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# Silkworm (*Bombyx mori* L.) fed with mulberry (*Morus alba* L.) leaves and cow's milk

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**ABSTRACT**

**Objective**: To evaluate the effect of cow's milk as a protein supplement to improve growth and expression of productive parameters in the silkworm (*Bombyx mori* L.).

**Design/Methodology/Approach**: Four treatments with mulberry foliage supplemented with milk were used, plus a control (which was fed with fresh leaf). Feeding was determined for the last two larval instars, resulting in the following groups: Treatment 1: V instar-TD, Treatment 2: V instar-DI, Treatment 3: IV and V instar-TD, and Treatment 4: IV and V instar-DI.

**Results**: Larvae fed with foliage supplemented with milk obtained an increase in growth rate and larval weight and formed their cocoons faster than the control. Additionally, there were differences in cocoon weight, silk percentage, and pupal weight, relevant traits for silk production.

**Study Limitations/Implications**: Very few works have been published about silkworms fed with mulberry foliage supplemented with milk.

**Findings/Conclusions**: Supplementation has a positive effect on growth and the evaluated traits, improving the yield of silk production.

**Key words**: Silkworm, milk, mulberry, economic parameters, supplementation.

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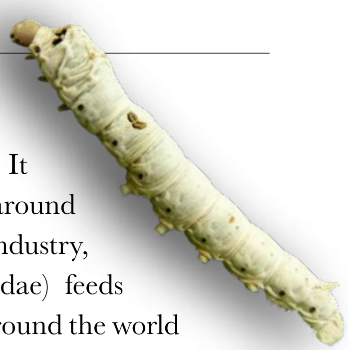

## INTRODUCTION

Mulberry (*Morus alba* L.) is native to temperate zones of Asia. It was first cultivated in regions of China, Japan, and the Himalayas around 4,500 years ago. It is the agronomic element of the sericulture industry, because the silkworm (*Bombyx mori* L.) (Lepidoptera: Bombycidae) feeds exclusive on its leaves. It is mainly used in more than 42 countries around the world in sericulture, for ecosystem improvement, and as animal and human food (Rodríguez *et al.*, 2012). Mulberry has one of the highest nutritive values among forages from plant origin; additionally, it has good biomass production and an important agronomic versatility, with an excellent potential to improve feed quality and increase animal production (Camayo *et al.*, 2021; Elizondo 2004). It is highly digestible and has an excellent crude protein (CP) content that reaches levels of 20 to 24% (Mejía, 2019; González and Milera, 2000). Therefore, the cell wall content —along

with the structural carbohydrates and ashes found in the foliage— has been considered as an excellent feed for high-yielding animals and can be offered fresh or dried as part of compound rations (Boschini, 2002). The crop is also valued both for its fruit (consumed fresh, juiced, or canned) and its medicinal properties as an infusion (mulberry leaf tea) (Kumar *et al.*, 2015).

The main use of mulberry is as food for the silkworm (*Bombyx mori* L.) (Lepidoptera: Bombycidae). This monophagous insect —considered as a model system among the lepidopterans, given its easy management and low cost— does not represent a biological hazard, and it is economically important for the sericulture industry, since it is vital for silk production worldwide (Konala *et al.*, 2013).

Insect nutrition plays a fundamental role in sericulture, since it improves the expression of the commercial characters of *B. mori* (Ruiz *et al.*, 2020; Prieto *et al.*, 2016), influencing the quality and quantity of production at an almost individual level (Ruiz *et al.*, 2020). Currently, efforts have been focused on research trials related to the enrichment of mulberry foliage, using proteins, carbohydrates, amino acids, vitamins, hormones, and antibiotics (among other nutrients) to encourge better productive responses (Mohamed and Helaly, 2018). In this sense, Bentea *et al.* (2011) point out that the use of these nutrients can improve the expression of such characteristics as larval and cocoon weight. Konala *et al.* (2013) and other authors also suggest that their inclusion in the diet of insects (particularly of *B. mori* L.) is important. Cow's milk can simultaneously provide bioactive compounds —*i.e.*, proteins, carbohydrates, fatty acids, minerals, and other nutrients that favor the growth and development of the life cycle— and peptides, polyamines, and enzymes that can potentially modulate several regulatory processes of the organism (Haug *et al.*, 2007). Therefore, this study evaluated the effect of milk as a protein supplement that improves growth and expression of productive parameters in silkworm (*B. mori*).

