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Impact of Glutamine addition on Biological and Economical Characteristics of the Silkworm Larvae *B. Mori* L.

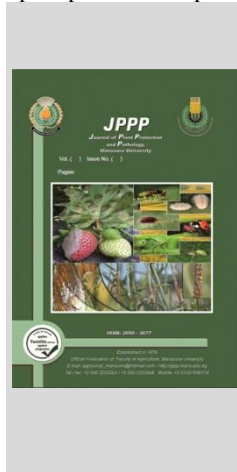
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ABSTRACT

Feeding is a critical aspect in silkworm rearing, as it directly influences its growth, development, and silk production. Glutamine is a building block for making proteins in the body. It's also synthesized other amino acids and glucose. The present study was conducted to evaluate the impact of glutamine powder as protein sources on some the biological and economical characters of mulberry silkworm, *Bombyx mori* L. It was added to mulberry leaves with different concentrations (1, 1.5 and 2 g/L water). This experiment was applied from the beginning of the fourth larval instar until hatching egg in spring 2023. The majority of the studied concentrations resulted in a significant improvement in the mulberry silkworm characteristics; the concentration 1g of glutamine showed a significant increase in larval and pupal weight, duration of larval stage, cocoon weight, cocooning percentage, cocoon length and silk ratio when compared with others concentration. Moreover, others concentration of glutamine was more effective than control. Therefore, adding glutamine to the diet of mulberry silkworm larvae *B. mori* can positively improve biological and economic characteristics. It can improve growth and development (the growth rate, weight gain, overall health of the silkworm larvae and higher Cocoon yield and quality). Finally, glutamine could be prescribed to the breeding programs to achieve high quantity and quality of cocoon yield for silk production.

Keywords: Glutamine, silkworm, rearing, Amino acid.

INTRODUCTION

In Egypt, the mulberry silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) is reared for cocoon only to silk production in spring season. Mulberry silkworm should be fed with good quality mulberry leaves for successful cocoon production (Vijaya *et al.*, 2009). Silk production is indirectly proportional to the nutritional treatments of mulberry leaves (Kerenhaph *et al.*, 2008). Nutrient supplementation is one of the ways to enrich growth rate in silkworm, *B. mori*. (Sengupta *et al.*, 1992 and Hossain *et al.*, 2015). Several studies on food supplementation and the development of artificial diets for silkworm rearing indicate that food supplementation has led to significant improvements in both commercial and biological aspects of sericulture. Supplementing the diet of silkworm larvae enhances growth, silk production, and overall rearing efficiency. However, attempts to rear silkworms using entirely artificial diets have had limited success, especially in commercial settings. Despite extensive research, artificial diets have not yet proven effective for large-scale silkworm rearing, likely due to their inability to fully replicate the nutritional benefits of natural mulberry leaves. (Goudar and Kaliwal, 2000 and Etebari and Matindoost 2005). Glutamine has been considered as a dietary supplement with a non-essential amino acid structure. The effect of mulberry leaves enriched with amino acids on the growth of *B. mori* has been studied by Radjabi (2010). The protein content of the silk gland, fat body, and muscles increase significantly when larvae were fed with ascorbic acid (Quraiza *et al.*, 2008). Around 70% of the silk proteins synthesized by silkworms

are obtained directly from the protein present in mulberry leaves (Bhattacharyya *et al.*, 2016). Adequate protein consumption is essential for the development of ovaries and eggs in adult female silkworms, as it is necessary for the secretion of juvenile hormones. Optimal proteins nutritional requirements vary by age, sex, physiological status and stress (Nation, 2001; Borah and Boro, 2020).

Several studies have found that supplementing with amino acids significantly improves the Production of raw silk. Mulberry Leaf Enrichment contains different amino acids, such as glycine (Saad *et al.* 2019), necessary amino acids (Arai and Ito, 1964), tyrosine phenylalanine and alanine (Nagarajan and Radha 1990), glycine, serine, and alanine (Mustafa and Elkaraksy (1990), aspartic acid (Kabila *et al.*, 1994) Glycine, Phenylalanine, Serine, and aspartic Acid (Vadivel, 1995), lanine and asparagine (Radjabi *et al.*, 2010), arginine and histidine (Chakrabarty). Serine (Gokul, 2015) and (Kaliwal and Chakrabarty 2012) have boosted the larvae, cocoon, reeling, and silk. Productivity-related metrics. Although the results are varied, several studies suggest that consuming glutamine can help treat some illnesses. In most cases, further research is required to fully grasp the potential benefits of glutamine. For this, the aim of this work to impact the effect of glutamine powder on the biological and economical characteristics of the mulberry silkworm larvae.

