

Nutritive effects of calcium carbonate (CaCO_3) on selected commercial traits of mulberry silkworm (*Bombyx mori* L.)

ABSTRACT

Silkworm (*Bombyx mori*) is the primary producer of silk. It has also been noted that minerals affect the quality of the silkworm. Calcium, a mineral with vital metabolic functions, might be involved in betterment of silkworms resulting in high quality silk production. Hence, this study was designed to evaluate the nutritive effects of Calcium carbonate (CaCO_3) on silkworms and subsequent silk production. Immediately after the completion of the third instar stage of silkworm (crossbreed race - BSR-95/14(M)SL), they were divided into the control group and treated groups. Both the treatment and control groups were replicated thrice with 60 larvae each. Then, the effect of Calcium carbonate was studied at 4th and 5th instar larvae of silkworm. The treated and the control group of 4th and 5th instar larvae of silkworm was fed four times a day with Mulberry leaves either dipped into different concentrations of Calcium carbonate (0.1%, 0.2%, 0.3%, 0.4% in distilled water) or dipped into distilled water only respectively. The results showed that the supplementation of Calcium Carbonate (CaCO_3) with Mulberry leaves resulted in significantly higher larval, egg, pupal, and cocoon characteristics compared to the control group. Among the different concentrations, 0.2% Calcium Carbonate supplementation showed the most valuable effect on silkworm. It can be concluded that enrichment of mulberry leaves with calcium carbonate is suitable for improvement of economic characteristics of silkworms, hence it might be suggested for usage in boosting farmers' yields.

Keywords: *Bombyx mori*, BSR-95/14(M)SL, Calcium Carbonate (CaCO_3), Mulberry leaves, Silk

1. INTRODUCTION

For the silkworm, *Bombyx mori* L., to grow and develop more effectively, nutrition is essential. The nutritional value of mulberry leaves and the larvae's diet have a significant impact on the quality of silk production, which in turn affects the creation of superior cocoons. Prior study has mostly focused on evaluating the leaves or their specific components efficacy [1]. The mulberry leaves provide all of the nutrition silkworms need. The leaves' nutritional state can be enhanced by adding vitamins and other nutrients to them [2]. Better silkworm larval growth and development were demonstrated by feeding them nutritionally enhanced leaves, which also had a direct impact on the amount and quality of silk produced [3]. By adding minerals and other elements to mulberry leaves, their nutritional value can be raised. Minerals are important components of silkworm diets because they function as co-factors in many enzyme systems and are essential for controlling the osmotic pressure of intracellular and extracellular fluids. The nutrition and health of the larvae determine the silkworm's conditions for producing silk. Numerous minerals have been utilized to increase these production factors, both in terms of quantity and

quality. Numerous investigations on the impact of minerals on silkworms were conducted by different researchers across varying time periods [4,5,6]. Methionine, L-serine, vitamin C, and vitamin B-complex all significantly enhance pupal and larval growth. [7]

Mulberry leaves can be fortified with minerals and other substances to improve their nutritional value. The protein, DNA, and RNA levels in the silk glands of the silkworm, *B. mori* Nistari race, are impacted by potassium iodide, cobalt chloride, calcium chloride, and potassium nitrate [8]. Calcium ions (Ca^{2+}) are essential signaling molecules that can either fully activate other enzymes engaged in signal transduction pathways or act directly as second messengers [9-10]. After being activated by recombinant prothoracicotropic hormone (PTTH), calcium is essential for promoting ecdysteroidogenesis in the silkworm prothoracic glands [11]. Moreover, it has been shown how the development of silkworms (*Bombyx mori* L.) and the production of silk are affected by the ideal levels of nitrogen, potassium, calcium, and copper [12]. The silkworm's most active eating period occurs in the late larval stage, when it builds up substantial fuel reserves in its tissues and displays special biochemical adaptations to preserve food supplies [13]. Applying CuSO_4 , ZnSO_4 , and FeSO increases food ingestion and absorption, growth, digestibility and efficiency, and larval metrics. [14]

Calcium is an important component that might affect various aspects of silkworm metabolism, but the information is still inadequate. Hence, the present study was designed to explore the effects of calcium carbonate supplementation with mulberry leaves on the silkworm's egg-laying, larval stages, pupa, and cocoon production.

