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Effects of Feeding Alanine-enriched Mulberry Leaves on the Economic Characters of the Silkworm Bombyx mori (Lepidoptera: Bombycidae) 【Research report】

桑葉添加丙氨酸對桑蠶經濟特性的影響【研究報告】

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Abstract

The effects of feeding alanine-enriched mulberry leaves on the larval weight, silk gland weight and cocoon characters of the silkworm Bombyx mori L. were studied. Fourth and fifth instar silkworms were fed once a day on mulberry leaves enriched with the amino acid alanine. Alanine was dissolved in distilled water and four concentrations of 10, 100, 500, and 1000 ppm were prepared. These solutions were then sprayed onto the fresh mulberry leaves, fed to the silkworms, and any changes in economic traits were recorded. In general, silkworms fed with alanine-enriched mulberry leaves did not show any improvement across the economic traits measured. Although the highest larval weight and effective rate of rearing percentage was observed in the silkworms treated with 1000 ppm alanine, there was no corresponding increase in the other traits. The untreated control fared better than the silkworms fed with alanine enriched leaves, indicating that alanine enrichment had no positive impact on the larval and economic traits of the silkworm, Bombyx mori L.

摘要

本研究以添加丙氨酸的桑葉餵食桑蠶Bombyx mori L.，來了解其對桑蠶幼蟲重量、絲腺重量、蠶繭特性所造成的影響。將丙氨酸溶於蒸餾水中，配置 10、100、500、1000 ppm 四種不同的濃度，噴灑在新鮮桑葉上，一天一次餵食第四齡及第五齡桑蠶幼蟲，並記錄在經濟特性上的所有變化。一般而言，用含有丙氨酸的桑葉來餵養桑蠶，並不會有任何經濟特性上的提升。儘管重量最重的幼蟲及有效的飼育比率都出現在 1000 ppm 的丙氨酸處理組，但是在其他處理組並沒有相同的趨勢。在對照組方面，桑蠶進食的狀況比實驗組良好。此實驗指出額外添加丙氨酸對於桑蠶幼蟲以及經濟效益沒有正向的提升效果。

Key words: alanine, economic characters, enrichment, silk glands, silkworm

關鍵詞: 丙氨酸、經濟性狀、增強食品營養、絲腺、桑蠶。

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Effects of Feeding Alanine-enriched Mulberry Leaves on the Economic Characters of the Silkworm *Bombyx mori* (Lepidoptera: Bombycidae)

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ABSTRACT

The effects of feeding alanine-enriched mulberry leaves on the larval weight, silk gland weight and cocoon characters of the silkworm *Bombyx mori* L. were studied. Fourth and fifth instar silkworms were fed once a day on mulberry leaves enriched with the amino acid alanine. Alanine was dissolved in distilled water and four concentrations of 10, 100, 500, and 1000 ppm were prepared. These solutions were then sprayed onto the fresh mulberry leaves, fed to the silkworms, and any changes in economic traits were recorded. In general, silkworms fed with alanine-enriched mulberry leaves did not show any improvement across the economic traits measured. Although the highest larval weight and effective rate of rearing percentage was observed in the silkworms treated with 1000 ppm alanine, there was no corresponding increase in the other traits. The untreated control fared better than the silkworms fed with alanine enriched leaves, indicating that alanine enrichment had no positive impact on the larval and economic traits of the silkworm, *Bombyx mori* L.

Key words: alanine, economic characters, enrichment, silk glands, silkworm

Introduction

The silkworm is an of economic insect used for silk production. Sericulture depends on mulberry leaves as the sole natural food of the silkworm *Bombyx mori* L., and the quality of the mulberry leaves has a direct bearing on the normal growth of the larvae and the quality of the cocoon (Horie *et al.*, 1967). The composition of mulberry leaves plays an important role in the growth and development of silkworms and other traits important to the economic production of these animals (Tazima,

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2001). Significant seasonal variations occur in the nutritional value and composition of mulberry leaves depending on factors such as the weather, pests and diseases as well as agricultural practices (Ito, 1978). This variation impacts, both qualitatively and quantitatively upon the silkworm cocoon crop. Enrichment of the mulberry leaves by nutrient supplementation is one of the strategies by which cocoon and silk productivity can be increased and the quality can be enhanced and maintained. Nutritional supplements can include minerals, vitamins, proteins, amino acids and sugars (El-Karaksy and Idriss, 1990; Kabila *et al*., 1994; Sarkar *et al*., 1995; Yasmin *et al*., 1995; Nirwani and Kaliwal, 1996, 1998; Zaman *et al*., 1996; Basit and Ashfagh, 1999; Goudar and Kaliwal, 1999; Etebari and Matindoost, 2005). These studies show that nutritional supplements have a significant impact on larval growth and cocoon production (Ito and Tanaka, 1962). It has been shown that 75% of mulberry leaf protein is directly converted into silk protein and 25% goes to the body tissues of the silkworm larvae (Tazima, 2001).

