NUTRITIVE EFFECTS OF CALCIUM CARBONATE ($CaCO_3$) ON SOME COMMERCIAL TRAITS OF MULBERRY SILKWORM, Bombyxmori L.

Comment [MF1]: NUTRITIVE EFFECTS OF CALCIUM CARBONATE ($CaCO_3$) ON SOME COMMERCIAL TRAITS OF MULBERRY SILKWORM, *BOMBYX MORI* LARVAE
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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.

Comment [MF2]: Silkworm products

Comment [MF3]: calcium

Keywords:*Bombyx mori, BSR-95/14(M)SL, Calcium Carbonate (CaCO3), 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 [4-6].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. moriNistari race, are impacted by potassium iodide, cobalt chloride, calcium chloride, and potassium nitrate [7]. Calcium ions (Ca2+) are essential signaling molecules that can either fully activate other enzymes engaged in signal transduction pathways or act directly as second messengers [8-9]. After being activated by recombinant prothoracicotropic hormone (PTTH), calcium is essential for promoting ecdysteroidogenesis in the silkworm prothoracic glands [10]. 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 [11]. 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 [12]. Calcium is an important component that might affects 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 the specified dosage. A control batch was established for the corresponding ages by feeding the larvae with distilled water. 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. 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:

Comment [MF4]: Mention how long the soaking period lasted

Comment [MF5]: Methods of measuring parameters need to cite a source 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.

Comment [MF6]: Need reference

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.

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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 [11]. This improvement, which was ascribed to the supply of higher food quality, was manifested in increased body length and weight[11].Similarly, it has been reported thatsupplementation of mineral salts comprising calcium, magnesium, natural phosphates, zinc, iron, manganese, and magnesium resulted in a considerable increase in larval weight[13].Another study showed that feeding the silkworm larvae with mulberry leaves sprayed with various minerals and amin acid resulted in a higher fifth instar larval weight[14]. Moreover,all groups that received mineral treatments showed an increase in total protein content[15].In addition to improving food consumption and utilization, the minerals given to silkworms caused the larvae to grow in length and weight[16]. The application of seleniumas dietarysupplements to silkworms (Bombyx mori L.) led to a considerable increase in larval weight when compared to the control group[17, 18].

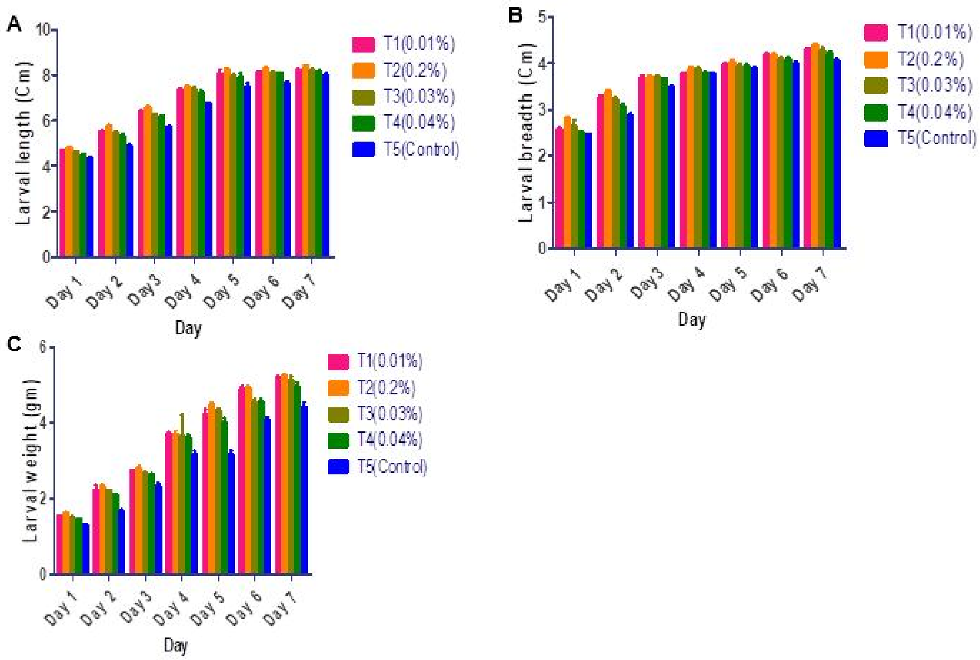

Figure1.Effect of Calcium carbonate on length,breadth and weight of5th instar larval of silkworms.