2. MATERIAL AND METHODS

The silkworm breed *Bombyx mori* chosen for the experiment was BSR-95/14(M)SL race of silkworm. This race was derived from the original Nistari stock, and the eggs were obtained from the germplasm bank of the Bangladesh Sericulture Research and Training Institute in Rajshahi. Once the larvae hatched, they were removed from the stock culture and provided with a sufficient supply of fresh mulberry leaves. The experiment involved the use of fourth and fifth instar larvae. Following the second instar, the larvae were adapted to the laboratory environment and separated into five experimental groups, one of which served as the control group. Throughout this period, the larvae received four feedings daily, and appropriate disinfection measures were upheld. Each of the 15 trays contained 60 larvae, arranged into five triplicate sets for the treatment at specified dosages. A control batch was established for the corresponding ages by feeding the larvae with distilled water only. The study material used in this experiment was Calbo® 500 (Calcium Carbonate), produced by Square Limited in Bangladesh. The calcium carbonate was crushed, dissolved in distilled water, and diluted to concentrations of 0.1%, 0.2%, 0.3%, and 0.4%. Fresh mulberry leaves were immersed in each concentration and left to air dry for 30 minutes in normal laboratory condition. The treated leaves of different concentrations were provided to the 4th and 5th instar larvae four times a day. The control group of silkworm larvae was fed mulberry leaves dipped in distilled water. This feeding regimen was maintained until the silkworms entered the cocoon stage. For all doses, the following parameters were measured: larval weight, length, and breadth; cocoon weight, length, and breadth; shell weight and ratio; pupal weight, length, and breadth; effective rearing rate; total number of eggs laid per female; egg hatching percentage; percentage of unfertilized, dead, and blue eggs.

To ascertain the significance of the variations between the treatment groups, the data were gathered and examined using statistical methods like ANOVA and student t test. The p-values are as follows: $p < 0.001$ implies very strong significance (***), $p < 0.01$ indicates strong significance (**), and $p < 0.05$ indicates moderate significance (*). and a value of 0.05 or above does not indicate significance.

3. RESULTS AND DISCUSSION

The data obtained from the experiment, which involved supplementing mulberry leaves with calcium carbonate to enhance silkworm larval, cocoon, and egg characteristics, are presented in Figures 1, 2,3 and 4. Following the application of CaCO_3 , the larvae's length (Fig.1A) and breadth (Fig.1B) in the fifth instar stage increased significantly. The p value of the statistical test (two-way ANOVA) indicates that the differences in larval length and breadth are statistically significant for all treatment groups. T5 had the smallest larval length and breadth, while T2 had the highest. Figure 1(C) shows the larval weight of 5th instar from day 1 to day 7. The 0.2% concentration of CaCO_3 showed the greatest impact. It demonstrates that all four calcium carbonate treatment groups had higher larvae weights than the control group and those differences were statistically significant. The results revealed that 0.2% CaCO_3 application resulted in an overall weight gain of 0.82 g. Comparable results reported by another study showed that the maximum food consumption and coefficient of utilization (which included 0.2% nitrogen, 0.3% potassium, 0.1% calcium, and 0.05% copper) considerably improves the performance of silkworm larvae . This improvement, which was ascribed to the supply of higher food quality, was manifested in increased body length and weight [12]. Similarly, it has been reported that supplementation of mineral salts comprising calcium, magnesium, natural phosphates, zinc, iron, manganese, and magnesium resulted in a considerable increase in larval weight [15]. Another study showed that feeding the silkworm larvae with mulberry leaves sprayed with various minerals and amino acid resulted in a higher fifth instar larval weight [16]. Moreover, all groups that received mineral treatments showed an increase in total protein content [17]. In addition to improving food consumption and utilization, the minerals given to silkworms caused the larvae to grow in length and weight [18]. The application of selenium as dietary supplements to silkworms (*Bombyx mori* L.) led to a considerable increase in larval weight when compared to the control group [19, 20].