Alanine is present at a level of 1.23% in dry mulberry leaves which is what a silkworm requires for its normal growth (Ito, 1978). In general, silkworms do not encounter alanine deficiency, and adding it to the silkworm diet has no negative effect (Hamamura, 2001). The amino acid alanine is regarded as a non-essential amino acid which is converted to aspartate or glutamate through transamination. Alanine plays an important role in glucose, tryptophan and organic acid metabolism. β-alanine is a derivative of B_5 vitamin (pentatonic acid) and coenzyme A (Chapman, 1998). Although quite a few studies have been conducted on amino acids supplementation, their results vary (Sarkar *et al.*, 1995; Yasmin *et al.*, 1995; Zaman *et al.,* 1996; Basit and Ashfagh, 1999; Etebari and Matindoost, 2005). Thus, in the present study a comprehensive effort was made to determine whether alanine supplementation influences the growth and the economic traits of the silkworm, *B. mori.*

Materials and Methods

Silkworm rearing

The eggs of bivoltine Japanese-Chinese hybrid silkworms (31 × 32) were obtained from the Iran Silkworm Research Center (Rasht, Iran), and reared in the Natanz Sericultural Center (Natanz, Isfahan, Iran) under standard conditions of 25°C with a RH of 75 ± 5% and a photoperiod of 16L: 8D as described by Lim *et al*. (1990). Fresh leaves of the Kenmochi variety of mulberry (*Morus alba*) were used for feeding the silkworms.

Treatments

L-alanine (Merck, Germany) was dissolved in distilled water and diluted to 10, 100, 500, and 1000 ppm concentrations. The different solutions were then sprayed onto fresh mulberry leaves and fed to larvae from the beginning of the fourth instar to the end of the fifth instar, once a day except during molting. One batch (250 larvae) of silkworms was fed with leaves sprayed with distilled water (distilled water control) and another batch was fed with normal leaves (control) in three replications. The amount of mulberry fed to each larvae or treatment group was not measured. The experiment was accomplished using a completely random design with three replications for each treatment and using 150 silkworms per replication.

Larval weight

To study the effect of feeding mulberry leaves enriched with L-alanine on the larval growth, the weight of the silkworm larvae were weighed on days 1, 3, 5, and 7 of the fifth instar using a digital balance (± 0.01 g). Thirty larvae from every replication were randomly selected and their mass recorded from which the average larval weight was then derived.

Silk gland weight

Three mature larvae were picked from each replication and were chilled in a refrigerator for a few minutes. With one longitudinal cut on the dorsal surface the larvae were cut open and their silk glands were isolated in 9% NaCl. The mass of the three silk glands was determined in a digital balance (± 0.01) and the average was recorded.

Cocoon, pupa and cocoon shell weights

One week after pupation, the cocoons were harvested and thirty cocoons in good condition were cut open from each batch. Male and female pupae were separated and cocoon, pupa and shell weights were recorded.

Cocoon shell percentage

The shell percentage of each cocoon was calculated as: Cocoon shell percentage = (shell weight/cocoon weight) × 100

Effective rate of rearing percentage

The effective rate of rearing (ERR%) was calculated by following the formula:

ERR percentage = (No. of cocoons harvested/No. of larvae retained) × 100

Statistical analysis

The data were subjected to analysis of variance (ANOVA) to determine if the differences found between treatments and the differences between treatments and the controls were significant. For analysis of variance, Duncan's multiple range function in SAS was used (SAS, 1997).

Results and Discussion

The effect of dietary supplementation of alanine on the larval weight and the economic characteristics are given in Tables 1 and 2. Figures 1 and 2 show the changes in the weight and ERR of the silk glands, respectively. The feeding of larvae with mulberry leaves enriched with alanine showed that the larval weight increased with the concentration mainly on the seventh day of the fifth instar, and in a gradual fashion. The maximum weight was recorded when a providing a dietary supplement of alanine at 1000 ppm. However, this difference was statistically not significant from that of the control (Table 1). At the same time, the weight of the silk gland was less in the treated silkworms than in the distilled water control and in the untreated control (Fig. 1). In fact, the maximum silk gland weight was recorded in the untreated control followed by the distilled water control. Among the treated groups, silkworms that were treated with a 500 ppm alanine concentration recorded the maximum silk gland weight.