## MATERIALS AND METHODS

The trial was conducted in 2019 at the Silkworm Laboratory of the Universidad Politécnica de Francisco I. Madero (UPFIM) (20° 15' 2" N and 99° 00' 1" W). Located at 1,995 m.a.s.l., the location has a temperate-cold climate, with 17 °C annual average temperature and 540 mm average annual precipitation (Rodríguez *et al.*, 2013).

**Biological material**. The biological material (Figure 1) was made up of populations of *B. mori* (kinshu×showa hybrid / Chinese×Japanese race). Larvae were reared in 40×40 cm plastic containers, at 24±4 °C and with a 55-65% relative humidity.

**Rearing method**. Mulberry (Kanva variety) leaves were dipped in milk and dried at room temperature before the first feeding of the day. Rations were offered three times a day (7:30 am, 12:00 pm, and 5:00 pm). Beddings and brood containers were cleaned daily to avoid health issues, eliminating feces and mulberry residues.

**Milk composition**. Ultra-pasteurized whole milk with added vitamins (A and D) was used. The nutritional composition per 250 ml was as follows: 7.8 g of protein, 8.8 g of lipids, 5.4 g of saturated fat, 13 g of carbohydrates, 13 g of sugars, 116 mg of sodium, 290 mg of calcium, 166 $\mu$g of vitamin A, 1.25 $\mu$g of vitamin D, and 162 kcal of energy content. Experimental design. For the purposes of the comparative analysis, larvae were divided

**Figure 1**. Larva of *Bombyx mori* L. kinshu×showa hybrid / Chinese×Japanese race.

into five groups. Group 1 was fed with fresh mulberry leaves every day, during the fifth instar, and was considered as the control. Group 2 was fed with mulberry leaves dipped in milk every day, during the fifth instar (Treatment 1: V instar - TD). Group 3 was fed with mulberry leaves dipped in milk on alternate days (1, 3, and 5) of the fifth instar (Treatment 2: V instar - DI); whereas, on days 2, 4, and 6, they were fed with fresh mulberry leaves. Group 4 was fed with mulberry leaves dipped in milk every day, during the fourth and fifth instar (Treatment 3: IV and V instar - TD). Group 5 was fed with mulberry leaves dipped in milk on alternate days (1, 3, and 5) from the fourth to the fifth instar (Treatment 4: IV and V instar - DI); whereas, on days 2, 4, and 6, they were fed with fresh mulberry leaves. Weights were recorded from day 1 to 5. A completely randomized design was used, with three replicates per treatment (with n=50 larvae each). ANOVA assumptions were verified individually for each variable. The independence of experimental errors was ensured through the random assignment of populations to each experimental unit. The assumption of normal distribution was analyzed using the Shapiro-Wilk test. The statistical software used was SPSS v. 25. The "F" value was considered significant at a 0.05 probability level and Tukey's multiple comparison test was performed at 5%.

**Productive traits evaluated**. Cocoon weight (g), bark weight (g), bark percentage (%), pupal weight (g), and fecundity (number) were defined as productive parameters related to silk quantity and relevant for silkworm rearing.

## RESULTS AND DISCUSSION

### Effect of fresh mulberry and mulberry supplemented with milk on larval growth

Larvae fed with mulberry foliage supplemented with milk showed a tendency to gain weight after the first day of handling; the highest growth and weight (3.175 g) was observed in Treatment 4. Additionally, in treatments where mulberry leaves were treated with milk during the IV and V instars, larvae decreased their consumption by the fifth day and matured early. The behavior observed with Treatment 1 was also expressed in larval weight, which was equal to the control on the fourth day of the V instar. Treatment 1 was the first group to initiate cocoon formation and the weight was reduced by the fifth day, the same as in Treatment 3 (Figure 2).