MATERIALS AND METHODS

The present study was carried out in the laboratory of Plant Protection Department, Faculty of Agriculture, Damietta University during the spring season 2023.

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1-Silkworm hybrid and protein resources

The eggs of the mulberry silkworm hybrid (HoxKKxG2xV2) were produced by the Sericulture and Agriculture Experiment Station in Bulgaria and supplied by the Sericulture Research Department of the Plant Protection Research Institute at the Agricultural Research Centre in Giza, Egypt.

2- Silkworm rearing technique:

Preparation for rearing:

The rearing room and appliances were completely cleaned, and the floor was washed with 5% bleaching powder solution. The whole room was disinfected by spraying with 2.5% sanitech and 0.5% slacked lime solutions (Dandin *et al.*, 2003).

Incubation of eggs:

The eggs were procured and incubated by adopting standard incubation and dark treatment practices (Krishnaswami, 1973).

Rearing room.

1- Stands and rearing equipment's were disinfected using formalin solution with 3% concentration.

Rearing technique:

Rearing of silkworm was carried out under laboratory condition (28.0+ 2.0 °C and 70 ± 5% R.H.).

Treatment: Leaves of mulberry were soaked in a food additive solution (glutamine powder, 1 g, 1.5 g, and 2 g /L water) for ten minutes and air dried. Then, the larvae were fed four times daily on mulberry *Morus alba* leaves with different treatments and leaves without food additives for control to estimate the economic and biological characters of the silkworm *Bombyx mori* L. under different treatments.

Experimental design:

The newly hatched *B. mori* larvae were divided into three groups (1000 larvae), Every group was divided into three subgroups. Each group content of 450 larvae (50 larvae for each replicant) and 100 larvae for control.

Studied parameters:

- 1- Weight (g) of larvae at the beginning, middle and end of the 4th and 5th instar before mounting.
- 2- Total larval duration (day) of 4th and 5th instar.

2- Cocoon characters:

- Weight of cocoon +pupa
- Fresh cocoon weight (g)
- Cocoon shell length (g).
- Wight of pupa (g).
- **Silk ratio= $\frac{\text{Weight of cocoon shell}}{\text{Weight of fresh cocoon}} \times 100$**
- **Cocoon percentage % = $\frac{\text{No. of cocoon harvest}}{\text{No. larvae retained.}} \times 100$**

• Duration of pupa

The cocoons were harvested seven days later. Fresh cocoons of each treatment were taken for measuring the cocoon indices. Also, adults' males and females were weighed for all treatments, fertility of egg (%), (total number

of fertile eggs /female) and Hatchability percentage (%) was estimated according to the following formula of (According to Lea (1996).

$$\text{Hatchability \%} = \frac{\text{Number of hatched larvae}}{\text{Number of fertilized eggs}} \times 100$$

Statistical analysis:

The collected data were analyzed statistically using a one-way ANOVA (Analysis of Variance) to determine whether there were significant differences between the control group and the experimental groups. Sigmaplot 15 (2024).

RESULTS AND DISCUSSION

Results

Biological Characteristics:

• Larval stage

Data in Table (1) showed the effect of different levels of glutamine treatments on the 4th larval duration of *B. mori* during spring rearing season of year 2023. The results showed that, there were highly significant differences between treatments. The highest values of larval weight were recorded for glutamine 1 g and followed by glutamine 1.5 and 2 g (0.385 and 0.595 g) in the middle and end of the 4th larval instar respectively, Moreover, the high average weight of the 5th larval instar was recorded for glutamine 1 g (0.531, 1.571 and 2.705) in beginning and middle and finally. On the other hand, the low value of larval weight was recorded for control (0.162, 0.301 and 0.462) in the beginning, middle, and finally of the 4th and (0.162, 1.458 and 1.985) in 5th larval instar respectively.

The data in Table (1) and Fig (1) indicated that the shortest larval duration was recorded for glutamine 1g, it was 6 days ,whereas, the other concentration of glutamine (1.5g. and 2g.) gave the same 4th larval duration 7 days and control was 8 days., while showed the effect of different food additives glutamine on the 5th larval duration glutamine 1g was long duration with 11 days while glutamine 1.5g and 2g were short duration with 10 days while the control gave the longest 5th larval duration with 12 days .