In Table 2, the cocoon traits of the males and the females silkworms are given separately. In males, the maximum cocoon weight was observed in those larvae that were fed with leaves enriched with 500 ppm alanine. The difference among the treatments was only minor and the difference among the controls was not significant. The result was similar in the case of pupa weight, shell weight and shell percentage. However, for the females, the maximum cocoon weight was recorded when they were fed leaves enriched with a 1000 ppm concentration of alanine. This was followed by the control and then the 100 ppm concentration of alanine. The female pupa weight had similar results with a maximum weight of 1.374 g at a concentration of 1000 ppm, and again with no significant difference compared to the untreated control. Cocoon shell weight was maximum for the 1000 ppm alanine concentration, but the cocoon shell percentage was the highest for the distilled water control. The effective rate of rearing was maximum in the untreated control and minimal in the larvae treated with 100 ppm. Among the treated silkworms, those treated with a concentration of 1000 ppm had the highest ERR% (Fig. 2).

Many studies have investigated the

Table 1. The effect of mulberry leaves enrichment with alanine on larval weight in the 5th instar of the silkworm, *Bombyx mori* L.

Treatments (ppm)	Larval weight during 5th instar (g) ± SE			
	1st day	3rd day	5th day	7th day
10	1.069 ± 0.056c	1.811 ± 0.113ab	2.743 ± 0.040ab	3.305 ± 0.169b
100	1.217 ± 0.034ab	1.780 ± 0.088ab	2.640 ± 0.020ab	3.470 ± 0.176ab
500	1.119 ± 0.015bc	1.753 ± 0.044ab	2.828 ± 0.155ab	3.565 ± 0.158ab
1000	1.166 ± 0.018abc	1.766 ± 0.066ab	2.588 ± 0.227b	3.945 ± 0.106a
No treatment control	1.254 ± 0.009a	1.961 ± 0.032a	3.060 ± 0.091a	3.725 ± 0.223ab
Distilled water control	1.187 ± 0.051abc	1.646 ± 0.105b	2.655 ± 0.155ab	3.633 ± 0.205ab

Means with the same letter in the columns are not significantly different at $p > 0.05$.

Table 2. The effect of mulberry leaves enrichment with alanine on the economic characteristics of the silkworm, *Bombyx mori* L.

Treatments (ppm)	Male				Female			
	Cocoon weight (g) ± SE	Pupa weight (g) ± SE	Cocoon shell weight (g) ± SE	Shell percentage ± SE	Cocoon weight (g) ± SE	Pupa weight (g) ± SE	Cocoon shell weight (g) ± SE	Shell percentage ± SE
10	1.372 ± 0.021a	1.005 ± 0.015a	0.366 ± 0.008a	26.70 ± 0.405a	1.450 ± 0.041c	1.195 ± 0.042b	0.254 ± 0.068b	17.35 ± 1.323a
100	1.360 ± 0.033a	1.030 ± 0.027a	0.330 ± 0.007a	24.30 ± 0.304a	1.707 ± 0.068a	1.361 ± 0.062a	0.346 ± 0.005ab	20.33 ± 0.476a
500	1.419 ± 0.040a	1.057 ± 0.028a	0.361 ± 0.014a	25.46 ± 0.483a	1.660 ± 0.019ab	1.325 ± 0.008a	0.334 ± 0.010ab	20.13 ± 0.404a
1000	1.365 ± 0.106a	1.018 ± 0.075a	0.346 ± 0.109a	24.79 ± 0.620a	1.723 ± 0.043a	1.374 ± 0.034a	0.348 ± 0.009ab	20.24 ± 0.211a
No treatment control	1.417 ± 0.015a	1.043 ± 0.021a	0.374 ± 0.005a	26.42 ± 0.701a	1.716 ± 0.023a	1.342 ± 0.021a	0.374 ± 0.007a	21.81 ± 0.417a
Distilled water control	1.284 ± 0.014a	0.938 ± 0.011a	0.345 ± 0.014a	26.88 ± 0.960a	1.542 ± 0.029bc	1.202 ± 0.016b	0.340 ± 0.017ab	22.02 ± 0.803a

Means with the same letter in the columns are not significantly different at $p > 0.05$.