Treatment of [CuSO_4 15 kg/ha + ZnSO_4 15 kg/ha + FeSO_4 30 kg/ha] showed a statistically significant increase on the PMxCSR2 hybrid variety of *Bombyx mori* larvae's performance and silk production traits. [14]

The 8 mg/ml concentration of vitamin mixture was found to be the most effective in silkworms (*Bombyx mori* L., Chinese hybrid 9f7x), with higher larval and pupal weights, lower mortality, a shorter fifth instar length, and an improved cocoon. A significant improvement was also observed in terms of silk productivity, cocoon and shell weights, and the ratio of cocoon shell.[21]

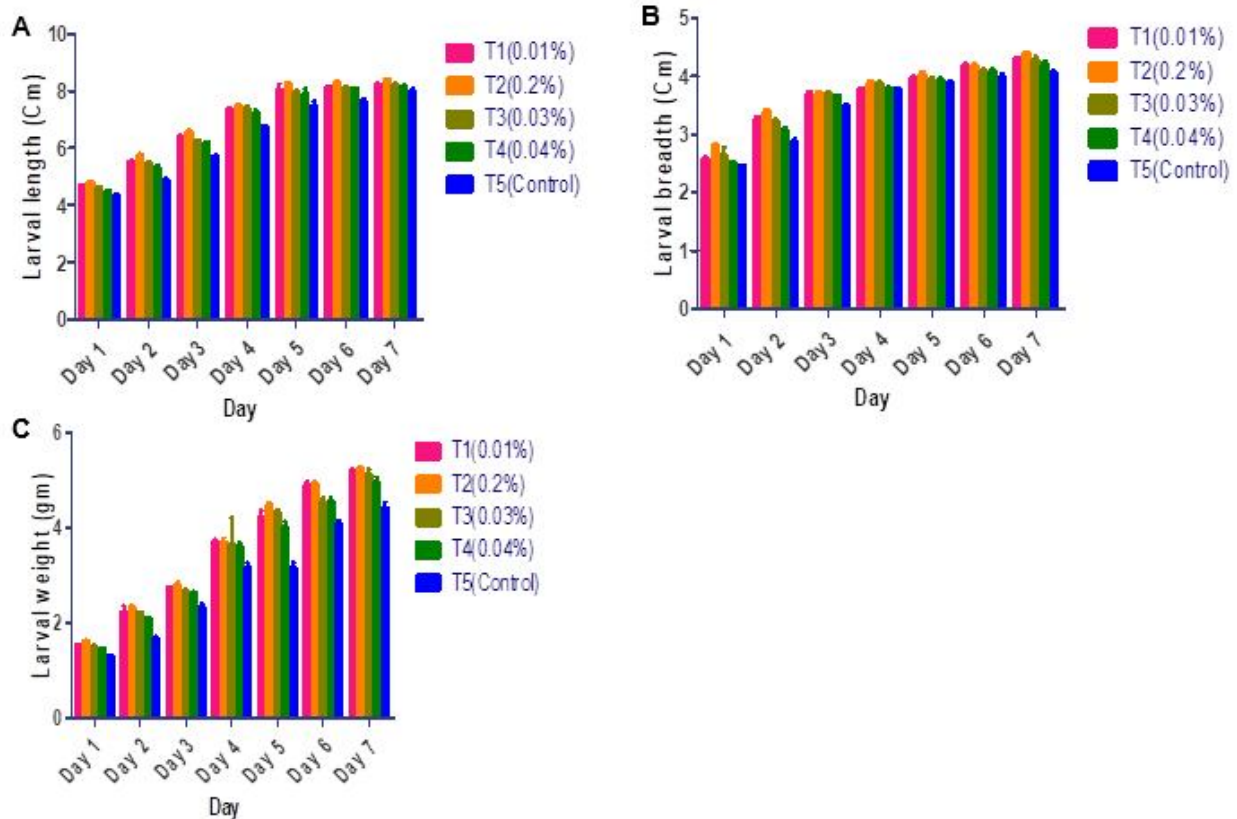


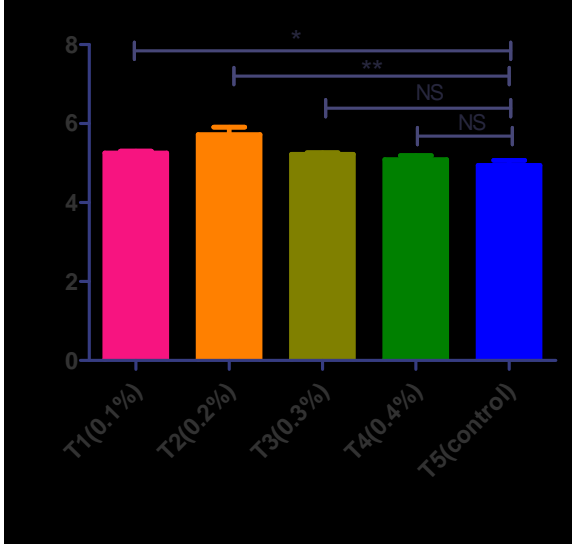
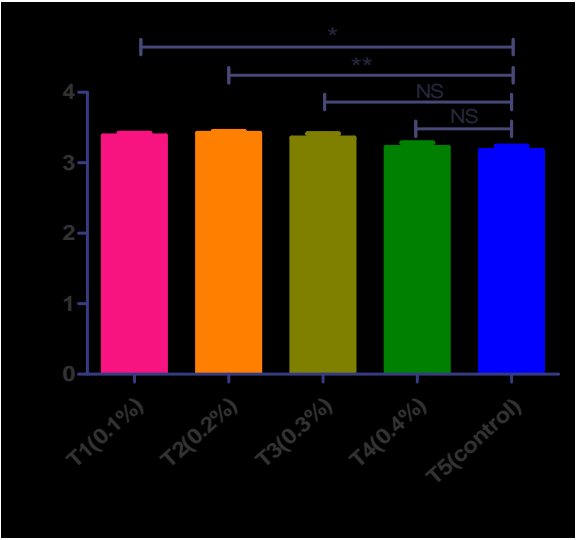
Figure 1. Effect of Calcium carbonate on length, breadth and weight of 5th instar larval of silkworms.

The traits of the shell and cocoon are depicted in Figure 2. The 0.1% and 0.2% concentration had a substantial impact on cocoon length, but the other two groups did not differ significantly from the control group (Fig.2). At the 0.2% concentration, the longest cocoon length (3.42 cm) was recorded. Both weight and breadth were strongly impacted at 0.1% and 0.2% (Fig. 2). Shell weight increased significantly at 0.1%, 0.2%, and 0.3% treatment of Calcium carbonate, but not significantly at 0.4% or the control group (Fig.2). The 0.2% concentration produced the highest pupal length, width, and weight measurements (2.93 cm, 4.39 cm, and