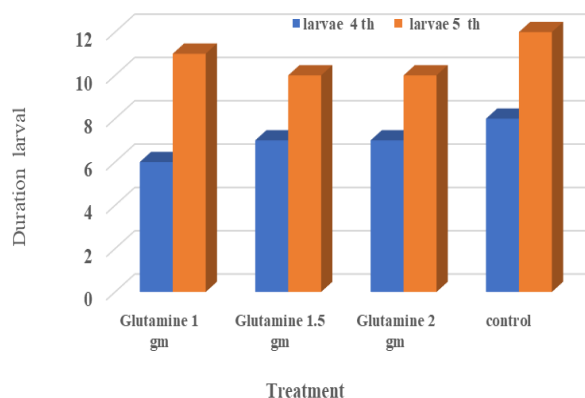


Fig. 1. Duration of larval stage during 4th and 5th instar in different concentrations of treatments.

Table 1. Effect of glutamine additive on the biological aspects of 4th and 5th instars of mulberry silkworm larva in spring 2023.

Treatment	Weight of larval instar (g)						Duration of larval stage (days)	
	Beginene 4 th	Mide of 4 th	End 4 th	Beginene 5 th	Mide of 5 th	End 5 th	4 th instar	5 th instar
Glutamine 1 g	0.177 ± 0.024 a	0.385 ± 0.061 a	0.595 ± 0.08 a	0.531 ± 0.04 b	1.571 ± 1.57 a	2.705 ± 0.05 a	6	11
Glutamine 1.5 g	0.171 ± 0.037 a	0.355 ± 0.015 b	0.580 ± 0.046 a	0.574 ± 0.04 a	1.503 ± 0.108 a	2.341 ± 0.156 b	7	10
Glutamine 2 g	0.179 ± 0.028 a	0.325 ± 0.018 c	0.52 ± 0.057 a	0.515 ± 0.04 b	1.509 ± 0.125a	2.209 ± 0.135 b	7	10
Control	0.162 a	0.301 c	0.462 b	0.162 c	1.458 a	1.985 c	8	12

• **Cocoon characters**

Data in Table (2) the results obtained that effect of food additives of glutamine with different concentration treatments on the cocoon weight of *B. mori* during spring 2023. The results showed that there were nonsignificant differences between treatments in the weight of cocoon and

pupa to gather. The highest value of cocoon weight was recorded for glutamine 1g (0.1946 g) and followed by glutamine 1.5 & 2g (0.1760 and 0.1794 g) respectively. whereas, the lowest value of cocoon weight was recorded in control. It was 0.1524 g.

Table 2. Influence of glutamine additive on some economic aspects of cocoon silkworm, *Bombyx mori* L .

Treatment	Weight of cocoon +pupae	Weight of cocoon	Pupa+malting	Pupa	Cocoon length cm	Pupation period	Silk ratio (%)
Glutamine 1 g	0.9310 a	0.1946 a	0.7541 a	0.6232 b	2.5592 a	11	20.94
Glutamine 1.5 g	0.8966 a	0.1760 b	0.7402 a	0.7222 a	2.3673 b	12	19.62
Glutamine 2 g	0.9082 a	0.1795 b	0.7484 a	0.7179 a	2.3224 b	12	19.82
control	0.8034 b	0.1524 c	0.6507 b	0.6419 b	2.1982 c	13	18.92

Letters in rows mean the significant differences between treatments and control group(p<0.05) according to Duncan’s test (Duncan, 1955).

There were highly significant differences between treatments. The highest value of cocoon length was recorded for glutamine 1g 2.595 cm, followed by glutamine 1.5 and 2g by 2.367 and 2.322 cm respectively, while the lowest value of cocoon length was recorded for control at 2.209 cm. Also, the results indicated that there were highly significant differences between treatments. The highest value of the percentage of silk ratio was recorded for glutamine 1g (20.94%). The percentage silk ratio of other concentrations and control were converging results (19.62 ,19.82 and 18.92 %) respectively in Tab (2). It means that the concentration of glutamine at 1gm achieved a high rate of silk ratio, higher than the normal rate of silk ratio (12 to 20 percent for the weight of the whole fresh cocoon).

On other hand, the results in Tab (2), showed that there were highly significant differences between treatments. The high value of pupae weight was recorded for glutamine 1.5 g and followed by glutamine 2, but the weight of pupa was similar in glutamine 1g and control. Cocooning percentage was recoded in tab (3), The results showed that, there were highly differences between treatments. The highest value of Cocooning percentage was recorded for glutamine 2g (86.5%). whereas, the lowest value of Cocooning percentage was recorded for glutamine 1g.