improvement in larval weight when feeding silkworms with amino acid enriched mulberry leaves (El-karaksy and Idriss, 1990; Sarkar *et al.*, 1995; Etebari and Matindoost, 2005). It has been demonstrated that mulberry leaves enriched with 5% alanine and 5% glycine enhanced the weight of the silkworm by 14% during the fifth instar (Sarkar *et al.*, 1995) and that 5% glycine contributed substantially to the increase in larval weight if they were fed on enriched mulberry leaves from the first day of the fifth instar. Zaman *et al.* (1996) demonstrated that mulberry leaves enriched with 0.2% nitrogen also contributed to the increase in silkworm larvae weight. The comparative gain in larval weight on the seventh day in any treatment in relation to other treatments is in response to the elevated protein synthesis and the same gain may be reflected in the weight of the silk glands as well. However, our findings did not show such a relationship. The silk gland weight and larval weight did not increase in tandem. Although the concentration of 1000 ppm resulted in the highest larval weight, this was not the case with the silk gland weight. Previous works suggested that L-alanine, L-aspartic acid and L-glutamic acid stimulate correspondingly but they did not mention a possible phagostimulatory effect of L-alanine on

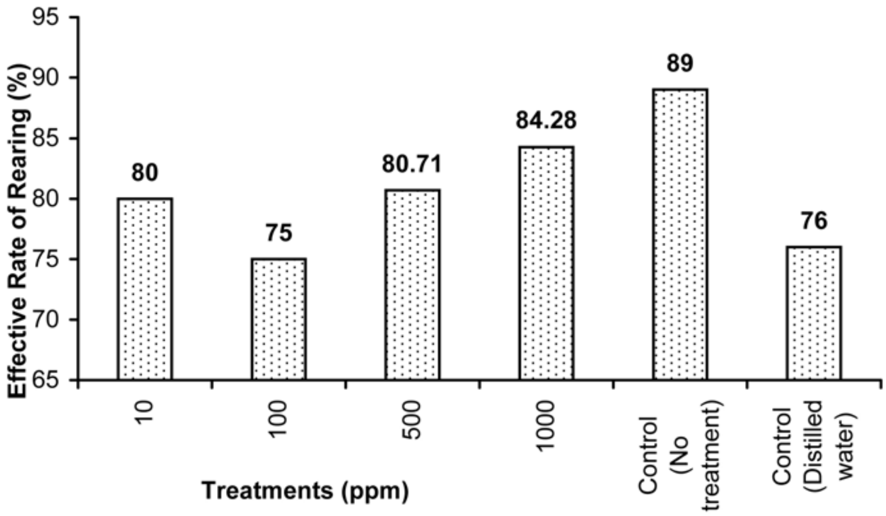

Fig. 1. The effect of mulberry leaves enrichment with alanine on the weight of the silk glands of the silkworm *Bombyx mori* L.

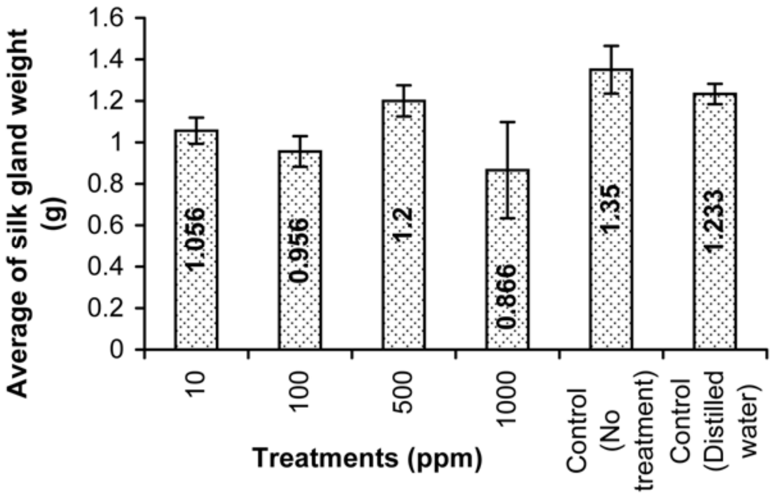

Fig. 2. The effect of mulberry leaves enrichment on ERR (%).

silkworm. The present study seems to indicate that L-alanine may not exert the same stimulatory effect in silkworms as in other insects already reported. The supplementation of the silkworm diet with selected amino acids at certain levels may be effective for improved growth, but a higher level of supplementation doesn't have a positive effect on silkworm growth and development as is evident from the present study.