2.06 g, respectively), while the control group showed the lowest values. Pupal length was significantly affected by the 0.1% & 0.2% concentration; the other two groups did not exhibit any significant differences (Fig.3). At 0.1% and 0.2% concentration, pupal weight and breadth showed significant changes (Fig.3). All groups, including the control, maintained excellent survival rates and low mortality, with the effective rate of rearing across all four concentrations demonstrating no significant differences in larval survival when compared to the control group but slightly higher at T2 group (Fig.3). Fortification of the fourth and fifth instar silkworms' diet with zinc Chloride was reported to greatly enhance the weight of their cocoons [22]. Higher amounts of zinc chloride supplementation were also reported to improve cocoon output and quality [23]. It was reported that administering 200 ppm of zinc chloride once daily in the morning to fifth-instar silkworms resulted in the highest pupal weight (1.62 g) [16]. The results of this study were in line with other studies that showed the use of zinc chloride, either alone or in combination, significantly increased the shell weight and shell ratio [22-25]. It was also reported that supplementation of $ZnCl_2$, $ZnSO_4$, mineral salt, or cobalt chloride with mulberry leaves caused improvement of all economic parameters of silkworm, including the number of green cocoons, cocoon weight, shell weight, floss protein, shell-cocoon ratio, floss-shell ratio, silk-shell ratio, raw silk weight, raw silk [26, 27]. Fifth instar bivoltine silkworm races and hybrids treated with a mineral mixture combining nickel sulfate and potassium bromide at different concentrations showed a considerable increase in cocoon weight [28].

Both biological and economic characteristics of *Bombyx mori* silkworm larvae was improved by feeding them glutamine. It increases the larvae's weight gain, growth, development, and general health, which raises the yield and quality of their cocoons. [29]

Supplementing 500 ppm nickel sulphate in the feed of silkworms, *Bombyx mori* L., showed a great improvement in growth performance and economic parameters such as larval weight, pupal weight, and cocoon traits compared to the control.[30]



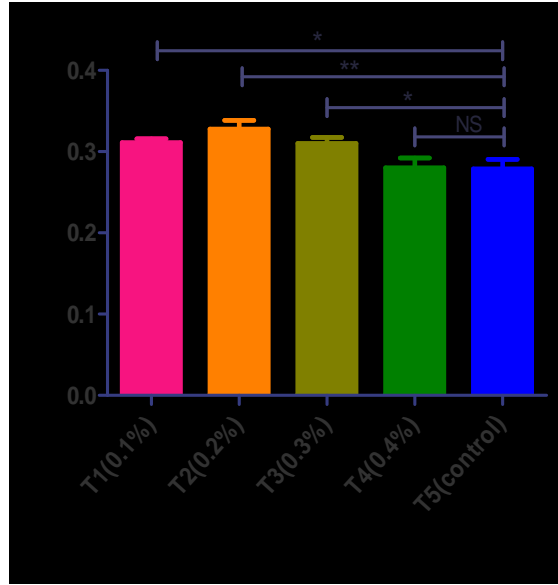
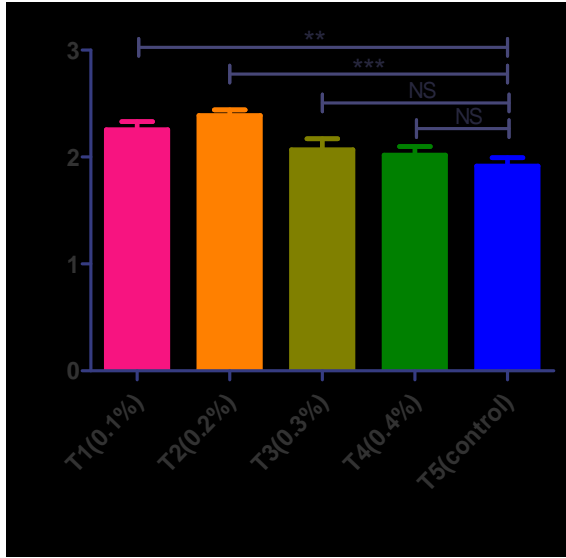


Figure 2. Effect of Calcium carbonate on the cocoon's length, breadth, and weight, and the weight of shell