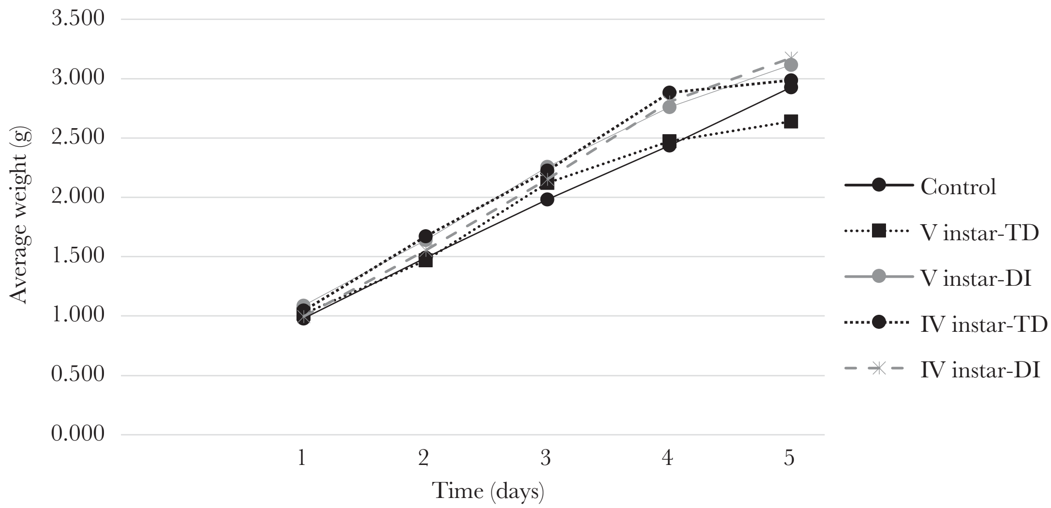


**Figure 2**. Relationship between the weight of larvae fed with fresh mulberry during the fifth instar days.

In general terms, the growth and weight of larvae fed with enriched foliage showed an increased trend compared to the control and, in this sense, Guevara *et al.* (2014) indicate that casein (the main protein component of milk) has a high content of essential amino acids. Casein has proven to be beneficial for the development of *B. mori*, probably because it contains large amounts of glutamic acid, which, just like aspartic acid, is required for the proper growth of larvae (Konala *et al.*, 2013). Other authors, including Mohamed and Helaly (2018), suggest that the increased growth and development could be caused by the high lipid and protein content of milk. Likewise, Hossain *et al.* (2015) and Byeon *et al.* (2005) point out that, during the fourth and fifth instars, nutrients used to enrich the foliage can have a positive influence on this trend.

**Effect of treatments on productive traits**

The results of the trial showed that cocoons formed by larvae fed with foliage supplemented with milk weighed more than those fed with fresh mulberry leaves (Table 1). Compared with control worms, the cocoon weights for Groups 3 (Treatment 2) and 5 (Treatment 4) increased by 27.5 and 24.7%, respectively; meanwhile, Group 4 (Treatment 3) increased by 18% and Group 2 (Treatment 1) increased by 12.5%.

Supplementation could induce an increase in larval weight. In this regard, Ramesh *et al.* (2018) points out that cocoons are selected by weight, because it functions as an indicator that predicts the approximate amount of silk that can be obtained —an aspect that largely depends on the quality and quantity of the mulberry foliage supplied.

Significant differences were found in the silk percentage variable. According to Tukey's multiple comparison test (5%), Treatment 1 and the control were grouped and showed similar results, while treatments 2, 3, and 4 —whose means did not represent differences, suggesting a similar behavior— formed the second group

These results suggest that milk has a high nutritional value, which could influence cocoon weight and directly affect silk percentage. In this context, Hossain *et al.* (2015) and Byeon *et al.* (2005) point out that foliage supplemented with milk does not hinder the

**Table 1**. Effect of milk on the productive traits of silkworms.

| Treatments | Cocoon weight (g) | Bark weight (g) | Silk percentage (%) | Pupal weight (g) | Fertility (No.) |
|---|---|---|---|---|---|
| 1 | 1.572 [bc] (±0.176) | 0.279 [a] (±0.033) | 17.831 [a] (±2.004) | 1.293 [bc] (±0.158) | 594.1 [a] (±90.5) |
| 2 | 1.782 [a] (±0.153) | 0.292 [a] (±0.024) | 16.452 [b] (±1.336) | 1.490 [a] (±0.143) | 556.7 [a] (±37.3) |
| 3 | 1.652 [ab] (±0.228) | 0.274 [a] (±0.026) | 16.759 [b] (±1.935) | 1.378 [ab] (±0.211) | 526.6 [a] (±35.5) |
| 4 | 1.742 [ab] (±0.155) | 0.293 [a] (±0.029) | 16.945 [b] (±2.197) | 1.448 [ab] (±0.189) | 520.7 [a] (±62) |
| Control | 1.397 [c] (±0.221) | 0.275 [a] (±0.048) | 19.819 [a] (±2.519) | 1.121 [c] (±0.189) | 521.1 [a] (±78.7) |

Note: a, b, c: means in each column with different letters show significant differences, according to Tukey (5%). Fecundity: number of eggs oviposited by a moth.

digestion of *B. mori*. Although lactose is the main carbohydrate component in milk, *B. mori* has the beta-glucosidase enzyme, active for cellobiose and lactose. Consequently, there are no negative effects to the metabolism of *B. mori*.

