thickness and tightness, in order to be eaten by silkworms easily (Koul, 1989). Mulberry (*Morus* species) leaf is the sole food and source of nutrition for the silkworm, *Bombyx mori* due to the presence of morin (Tribhuwan and Mathur, 1989).

Recently, much research has been done on the diet supplementation of mulberry leaves fed to silkworms. These supplementations include vitamins such as ascorbic acid, thiamin, niacin, folic acid, multi-vitamins and vitamin C (Nirwani and Kaliwal, 1998; Saha and Khan, 1996; Etebari and Fazilati, 2003; Etebari et al., 2004 and Balasundaram et al., 2008). Although some of the compounds have shown significant results, enrichment has not always caused the improvement of biological characteristics of the silkworm. Etebari et al. (2004) have reported the yield decrease, when ascorbic acid concentration is enhanced in silkworm diet. Dietary supplementation of copper sulphate, nickel chloride and potassium iodide increased the physiological parameters.
Feeding patterns have also been observed in other lepidopteran insects such as the tobacco hornworm, *Manduca sexta* (Reynolds et al., 1986; Bowdan, 1988; Timmins et al., 1988; Bernays and Woods, 2000), the woolly bear caterpillar, *Grammia geneura* (Bernays and Singer, 1998) and the corn earworm, *Helicoverpa armigera* (Barton Browne and Raubenheimer, 2003). In addition, feeding patterns of *Manduca* larvae raised on artificial diet were found to be different from those of caterpillars fed plant leaves (Reynolds et al., 1986; Bernays and Woods, 2000). The quality and quantity of the mulberry leaves fed during rearing, decide the success of silkworm crop. Hence, the choice of mulberry leaves suitable for healthy growth of silkworm is one of the most important factors in Sericulture. It feeds continuously during five instars of larval period to spin cocoon. Cocoon characters, both qualitative as well as quantitative, depend largely on the quality and quantity of leaves (Koul, 1989; Chenthilnayaki et al., 2004; Balasundaram et al., 2008). The silkworm larvae are attracted by three stimulants in mulberry leaves viz., the attractant, biting factor and swallowing factor (Hamamura and Naito, 1961). Leaf consumption directly affects the silk producing capacity of the silkworm (Muthukrishnan et al., 1978).

Sumioka et al. (1982) have observed that the leaf consumption influenced the body weight which inturn influences the silk output. Therefore, a very simple and clear piece of logic to boost the production of the silk, improved quality of leaf or mulberry variety has to be used for silkworm rearing. Thus, for each instar, the increase in fresh and dry weights of the larvae, fresh and dry weights of food eaten and digested and dry weight of feces produced were recorded (Rath et al., 2003). There is a need to recognize and integrate the physiological and nutritional requirements of the silkworm hybrids under ecological conditions to the silkworm breeding and management programmes to make them need-based (Nagaraju, 2002).

The *Bombyx mori* larval feeding behaviour through continuous observations of larvae throughout larval development. In addition to this analysis, the behaviours of starved larvae were also observed to determine how diet-deprivation affects feeding behaviour. Finally, the study examined defecation and physical stimulation in *Bombyx* larvae as possible triggers for feeding (Simpson, 1995). Enrichment of mulberry leaves with vitamin E did not have significant effect on food consumption in silkworm larvae (Mosallanejad et al., 2002). The relationship between the environment and genes has considered bidirectional with food consumption efficiency on gene expression varies depending on the genetic background of an organism and expressed physiological or nutritional unit in gene regulation studies (Giacobino et al., 2003; Milner, 2004; Kang, 2008; Ogunbanwo and Okanlawon, 2009).

**Materials and Methods**

**Silkworm Rearing**

The first day of V instar of popular Indian bivoltine hybrid (CSR₂×CSR₂) silkworm *Bombyx mori* (Local Bivoltine) race were collected from Silkworm Culture Centre at 2nd Agraharam, Salem in Tamilnadu. The larvae were reared simultaneously both in control and experimental groups separately on mulberry leaves dipped in silver nanoparticles solution in the laboratory. Proper environmental conditions provided to the silkworms with photoperiod of 12:12 h light and darkness as recommended by Krishnaswamy et al. (1973). The first day of V instar larvae were placed at ambient temperature of 25 ± 27°C and relative humidity of 70 to 80%. The larvae were reared in card board boxes measuring 22×15×5 cms covered with nylon net and placed in an iron stand with ant wells (Govindan, et al., 1981).