Table 3. Percentage of Cocooning, loss of cocoons and Hatchability (%) and Numbers of egg (egg /female) in different treatments.

Treatment	Cocooning percentage (%)	Percentage of loss of cocoons (%)	Numbers of egg (egg/female)	Hatchability percentage (%)
Glutamine 1 g	52.9	47.1	179	100
Glutamine 1.5 g	83.5	16.5	390.2	92.5
Glutamine 2 g	86.5	13.5	179.4	100
control	72.4	27.6	330.8	52.7

• **Adult characters**

The results in Fig (2) demonstrated the difference in weight between males and females under different treatments. High weight of females were in glutamine 1g and control (0.322 & 0.315 g) respectively while the highest weight of male was in glutamine 1.5 g (0.128 g) and the lowest weight was in control (0.066gm). Conversely, the mean numbers of eggs per female were different in different treatment, high mean numbers of egg was in glutamine 1.5g (390.2 egg/ f) followed by control (330 egg/ f) but less mean

of numbers egg was in glutamine1 &2 g .on other hand the high hatching percentage were 100% in glutamine 1&2 g and hatching percentage were 92.5 &52.7 % in glutamine 1.5 and control in Tab (3) .

This result showed that, although mean numbers of egg was low in concentrations 1 and 2 of glutamine, the egg hatching rate was high and reached 100 in both concentrations in tab (3). These results are due to crowding in the number of eggs or their vitality.

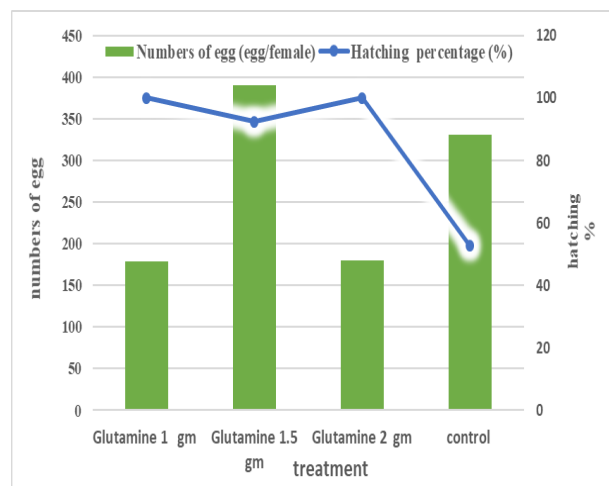


Fig. 2. Numbers of egg (egg/female) and Hatchability percentage (%) in different treatments of glutamine.

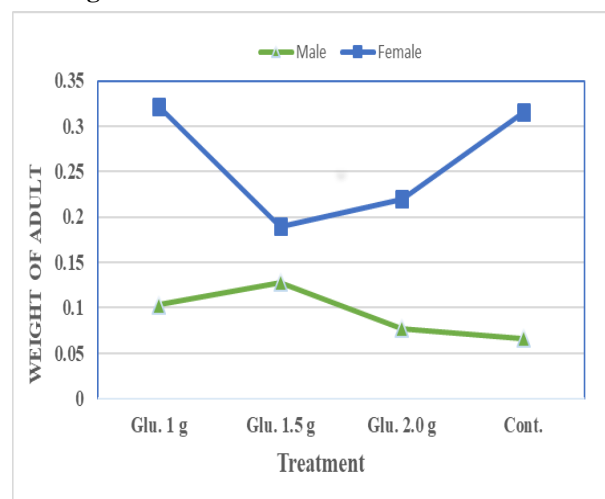


Fig. 3. Effect of different treatments of glutamine on weight of female and male.