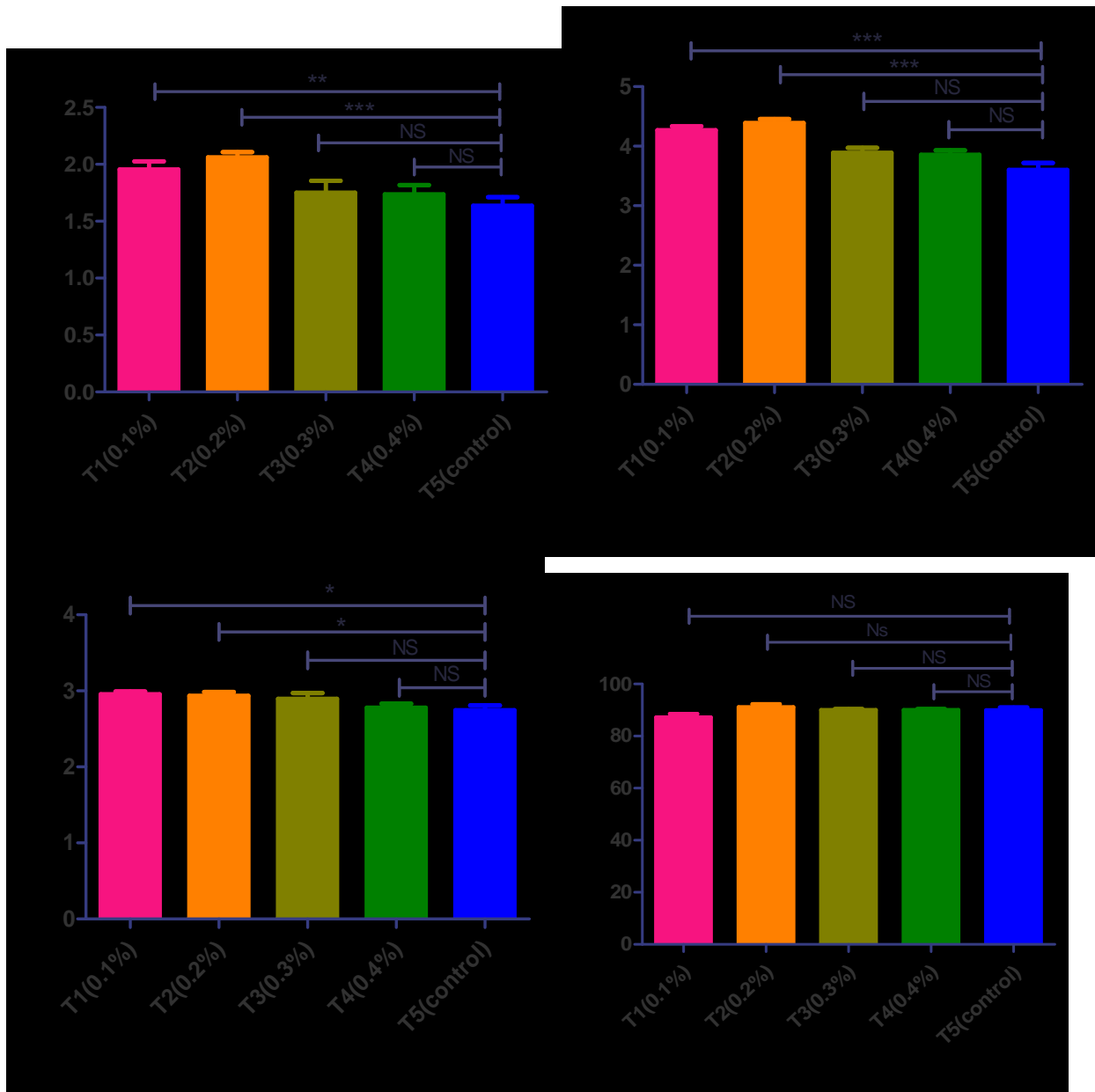
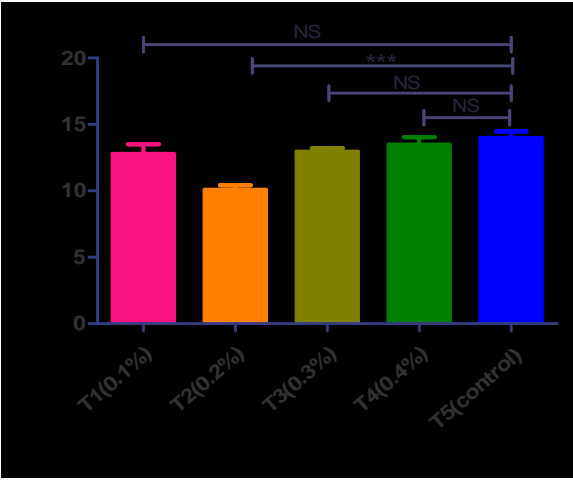
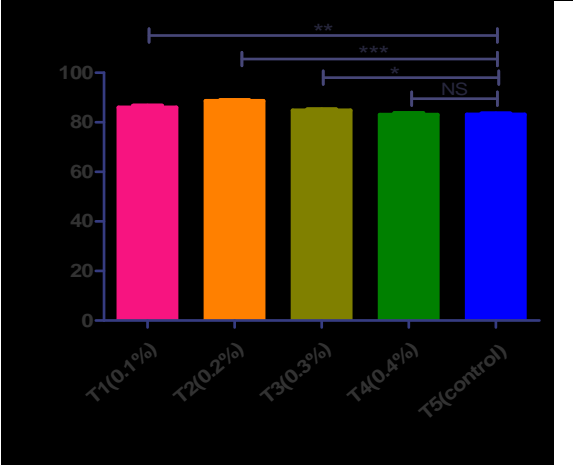