Significant differences were found in pupal weight. Treatment 2 had the best response. In this regard, Mohamed and Helaly (2018) and Sekar *et al*. (2016) indicate that feeding the larvae of *B. mori* L. in the fifth instar mulberry foliage supplemented with milk or other nutrients improves some biological characteristics, possibly as a result of the high content of nutrients (proteins and lipids) that increases their growth and development. Manuelian *et al*. (2018) and Haug *et al*. (2007) report that casein contains fatty acids, cholesterol, sugars, vitamins, and minerals (Ca, Mg, P, K, and Na) and, according to Konala *et al*. (2013), they stimulate the feeding efficiency of *B. mori* L. Regarding bark weight and fecundity, no significant differences were found between the control and the treatments used.

## CONCLUSIONS

This study determined that foliage supplemented with milk has a positive effect on the growth and weight of the larvae of *B. mori*. In addition, it promoted the early maturation of the larvae —*i.e.*, it accelerated the beginning of cocoon construction, an indicator of the growth rate acceleration. This is a highly relevant aspect, since it would reduce costs as a consequence of the decrease in the amount of foliage supplied, therefore reducing the labor used per brood. Likewise, cow's milk induced a significant increase on productive characters such as cocoon weight, silk percentage, and pupal weight, which are directly related to the increase in yield for silk production. Therefore, the results suggest that *B. mori* larvae can be fed with mulberry foliage supplemented with bovine milk and, in this way, increase silk production. However, the offspring should be fed constantly and its effect should be evaluated for each generation.

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## REFERENCES

Bentea, M., Marghitas, L. A. y Sara, A. 2011. The effect of some additives on the bioproductive performances of silkworm *Bombyx mori* L. *Anim. Sci. Biotechnol., 44*(1): 9-12. https://core.ac.uk/reader/25520850

Boschini, C. F. 2002. Nutritional quality of mulberry cultivated for ruminant feeding. In Mulberry for Animal Production, M.D. Sanchez (ed). FAO Animal Production and Health Paper, 171-182. https://www.fao.org/3/X9895E/x9895e0g.htm

Byeon, G. M., Lee, K. S., Gui, Z. Z., Kim, I., Kang, P. D., Lee, S. M., Sohn, H. D. y Jin, B. R. 2005. A diggestive beta-glucoside from silkworm, *Bombyx mori*: cDNA cloning, expression and enzymatic characterization. *Comp. Biochem. Phys. Part B, Biochem. Mol. Biol., 141*(4):418-427. https://doi.org/10.1016/j.cbpc.2005.05.001

Camayo, M., Montes, C., y Anaya, M. 2021. Efecto del abono orgánico líquido mineralizado en la producción de biomasa de morera (*Morus alba*). *Biotecnología en el Sector Agropecuario y Agroindustrial, 19*(1), 230-243. https://doi.org/10.18684/bsaa(19)230-243

Elizondo, J. 2004. Calidad nutricional y consumo de morera (*Morus alba*), Ramio (*Bohemeria nivea* (L) GAUD) y sorgo negro forrajero (*Sorghum almum*) en cabras. *Agronomía Mesoamericana 15*(2):209-213. https://www.redalyc.org/pdf/437/43715211.pdf

González, E., y Milera, M. 2000. Mulberry in livestock feeding systems in Cuba: Forage quality and goat growth. 2000. www.fao.org/WAICENT/FAOINFO/AGRICULT/ AGAP/ FRG/MULBERRY/Papers/HTML/Gonzalez.htm

Guevara, L., Cuartas, D., y Llano, F. 2014. Kappa caseína de la leche: aspectos bioquímicos, moleculares, productivos y nutricionales. *Revista Médica de Risaralda, 20*(1): 29-33. http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0122-06672014000100007&lng=en&tlng=es.