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 significantlyaffected 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[19]. Higher amounts of zinc chloride supplementation were also reported to improve cocoon output and quality[20]. 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)

[14]. The results of this studywere 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[19-22].It was alsoreported that supplementation of$ZnCl_2$, $ZnSO_4$,mineral salt, or cobalt chloridewith 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 [23, 24]. 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[25].

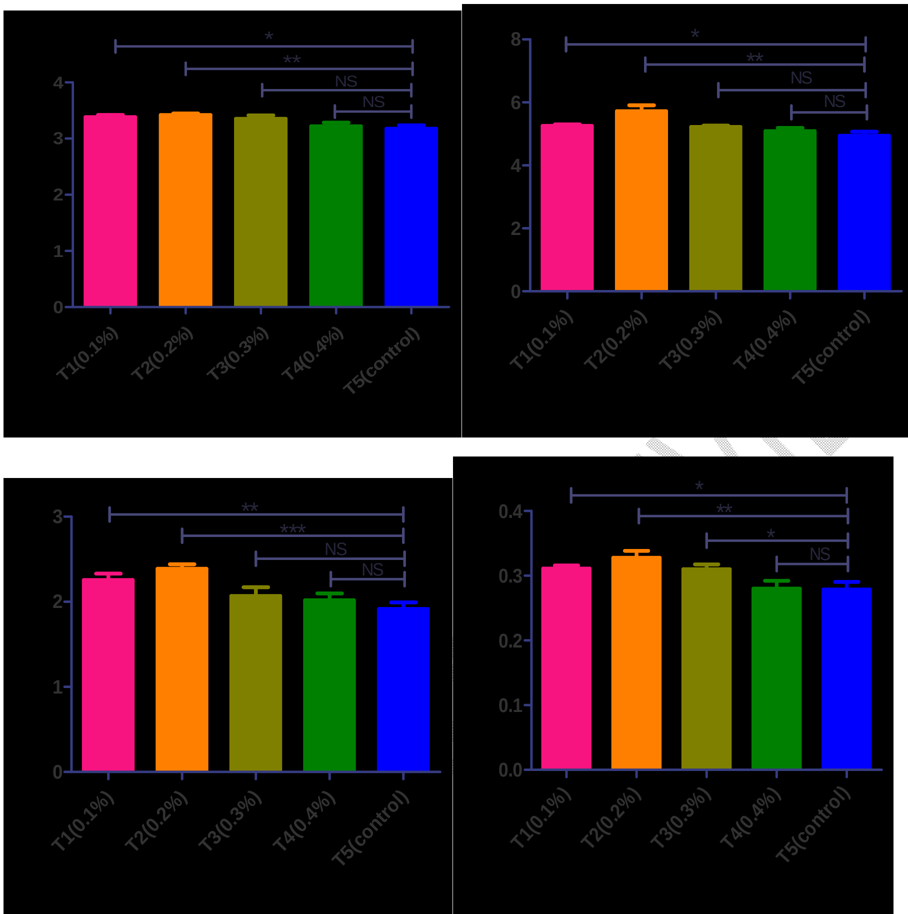

Figure2.Effect of Calcium carbonate onthe cocoon's length, breadth, and weight, and the weight of shell