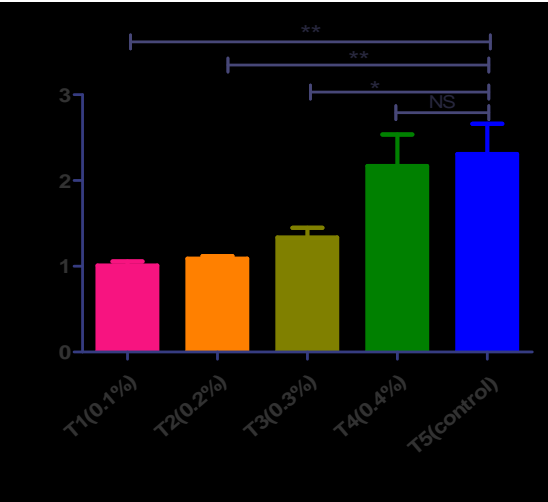
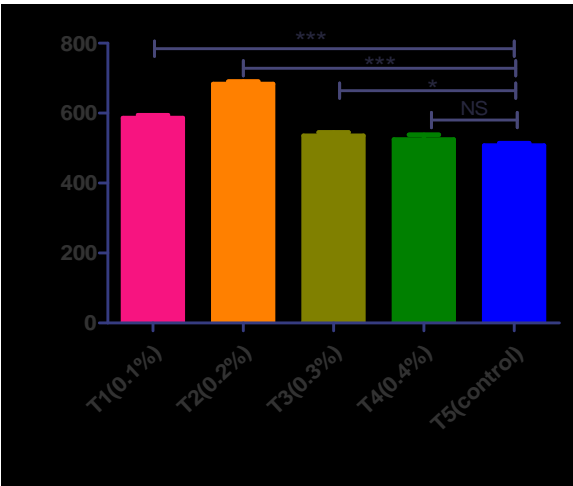