**Preparation of *Morus sinensis* Leaf Extract**

Fresh leaves of *Morus sinensis* were collected from faculty of Agriculture, Annamalai University, Annamalainagar, Tamilnadu, India. The fresh leaf of *Morus sinensis*, mulberry leaves were washed several times with...
deionized water. 25 gm of leaves were finely cut and stirred with 100 ml deionized water at 40°C for 20 minutes and filtered with Whatman No. 1 filter paper to get the extracts (dark green in colour) in separate conical flask. The prepared leaf extract was used as a reducing agent and stabilizing agent for Silver Nitrate to silver nanoparticles (Mallikarjuna et al., 2011).

Green Synthesis of Silver Nanoparticles from *Morus sinensis* Leaf Extract

Silver Nitrate (AgNO₃) was purchased from Qualigens Fine Chemicals, Mumbai, India. 100 mL of aqueous solution of AgNO₃ (0.7×10⁻³ M) and stirring continued for one minute. After stirring, the solution heated at 70°C for 15 minutes. After boiling of the solution, 5 ml of *Morus sinensis* leaf extract was added drop wise to 100 ml aqueous solution of AgNO₃. During this process, solution was mixed vigorously until colour change is evident (dark brown in colour) the formation of silver nanoparticles colloidal. Then the solution was removed from the heating mantle and stirred until cooled at room temperature (Lee and Meisel, 1982).

Feed Efficacy (Physiological Traits)

The quantity of MR₂ mulberry leaf offered to the entire groups was similar and *Bombyx mori* larvae were fed five times a day. The left over mulberry leaves and litter were weighed daily and recorded. Similarly, initial and final weights of the V instar larvae were recorded in control group and silver nanoparticles treated groups. Fresh leaves were cut into two halves; one half was used to determine the initial water content. Three V instar larvae in control and Silver nanoparticles treated groups were dried in a hot air oven to constant weight to determine the dry weights. Based on these weights, the physiological parameters like food consumption (FC), food utilization (FU), approximate digestibility (AD), consumption index (CI) and coefficient of food utilization (CFU) were calculated (Arsenev and Bromlej, 1957).

Food Consumption (FC) was calculated by following formula

\[ FC = \text{Dry weight of leaves offered} - \text{Dry weight of residual leaves} \]

Food Utilization (FU) was calculated by following formula

\[ FU = \frac{\text{Weight of food consumed} - \text{Weight of faecal matter}}{\text{Dry weight of food eaten}} \]

Approximate Digestibility (AD) was calculated by following formula

\[ AD = \frac{\text{Dry weight of food eaten} - \text{Dry weight of faecal produced}}{\text{Dry weight of food eaten}} \times 100 \]

Food Consumption Index (FCI) was calculated by following formula

\[ FCI = \frac{E}{T \times A} \]

Where,

\[ E = \text{Dry weight of food eaten}, \]
\[ T = \text{Duration of Experimental period} \]
\[ A = \text{Mean dry weight of animal during experimental period} \]

Co-efficient of Food Utilization (CFU) was calculated by following formula

\[ \text{CFU} = \frac{\text{Dry weight of food consumed} - \text{Dry weight of faeces}}{\text{Dry weight of food consumed}} \times 100 \]

Selection of the Effective Concentration of Silver Nanoparticles

The prepared silver nanoparticles colloidal solution was diluted to 25%, 50%, 75% and 100% concentrations. Fresh MR₂ mulberry leaves were separately soaked with each concentration for 15 minutes and then were dried in air for 10 minutes. The Silver nanoparticles treated leaves were used for feeding the V instars larvae of silkworm *Bombyx mori* (Suleman, 1999).