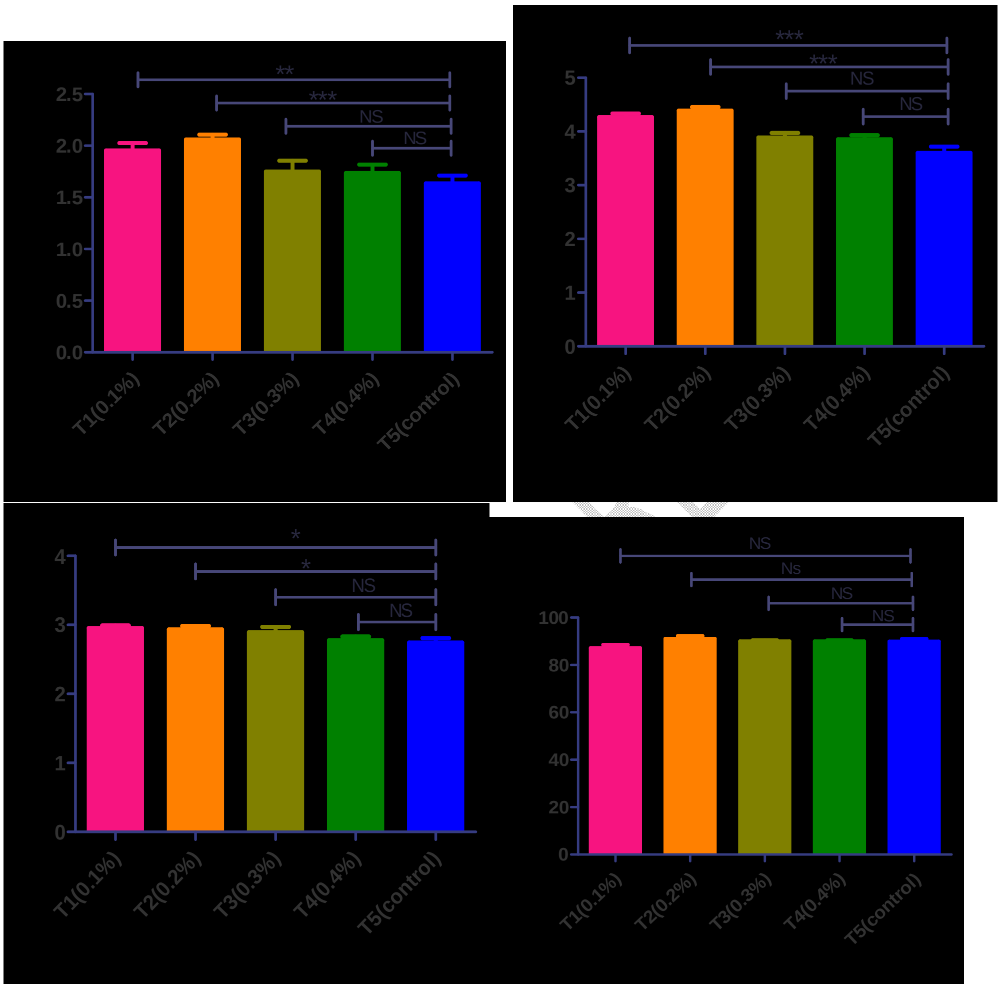

Figure3.Effect of Calcium carbonate on the pupa's length, breadth, and weight, and effective rate of silkworm rearing.

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 raring 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[24],cobalt [26], ferrous and magnesium sulfate[27], potassium nitrate[28], potassium bromide and nickel sulfate [25] and potassium and magnesium carbonates[29] .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[30].