Discussion

Amino acids from leaves are vital for silkworm larvae's growth, development, and cocoon formation. Key essential amino acids include arginine, histidine, leucine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, along with aspartic and glutamic acid. These nutrients are crucial for the larvae's health, as they cannot produce sufficient amounts of these essential amino acids on their own. As a result, they must obtain these amino acids through their diet. The essential amino acids required by silkworms are the same as those needed by other insects. Without these essential amino acids, silkworms cannot grow (Shamsuddin, 2009). Silkworms need twelve essential amino acids plus proline, which is semi-essential since some growth can occur without it. Non-essential amino acids like alanine, cystine, glycine, serine, and tyrosine are also important for their growth. Additionally, using food additives yields better growth results compared to not using them. Using food additives enhances the duration of the fourth and fifth larval stages in silkworms. This aligns with findings by Ito and Inokuchi (1992), which showed that asparagine and glutamine effectively restore normal physiological patterns in *Bombyx*, indicating their efficient utilization. Additionally, He *et al.*, (2021) found that specific concentrations of lactic acid in the diet also promote increased larval growth. These results support the importance of these compounds in the metabolic processes of silkworms. Recent findings by Muruges *et al.*, (2022) indicated that larvae fed on mulberry leaves enriched with Glycine, Alanine, and Serine showed improvements in biological traits and cocoon parameters. The previous results are consistent with those found by Maryam *et al.* (2012) and Mavajpour *et al.* (2008), who observed a significant increase in hatchability when silkworm larvae were reared during the spring season. Additionally, these findings align with those of Mostafa (2024), who reported that higher concentrations of hen egg albumen were significantly inversely related to cocoon production. Glutamine supplementation enhances the growth rate, weight gain, and overall health of the silkworm larvae. It plays a vital role in protein synthesis and energy production, leading to improved larval development. Supplementing silkworm diets with amino acid solutions at specific, optimal levels effectively enhances their growth and development. However, when the supplementation exceeds this optimal level, it ceases to have beneficial effects and may even become detrimental, indicating that only moderate supplementation is advantageous.

CONCLUSION

Adding glutamine to the diet of silkworm (*Bombyx mori* L.) larvae can positively impact both biological and economic aspects. The concentration of glutamine (1g /L) gave the best results in both the biological and economic characteristics of raising silkworms.

REFERENCES

- Borah, S. D., and Boro, P. (2020). A review of nutrition and its impact on silkworm. *Journal of Entomology and Zoology Studies*, 8(3), 1921-1925.
- Dandin, S. B., Kumar, N. S., Basavaraja, H. K., and Reddy, N. M., (2003). Effect of high temperature and high humidity on the quantitative traits of parents, foundation crosses, single and double hybrids of bivoltine silkworm, *Bombyx mori* L. *International Journal of Industrial Entomology*, 6(2), 197-202.
- Etebari, K., Mirhoseini, S. Z., and Matindoost, L. (2005). A study on interspecific biodiversity of eight groups of silkworm (*Bombyx mori*) by biochemical markers. *Insect Science*, 12(2), 87-94.
- Gokul, M. (2015). Effect of exogenous supplementation of amino acids and minerals on economic traits of silkworm, *Bombyx mori* L. M.Sc. (Ser.) Thesis, Tamil Nadu Agricultural University, Coimbatore, p. 101.
- He, Z., Fang, Y., Li, D. C., Chen, D. S., and Wu, F. (2021). Effect of lactic acid supplementation on the growth and reproduction of *Bombyx mori* (Lepidoptera: Bombycidae). *Journal of Insect Science*, 21(2), 7.
- Hossain, M. S., Uddin, M. A., Islam, M. S., and Alim, M. A. (2015). Effect of cow milk on the growth and economic traits of silkworm (*Bombyx mori* L.). *Int. J. Scientific & Engineering Res*, 6(3), 517-520.
- Ito, T., and Inokuchi, T. (1992). Nutritive effects of asparagine and glutamine on the silkworm, *Bombyx mori*. *Appl. Entomo. and zoology*, 27(4), 575-586.
- Kabila, V.; Subburathinumm K.M. and Chetty, J.S. (1994). Growth and economic characters of silkworm, *Bombyx mori* L. on feed enriched with neutralized aspartic acid, *Ind. J. Sericulture*, 33 (1): 80-81.
- Kaliwal, B. B. & Chakrabarty, S., (2012). Application of arginine, histidine and their mixture on economic traits of the silkworm, *Bombyx mori* L. *DAV International Journal of Science*, 1(2), 107-111.
- Kerenhap, R. S.; Das, P.K.; Singh, G.B. and Datta, R.K. (2008). Response of irrigated mulberry (*Morus alba* L.) to inoculation under graded doses of phosphorus. *Plant and Soil*. 170 (2): 331-337.
- Krishnaswamy, S., Narasimhanna, M.N. Suryanaryan, S.K. and Kumar Raja, S. (1973). *Sericulture Manual 2. Silkworm Rearing*. F.A.O. Agricultural Services Bulletin Rome, 15(2): 1-131.
- Lea, H. Z. (1996). Basic principles and practical techniques of silkworm Breeding department of biology. Kanwon National University Chunchon. Korea.
- Maryam, K.; Alireza, S. and Shahbodin, G. (2012). Study of the reaction of silkworm hybrid and line varieties against individual selection in spring and autumn seasons {2012}. In . *Agris.Sien*.11:9 , 1461-1471.
- Mavajpour, M.; Mirhosseni, S. Z, Ghanipour, M. and Seidavi, A.R. (2008). Effect of different environmental condition on performance of silkworm, *Bombyx mori* L. *Plant Protec J*. 22:35-45.
- Moustafa, M. N. (2024). Potential of Egg Albumen as a Source of Amino Acids and Protein Supplement on the Production of Silkworm *Bombyx mori* L. *Catrina: The International Journal of Environmental Sciences*, 30(1), 31-39.
- Muruges, K. A., Aruna, R., and Chozhan, K. (2022). Influence of amino acids on the economic characters of silkworm, *Bombyx mori* L. *Madras Agricultural Journal*, 108(september (7-9), 1.