The *Bombyx mori* larvae were divided into two groups (Control and Treated). The treated group divided into four sub groups (T₁, T₂, T₃ and T₄) this sub groups were treated with different concentrations of silver nanoparticles (25%, 50%, 75% and 100%) efficacy of these concentrations were compared to control group and to find out the physiological traits. The control and silver nanoparticles treated MR₂ mulberry (*Morus sinensis*) leaves were fed by silkworm *Bombyx mori*, five feedings/per day.

Experimental Groups

The V instars of *Bombyx mori* larvae fed with the following MR₂ mulberry leaves. Control group (C) larvae fed with normal mulberry leaves, group T₁ larvae fed with 25% silver nanoparticles treated MR₂ mulberry leaves, group T₂ larvae fed with 50% silver nanoparticles treated MR₂ mulberry leaves, group T₃ larvae fed with 75% silver nanoparticles treated MR₂ mulberry leaves and group T₄ larvae fed with 100% silver nanoparticles treated MR₂ mulberry leaves. (Rasool, 1995).

Observation of Feeding Behavior of Larvae

Only populations of larvae in synchronous growth were observed. During observations, each larva was placed in a plastic container facing a 3 cm² block of artificial diet. Larvae were spaced within the container so as not to disrupt the feeding behaviour of other animals. (Shinji Nagata and Hiromichi Nagasawa, 2006).

Statistical Analysis

All the data were analyzed by one way analysis of variance (ANOVA) followed by Duncan’s multiple range test (DMRT) using a commercially available statistics software package (SPSS® for Windows, V. 16.0, Chicago, USA). Results were presented as mean ± standard deviation (SD).
Table 1. Feed efficacy (Physiological traits) of V instar of Bombyx mori larvae fed with control and different concentrations of silver nanoparticles treated MR2 mulberry leaves

<table>
<thead>
<tr>
<th>Experimental Groups and Concentration</th>
<th>Food Consumption (gm)</th>
<th>Food Utilization (gm)</th>
<th>Approximate Digestibility (%)</th>
<th>Food Consumption Index (%)</th>
<th>Co-efficient of Food Utilization (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>48.1000±1.1662</td>
<td>45.4000±0.1511</td>
<td>87.1017±0.3680</td>
<td>39.4550±0.10183</td>
<td>86.3200±0.5906</td>
</tr>
<tr>
<td>AgNps (T1) 25%</td>
<td>52.7667±0.9709</td>
<td>49.4833±1.6497</td>
<td>90.4133±1.2167</td>
<td>43.5850±1.1605</td>
<td>91.2900±0.8795</td>
</tr>
<tr>
<td>AgNps (T2) 50%</td>
<td>47.8833±1.0980</td>
<td>42.0367±0.6018</td>
<td>86.4983±0.2385</td>
<td>37.9633±1.3564</td>
<td>85.8300±0.3782</td>
</tr>
<tr>
<td>AgNps (T3) 75%</td>
<td>46.9833±0.3817</td>
<td>42.9133±3.6205</td>
<td>86.7317±0.2577</td>
<td>36.1850±1.8720</td>
<td>84.8150±0.9484</td>
</tr>
<tr>
<td>AgNps (T4) 100%</td>
<td>46.5500±0.8167</td>
<td>45.0750±1.2701</td>
<td>86.2017±0.2190</td>
<td>35.7083±1.4203</td>
<td>84.7633±0.5464</td>
</tr>
</tbody>
</table>

Values are Mean ± Standard Deviation of six observations. Values in the same column with different superscript letters (a, b & c) differs significantly at P<0.05 (DMRT).

< 0.05 was regarded as statistically significant (Sokal and Rohlf, 1981).

Results

Feed efficacy characters like Food Consumption (FC), Food Utilization (FU), Approximate Digestibility (AD), Food Consumption Index (FCI) and Co-efficient of Food Utilization (CFU) data of V instar larvae of Bombyx mori fed with control MR2 mulberry leaves and different concentrations of Silver nanoparticles treated MR2 mulberry leaves were presented in Table 1.

Table 1 shows that the food consumption (FC) data of V instar larvae of Bombyx mori fed with control and Silver nanoparticles treated MR2 mulberry leaves. The food consumption (gm) of group ‘C’ larvae (48.1000 ± 1.1662 gm), group T1 larvae (52.7667 ± 0.9709 gm), group T2 (47.8833 ± 1.0980 gm) larvae, group T3 (46.9833 ± 0.3817 gm) and group T4 (46.5500 ± 0.8167 gm), respectively. In these five observations, the 25% (group T1) Silver nanoparticles treated larvae food consumption (gm) was significantly increased than the other four groups (‘C’, T2, T3 and T4).