Haug, A., Hostmark, A. T. y Harstad, O. M. 2007. Bovine milk in human nutrition- a review. *Lipids Health Dis.,* 25: 6–25. https://doi.org/10.1186/1476-511X-6-25

Hossain, S., Uddin, A., Islam, S. y Alim, A., 2015. Effect of cow milk on the growth and economic traits of silkworm (*Bombyx mori* L.). *Int. J. Eng. Res., 6*(3):517-520. https://www.ijser.org/paper/Effect-of-Cow-Milk-on-the-Growth-and-Economic-Traits-of-Silkworm-Bombyx-mori-L.html

Konala, N., Abburi, P., Reddy, V. y Mamillapalli, A. 2013. The Effect of Bovine Milk on the Growth of *Bombyx mori*. *J. Insect Sci., 13*(98): 1-7. https://doi.org/10.1673/031.013.9801

Kumar, V., Gautam, C., Shobha, N. y Shankar, R. 2015. Use of mulberry leaves as supplementary food in cows and goats to improve milk production. *Int. J. Appl. Eng. Res., 1*(13): 81-84. https://www.allresearchjournal.com/archives/2015/vol1issue13/PartB/1-12-94.pdf

Manuelian, C. L., Penasa, M., Visentin, G., Zidi, A., Cassandro, M., y De Marchi, M. 2018. Mineral composition of cow milk from multibreed herds. *Animal Science Journal.* https://doi.org/10.1111/asj.13095

Mejía, H. 2019. La morera (Morus sp) como alternativa en sistemas silvopastoriles. *Rev. Iberoam. Bioecon. Cambio Clim., 5*(9), 1157–1163. https://doi.org/10.5377/ribcc.v5i9.7951

Mohamed, W., y Helaly, Y. 2018. Evaluation of two food additives on Bombyx mori L. characters. *Journal of Entomology and Zoology Studies, 2018; 6*(2): 3119-3123. https://www.entomoljournal.com/archives/2018/vol6issue2/PartAJ/6-1-406-620.pdf

Prieto, M., Díaz, M., Pérez, M., y Martín, D. 2016. Influencia de tres variedades de Morus alba L., en el crecimiento de Bombyx mori L. *Pastos y Forrajes, Vol. 39*(3): 132-137. https://www.redalyc.org/journal/2691/269148030008/html/

Ramesh, V., Pushparaj, K., Ganesh, P., y Rajasekar, P. 2018. Nutritional supplementation of amino acid l-serine on silkworm *Bombyx mori* (L.) larvae in relation to growth rate and silk production. *Research Journal of life sciences, Bioinformatics, pharmaceutical and chemical sciences, 4*(5), 1-12. https://doi.org/10.26479/2018.0405.24

Rodríguez, O. A., Martínez, M. A., Ventura, M. A., y Vargas, M. J. 2012. Adaptación de tres variedades de morera (*Morus* spp.) en el estado de Hidalgo. *Revista Mexicana de Ciencias Agrícolas*, Vol.3 Núm. 4, p. 671-683. https://cienciasagricolas.inifap.gob.mx/index.php/agricolas/article/view/1422/1255

Rodríguez, O. A., Martínez, M. A., Ventura, M. A., Vargas, M. J., Muhamed E. y Lara, V. F. M. 2013. Evaluación de variedades de morera en la alimentación del gusano de seda (*Bombyx mori*) en Hidalgo, México. *Revista Mexicana de Ciencias Agrícolas*, Vol. 4, Núm.5: 701-712. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S2007-93420130005000004

Ruiz, A., Caballero, B., Martínez, Y., Vega, R., Valdés, A., y Pérez, M. 2020. Analysis of the Energy Balance in the *Morus alba-Bombyx mori* System in Cuba's Sericulture. *International Journal of Agricultural Economics, 5*(1): 30-35. https://doi.org/10.11648/j.ijae.20200501.14

Sekar, P., Kalpana, S., Ganga, S., George John, Kannadasan, N., y Krishnamoorthy, R. 2016. Studies on the growth parameters (length & weight) and cocoon production in *Bombyx mori*, fed on mulberry leaves fortified with a putative Probiont, *Lactobacillus casei*. *International Journal of Current Research, 8*(04), 29127-29132. http://www.journalcra.com/sites/default/files/issue-pdf/14134.pdf