- Mustafa, S. M and Elkaraksy, I. A. (1990). The effect of certain aminoacids on silkworm, *Bombyx mori* L. *Agricultural Research Review.*, 66(1): 105-109.
- Radjab, R. (2010). Effect of mulberry leaves enrichment with amino acid supplementary nutrients on silkworm, *Bombyx mori* L. at north of Iran. *Academic journal of Entomology*, 3(1), 45-51.
- Saad, M. S. I., Helaly, W. M. M. and Sheikh, E. A. E. (2019). Biological and physiological effects of pyriproxyfen insecticide and amino acid glycine on silkworm, *Bombyx mori* L. *Bulletin of the National Research Centre.*, 43:145-151.
- Sengupta, K., Singh, B.D. and Mustafi, J.C. (1992) Role of vitamins in silkworm nutrition. *Indian J. of Sericulture.* 11(1): 11-19, 1992.
- Shamsuddin, M. (2009). *Silkworm physiology: a concise textbook.* Daya publishing house, Delhi-110035. 2009, 1-212.
- Vadivel, K. (1995). Effect of supplementation of amino acids on economic characters of silkworm *Bombyx mori* L (Doctoral dissertation, Tamil Nadu Agricultural University, Coimbatore).
- Vijaya, D.; Yeledhali, N. A.; Ravi, M. V.; Nagangoud, A. and Nagalakar, V. P. (2009). Effect of fertilizer levels and foliar nutrients on M-5 mulberry leaf nutrients content quality and cocoon production. *Karnataka. J. Agric Sci.* 22 (5): 1006-1012.

تأثير المكملات الغذائية بالجلوتامين على الخصائص البيولوجية والاقتصادية ليرقات دودة الحرير التوتية

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الملخص

أجريت الدراسة الحالية لتقييم تأثير مسحوق الجلوتامين كمصدر للبروتين على بعض الخصائص البيولوجية والاقتصادية لدودة القز التوتية. تمت الدراسة في كلية الزراعة، جامعة دمياط معمل تربية ديدان الحرير. حيث تم إضافة مسحوق الجلوتامين إلى أوراق التوت بتركيزات مختلفة (1، 1.5 و 2 جم / لتر ماء) وتخزينها على أربع فترات من بداية العمر اليرقي الرابع حتى نهاية العمر الخامس خلال موسم الربيع 2023. وتم قياس الخصائص البيولوجية والاقتصادية ليرقات ديدان الحرير. وظهرت النتائج فعالية جميع التركيزات المختلفة للجلوتامين في زيادة الخصائص البيولوجية والاقتصادية لديدان الحرير. وقد أظهر تركيز 1 جم من الجلوتامين زيادة كبيرة في كلا من وزن اليرقات والعذارى، قصر مدة الطور اليرقي، وزن الشرنقة، نسبة الشرنقة، طول الشرنقة، نسبة تكون الحرير ونسبة القسق مقارنة بالتركيزات الأخرى. بالإضافة الي ذلك كانت التركيزات الأخرى للجلوتامين أكثر فعالية من الكنترول او اليرقات الغير معاملة. ويمكن استنتاج أن إضافة الجلوتامين إلى النظام الغذائي ليرقات دودة الحرير التوتية يمكن أن يؤثر بشكل إيجابي على كل من الجوانب البيولوجية والاقتصادية لها.