Table 1 shows that the food utilization (FU) data of V instar larvae of Bombyx mori fed with control and Silver nanoparticles treated MR2 mulberry leaves. The food utilization (gm) of group ‘C’ larvae (45.4000 ± 0.1511 gm), group T1 larvae (49.4833 ± 1.6497 gm), group T2 (42.0367 ± 0.6018 gm) larvae, group T3 (42.9133 ± 3.6205 gm) and group T4 (45.0750 ± 1.2701 gm), respectively. In these five observations, the 25% (group T1) Silver nanoparticles treated larvae food utilization (gm) was significantly increased than the other four groups (‘C’, T2, T3 and T4).

Table 1 shows that the food consumption index (FCI) data of V instar larvae of Bombyx mori fed with control and Silver nanoparticles treated MR2 mulberry leaves. The food consumption index (%) of group ‘C’ larvae (39.4550 ± 1.0189), group T1 larvae (43.5850 ± 1.1605 %), group T2 (37.9633 ± 1.3564 %) larvae, group T3 (36.1850 ± 1.8720 %) and group T4 (35.7083 ± 1.4203 %), respectively. In these five observations, the 25% (group T1) Silver nanoparticles treated larvae food consumption (%) was significantly increased than the other four groups (‘C’, T2, T3 and T4).

Table 1 shows that the approximate digestibility (AD) data of V instar larvae of Bombyx mori fed with control and Silver nanoparticles treated MR2 mulberry leaves. The approximate digestibility (%) of group ‘C’ larvae (87.1017 ± 0.3680 %), group T1 larvae (90.4133 ± 1.2167 %), group T2 (86.4983 ± 0.2389 %) larvae, group T3 (86.7317 ± 0.2577 %) and group T4 (86.2017 ± 0.2190 %), respectively. In these five observations, the 25% (group T1) Silver nanoparticles treated larvae approximate digestibility (%) was significantly increased than the other four groups (‘C’, T2, T3 and T4).

Table 1 shows that the co-efficient of food utilization (CFU) data of V instar larvae of Bombyx mori fed with control and Silver nanoparticles treated MR2 mulberry leaves. The co-efficient of food utilization (%) of group ‘C’ larvae (86.3200 ± 0.5906 %), group T1 larvae (91.2900 ± 0.8795 %), group T2 (85.8300 ± 0.3782 %) larvae, group T3 (84.8150 ± 0.9484 %) and group T4 (84.7633 ± 0.5464 %), respectively. In these five observations, the 25% (group T1) Silver nanoparticles treated larvae co-efficient of food utilization (%) was significantly increased than the other four groups (‘C’, T2, T3 and T4).

Discussion

According to Soo-Hoo and Frankel, (1966) the diminishing consumption rate of less preferred food was partially compensated by increased assimilation efficiency. According to Mathavan and Krishnan, (1976) assimilation efficiency did not vary significantly as a function of reduced food consumption. It has been reported that cocoon weight and pupal weight were directly proportional to the concentration of JH and the feeding period (Akai et al., 1985 and Chowdhary et al., 1990). Ashfaq et al. (2001) have mentioned that silkworm fed with M. nigrina showed high food consumption, coefficient of nutrition utilization, larval size, larval weight and cocoon weight that may provide important factors for increasing silk tenacity and elongation. The growth and development of silkworm is under the continuous influence of factors operating within and outside of the body.
Ascorbic acid had effect on the growth of silkworm (Javed and Gondal, 2002). Mulberry leaves with the combination of Nitrogen (0.2%) which enhances the growth and silk production (Javed and Gondal, 2002). Annway protein supplemented (10%) mulberry leaf significantly improved larval growth and economic characters of silkworm (Amala Rani et al., 2011). Verma and Atwal, (1963) have observed that feeding mulberry leaves supplemented with distilled water alone slightly increased the weights of larva, pupa and cocoon shell. According to Soo-Hoo and Frankel, (1966) the diminishing consumption rate of less preferred food was partially compensated by increased assimilation efficiency. According to Mathavan and Krishnan, (1976) assimilation efficiency did not vary significantly as a function of reduced food consumption. It was reported that cocoon weight and pupal weight were directly proportional to the concentration of JH and the feeding period (Akai et al., 1985 and Chowdhary et al., 1990).