Figure 3. Effect of Calcium carbonate on the pupa's length, breadth, and weight, and effective rate of silkworm rearing.

The effects of varying calcium carbonate concentrations on the overall number of silkworm eggs, hatching rates, and the proportions of distinct egg types are shown in Figure 4. All treatment groups except T4 showed a statistically significant increase in the overall number of eggs (Fig.4). 0.2% concentration of Calcium carbonate showed the highest count at 683.6, while the control group had 507.8 (Fig. 4). Additionally, the hatching rate increased dramatically, peaking at 88.62% at the 0.2% concentration. Except for the 0.4% concentration, this improvement was statistically significant in every group (Fig.4).

But, the percentages of unfertilized eggs in the other three groups (T1, T2, T3) were significantly lower. The control group had the highest percentage of unfertilized eggs (2.3%) (Fig.4). The T2 groups had the lowest percentage of dead eggs (10.07%), and difference was statistically significant, but differences for the other three groups were not statistically significant (Fig.4). When compared to the control group, T1, T2 and T3 had considerably fewer blue eggs than the other group, and differences were statistically significant (Fig.4). These findings suggest that the 0.2% concentration was highly effective in reducing the proportions of dead, blue and unfertilized eggs. The positive impact of mineral nutrition on the silkworm economic traits such as cocoon weight, shell weight, fecundity, larval duration, effective rearing rate, silk filament length and weight, denier cocoon- shell ratio has been well documented with reference to nutritional role of several mineral salts such as cobalt chloride [27], cobalt [31], ferrous and magnesium sulfate [32], potassium nitrate [33], potassium bromide and nickel sulfate [28] and potassium and magnesium carbonates [34] . The results of another study showed that the leaves that treated with 0.2% Zinc sulfate significantly increased the body weight and the total amount of blood amino acid , whole cocoon weight, cocoon shell weight, total number of the spawned eggs and the normal eggs [35].



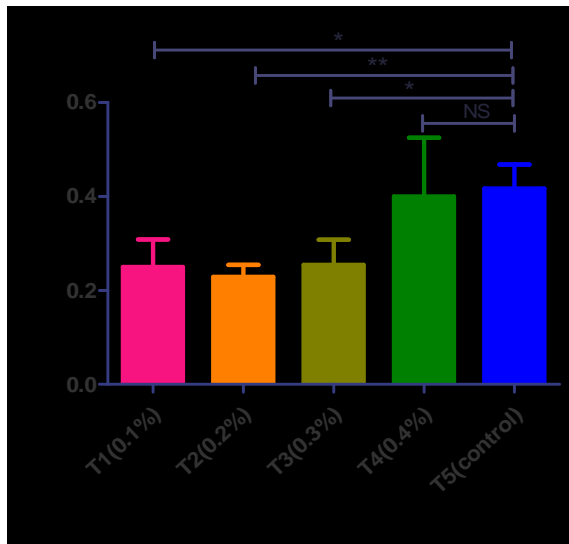


Figure 4. Effect of Calcium carbonate on the overall number of eggs, and percentage of egg hatching, unfertilized eggs, dead eggs, and blue eggs.

4. CONCLUSION

According to the study, supplementing **mulberry** with calcium carbonate at concentrations of 0.1% to 0.2% gradually enhances the biological and economic characteristics of *Bombyx mori*, but performance starts to deteriorate at concentrations of 0.3% to 0.4%. In particular, 0.2% calcium carbonate dramatically lowers the rates of dead and unfertilized eggs while increasing egg production, cocoon and pupal characteristics, larval length, breadth and weight. These results demonstrate that 0.2% calcium carbonate **in mulberry** is a useful dietary supplement for increasing sericulture silk yield.

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Authors' contributions

This work was carried out in collaboration among all authors. The manuscript was finalized by Md Fazlul Haque. All authors read and approved the final manuscript.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The author(s) hereby declare that limited use of generative AI technologies, such as Large Language Models (e.g., ChatGPT-4 and Quillbot), was utilized solely for assistance during writing. No text-to-image generators have been used in the creation of this work.

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