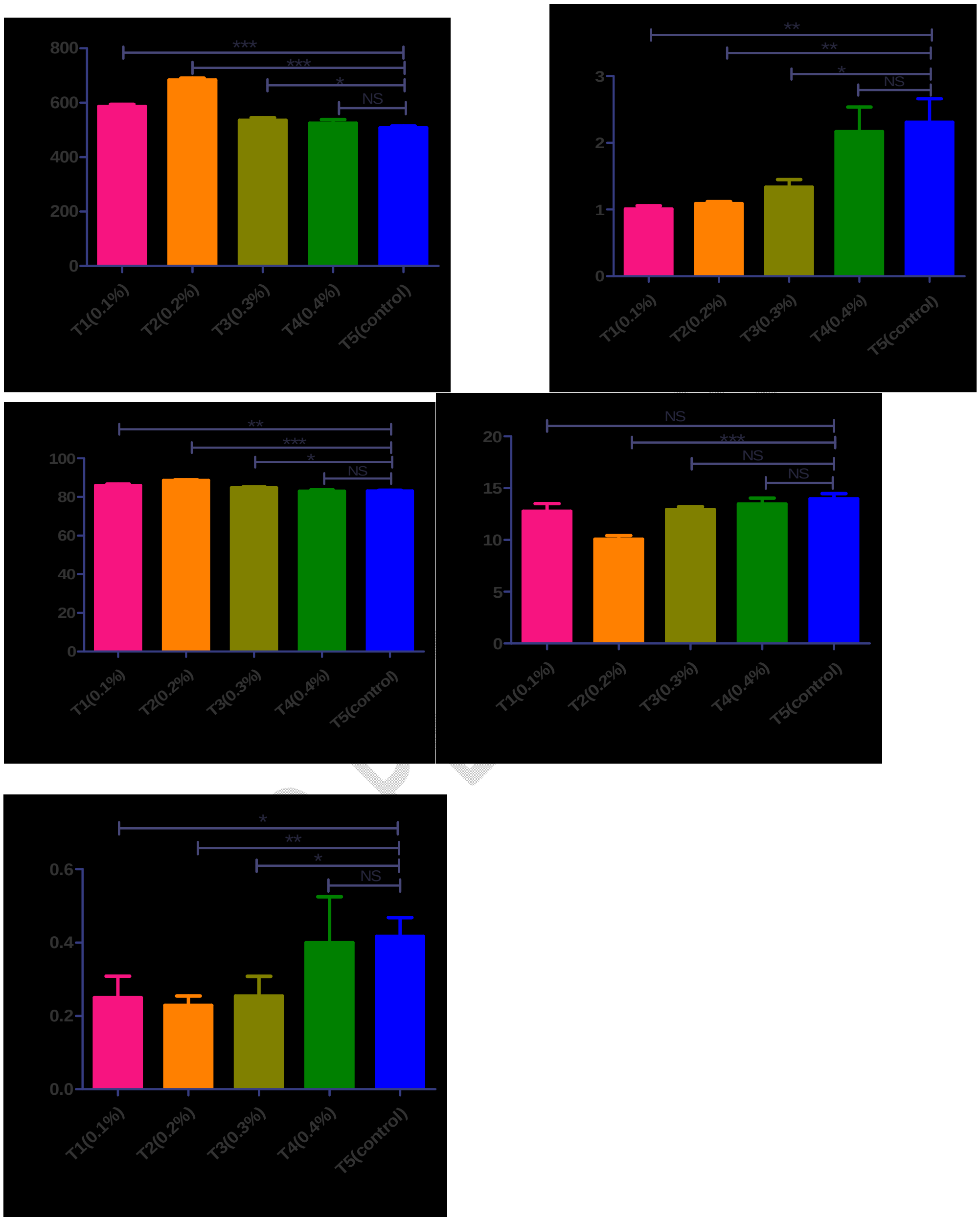

Figure4. Effect of Calcium carbonate onthe overall number of eggs,and percentageof egg hatching,unfertilized eggs, dead eggs, and blue eggs.

4. CONCLUSION

According to the study, supplementing 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 is a useful dietary supplement for increasing sericulture silk yield.

REFERENCES

Comment [MF8]: There are no recent sources Therefore, it is better to cite modern sources

1. Hanson B, Lindblom SD, Loeffler ML, Pilon-Smits EAH. Selenium protects plants from phloem-feeding aphids due to both deterrence and toxicity. New Phytologist. 2004 Jun;162(3):655–62.
2. Kanafi RR, Ebadi R, Mirhosseini SZ, Seidavi AR, Zolfaghari M, Etebari K. A review on nutritive effect of mulberry leaves enrichment with vitamins on economic traits and biological parameters of silkworm Bombyx mori L. Invertebrate Survival Journal. 2007;4(2):86–91.
3. Brandão-Neto J, Stefan V, Mendonça BB, Bloise W, Castro AVB. The essential role of zinc in growth. Nutrition research. 1995;15(3):335–58.
4. Khan MA, Akram W, Ashfaq M, Khan HAA, KIM YK, LEE J. Effects of optimum doses of nitrogen, phosphorus, potassium and calcium on silkworm, Bombyx mori L., growth and yield. Entomol Res. 2010;40(6):285–9.
5. Banu NA. Effect of Salt, Nickel Chloride Supplementation on the Growth of Silkworm, Bombyx mori L.(Lepidoptera: Bombycidae) Md. Rezuanul Islam," Md. Abdul OhayedAli,"Dipak Kumar Paul," Shaheen Sultana. Journal of Biological Sciences. 2004;4(2):170–2.
6. Bhattacharya A. Influence of themineral potassium Kochi and Kaliwal 23 permanganate on the economic parameters of the silkworm, Bombyx mori L. In: Proc National Conference on Sericulture for Global Competitiveness. 2005. p. 338–40.
7. Dasmahapatra AK, Chakraborthi MK, Medda AK. Effect of potassium iodide, cobalt chloride, calcium chloride and potassium nitrate on protein, RNA and DNA content of silk gland of silkworm,(Bombyx mori L.) Nistari race. Sericologia. 1989;29:355–9.
8. Berridge MJ. Inositol trisphosphate and calcium signalling. Nature. 1993;361(6410):315–25.
9. Putney Jr JW. Excitement about calcium signaling in inexcitable cells. Science (1979). 1993;262(5134):676–8.