As dietary or nutrient factors and related metabolic interactions has direct and indirect influence on specific gene regulation and expression (Hikhtar and Hussain, 2002; Phillips et al., 2008). Such interactions and variations in the field of nutrigenetics could be applied to choose the silkworm breeds based on their nutritional efficiency parameter as biomarkers. The majority of silkworm germplasm breeds were evaluated based on the feeding habit and adaptability for the commercial rearing on artificial diet that can feed on low cost artificial diet lacking mulberry (Mano et al., 1991; Zhang et al., 2002). Further, it was established that silkworm derives over 70% of the protein from the mulberry leaves and in V instar upto 96% of ingested protein is used for silk protein synthesis and variation in the quantity or quality of nutrition have profound effect on insect development (Fukuda and Higuchi, 1963). In sericulture, nutritional requirement and its conversion efficiency contribute directly or indirectly on the cost benefit ratio of silkworm rearing. It was considered as an important physiological criterion for evaluating the superiority of silkworm breeds. In silkworm, 97% of the total food intake during the last two instars and the feed utilization study confined to V instar larvae as 80-85% of the total leaves consumed in this instar as silkworm very active metabolically at this stage (Rahmathulla et al., 2005).

Javaid (1991) and Nadeem (1996) who have reported that silkworm larvae fed on mulberry leaves supplemented with mineral nutrients gave good food consumption and coefficient of utilization but low mortality. Rehman (1997) has found similar impact of different doses nitrogen and concluded that higher doses resulted in decline food consumption and coefficient of utilization. Javed and Gondal, (2002) have reported that the larvae which were offered mulberry leaves treated with 0.2% N + 0.150% ascorbic acid showed lower mean values of body weight, body length, food consumption, coefficient of utilization and cocoon shell ratio but higher mortality rate. Sarkar and Fujita (1994) have reported that the low intake of food results led to reduced larval period. This finding clearly indicates that the varieties with high conversion efficiencies may reduce the larval span and consequently less quantity of the food is needed to support optimal growth.

The result obtained from the study revealed a highly significant variance on nutritional traits between the leaf variety such as MR1 and MR2 treated with silver nanoparticles (Silver nanoparticles). Similar results have been reported by Magadum et al. (1996) and Gokulamma and Reddy, (2005). Efficiency of the nutrition almost nullified by the increase in consumption result in increased production of cocoon, shell and understood that dietary factors and related metabolic interactions has direct and indirect influence on specific gene expression. Feed conversion efficiency contributes directly and indirectly to the major chunk of the cost benefit ratio of silkworm rearing and considered as an important physiological criterion for evaluating the superiority of silkworm breeds. Previously Vyjayanthi and Subramanyam, (2002) have stated that multivoltine silkworms had significantly higher rates of feeding, assimilation and conversion with increased efficiency of conversion of ingested and digested food to body substance when compared with bivoltine silkworms. Food utilization parameters have been studied in many insects (Rath et al., 2003). The nutritive value of mulberry leaves depends on various agro-climatic factors and any deficiency of nutrients in leaves affect silk synthesis by the silkworm. Nutritional management directly influences the quality and quantity of silk production (Hiware, 2006). Vyjayanthi and Subramanyam, (2002) have stated in the silkworm, Bombyx mori, feeding behavior depends on the niche, amount of food offered, quality of food, age and health of the larva. As most phytophagus lepidoptera are voracious feeders any imbalance in the inputs from various factors affect food intake and result in poor larval development (Waldbauer, 1968; Vyjayanthi and Subramanyam, 2002). It may be concluded from this study that feed efficacy of Bombyx mori was comparatively more when the worm fed with 25% Silver nanoparticles treated MR2 mulberry leave than the control MR2 leaves and other groups.

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