10. Gu SH, Chow YS, O'Reilly DR. Role of calcium in the stimulation of ecdysteroidogenesis by recombinant prothoracicotropic hormone in the prothoracic glands of the silkworm, Bombyx mori. Insect BiochemMol Biol. 1998;28(11):861–7.
11. Ashfaq M, Ahmad N, Ali A. Effects of optimum dosages of nitrogen, potassium, calcium and copper on silkworm, Bombyx mori L. development and silk yield. South Pac Study. 1998;18:47–50.
12. Etebari K, Kaliwal BB, Matindoost L. Different aspects of mulberry leaves supplementation with various nutritional compounds in sericulture. Int J IndustEntomol. 2004;9(1):15–28.
13. Vishwanath AP, Krishnamurthy K. Effect of foliar spray of micronutrients on the larval development and cocoon characters of silkworm (Bombyx mori L.). Indian journal of sericulture. 1982; 21: 1-6.
14. Gokul M. Effect of exogenous supplementation of amino acids and minerals on economic traits of silkworm. Bombyx mori. 2015;101.
15. Etebari K, Fazilati M. Effects of feeding on mulberry's supplementary leaves with multi-mineral on some biological and biochemical characteristics of silkworm (Bombyx mori). Isfahan University of Technology-Journal of Crop Production and Processing. 2003;7(1):233–44.
16. Khan MA, Akram W, Ashfaq M, Khan HAA, KIM YK, LEE J. Effects of optimum doses of nitrogen, phosphorus, potassium and calcium on silkworm, Bombyx mori L., growth and yield. Entomol Res. 2010;40(6):285–9.
17. Bențea M, Mărghitaș LA, Șara A, Dezmirean D, Gabor E, Vlaic B, et al. The effect of selenium and zinc supplementation on growth and reproduction of the mulberry silkworm (Bombyx mori L.). ProEnvironmentPromediu. 2011;4(8): 302-307.
18. Jiang L, Peng LL, Cao YY, Thakur K, Hu F, Tang SM, et al. Effect of Dietary Selenium Supplementation on Growth and Reproduction of Silkworm Bombyx mori L. Biol Trace Elem Res. 2020 Jan 1;193(1):271–81.
19. Kaliwal BB, Hugar II. Effect of Zinc Chloride on Commercial Traits of the Bivoltine Silkworm, Bombyx mori L. Int J IndustEntomol. 2003;6(1):75–9.
20. Kavitha S, Sivaprasad S, Saidulla B, Yellamma K. Effect of zinc chloride and zinc sulphate on the silkworm, Bombyx mori growth tissue proteins and economic parameters of sericulture. The Bioscan. 2012;7(2):189–95.
21. Ashfaq M, Afzal W, Hanif MA. Effect of Zn(II) deposition in soil on mulberry-silk worm food chain. Afr J Biotechnol [Internet]. 2010;9(11):1665–72. Available from: http://www.academicjournals.org/AJB
22. Hugar II, Nirwani RB, Kaliwal BB. Effect of zinc chloride on the bio-chemical changes in the fat body and haemolymph of the bivoltine silkworm, Bombyx mori L. Sericologia. 1998;38(2):299–308.

23. Wani Y, Bashir M, Rani S, Rather RA, Shafi S. Effect of zinc on the larval growth and quality cocoon parameters of silkworm (Bombyx mori L.): A review. ~ 31 ~ International Journal of Fauna and Biological Studies. 2018;5(4):31–6.
24. Chakrabarti MK, Medda AK. Effect of cobalt chloride on silkworm (Bombyx mori L.) Nistari race [India]. Science and Culture (India). 1978;44(9):406-408.
25. Kochi SC, Kaliwal BB. Synergetic Effect of Minerals Mixture of Potassium Bromide and Nickel Sulphate on the Economic Traits of CSR2, CSR4 and CSR2 × CSR4 Crossbreed Races of the Silkworm, Bombyx mori L. Int J IndustEntomol. 2005;10(2):107–17.
26. Narasimhamurthy C V, Govindappa S. Effect of cobalt on silkworm growth and cocoon crop performance. Indian J Seric. 1988;27(1):45–7.
27. Nirwani RB, Kaliwal BB. Effect of ferrous and magnesium sulphatesupplementetion on some commercial characters of B. mori L. Bull Seric Res. 1995;6:21–7.
28. Goudar KS, Kaliwal BB. Effect of Potassium Nitrate Supplementation on Economic Parameters of the Silkworm Bombyx mori L. Int J IndustEntomol. 2000;1(1):47–52.
29. Chakrabarty S, Kaliwal BB. Supplementation with Potassium carbonate, magnesium carbonate and their synergetic effects on the economic traits of the silkworm, Bombyx mori L. World Journal of Science and Technology. 2011;1(5):10–23.
30. Zhangji Y, Shixian H, Yongqiang H. Effects of zinc on the growth and cocoon quality of silkworm, Bombyx mori. Xi Nan Nong Ye Xue Bao. 1997;10(2):81–4.