Usually, heavier silkworms produce heavier cocoons with a direct corresponding positive influence on the weight of the cocoon shell. Silk glands attain maximum growth towards the end of the fifth instar owing to fibroin synthesis (Kirimura, 1962) and it is obvious that the silk gland weight is one of the important parameters for assessing the silk production potential of the larvae. Positive effects of feeding silkworms with mulberry leaves enriched with nutritional supplements on silk glands weight were reported by some researchers (Muniandy *et al*., 1995; Nirwani and Kaliwal, 1996, 1998). Filibon (Vitamins + Mineral elements) at 3% concentration enhanced larval weight by 27% which caused an the silk glands weight to increase by 108% (Muniandy *et al*., 1995). In silkworms, silk fibroin is derived mainly from four amino acids: alanine, serine, glycine and tyrosine (Kirimura, 1962) which come from their dietary source of protein and amino acids (Ito, 1983). Silkworms obtain 72-86% of their amino acids from mulberry leaves. More than 60% of the absorbed amino acids is used for silk production (Lu and Jiang, 1988). It is also reported that the fibroin-sericin ratio in the cocoon shell depends on the amino acid composition of the diet. In the present study, even though the 1000 ppm alanine concentration resulted in the maximum larval weight, the same was not seen in the silk gland. This shows that in the silkworm, dietary supplementation of alanine does not enhance the cocoon/shell weight despite the increased larval weight. This may probably be due to the fact that a proper increase in both the silk gland weight and the shell weight may depend on a proper balance among the required amino acids. The addition of any one amino acid may not necessarily evoke a positive response in terms of increased cocoon/shell weight. It may be that a balance between essential and non-essential amino acids is important in the silkworm diet. The effect on the silk gland may also depend on the types of amino acid supplemented. For example, enriching mulberry leaves with B_{12} which is not available in mulberry leafs can increase the synthesis of the proteins and the nucleic acid in the silk glands (Das and Medda, 1988). This enhancement will then lead to an increase in the weight of the silk glands along with the general effect of increased larval weight. However, the isolated supplementation of alanine does not seem to stimulate an enhanced synthesis of silk protein. This observation correlates with the findings of Khan and Saha, 1995 who reported that alanine supplementation at 100 and 1000 ppm did not augment the larval and cocoon traits.

The main reason of nutritional supplementation for silkworms is to enhance the economic traits such as cocoon weight, cocoon shell weight and cocoon shell percentage. There are numerous reports containing the positive effects of nutritional supplementation on the economic traits of silkworms (Kabila *et al*., 1994; Nirwani and Kaliwal, 1998; Etebari, 2002; Rajabi *et al*., 2007). Etebari (2002) reported that a treatment with 2% ascorbic acid increased the cocoon weight and the shell weight of both male and female silkworms. They also reported that mulberry leaf enrichment with various concentrations of thiamine leads to significant increases in the larval period, cocoon weight, cocoon shell weight and fecundity (Nirwani and Kaliwal, 1998). Kabila *et al*. (1994) indicated that the addition of aspartic acid in concentration of 1 or 2% to mulberry leaves increased the economic characteristics of the silkworms. It has been reported that silkworm larvae fed on 0.2% of nitrogen increased cocoon shell weight (Zaman *et al*., 1996). Mulberry leaves enriched with 0.2% N and 0.15% Mg increased cocoon shell weight by 94% (Zaman *et al*., 1996). However, in the present experiment, alanine did not improve the economic characteristics. This may be related to the results of Fukuda (1959) who stated that the quantitative requirements for amino acids are different for male and female silkworms and may depend on the season. Despite the fact that the fortification of mulberry leaves with amino acids can improve production efficacy, it is obvious that such positive effects cannot be guaranteed (Etebari, 2002). A high level of essential amino acids in the diet is suitable for silk production; however, a high level of non-essential amino acids has a negative effect. The results of the present study are in agreement with this concept. This is evident from the results of this study, since the treated silkworms did not show any improvement in the ERR (%) while it was maximal in the untreated control. According to our results, it is clear that supplementing the diet of silkworms with alanine does not have any positive impact on the economic traits, at least not under the climate conditions of Natanz in Isfahan.

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桑葉添加丙氨酸對桑蠶經濟特性的影響

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摘　　要

本研究以添加丙氨酸的桑葉餵食桑蠶 *Bombyx mori* L.，來了解其對桑蠶幼蟲重量、絲腺重量、蠶繭特性所造成的影響。將丙氨酸溶於蒸餾水中，配置 10、100、500、1000 ppm 四種不同的濃度，噴灑在新鮮桑葉上，一天一次餵食第四齡及第五齡桑蠶幼蟲，並記錄在經濟特性上的所有變化。一般而言，用含有丙氨酸的桑葉來餵養桑蠶，並不會有任何經濟特性上的提升。儘管重量最重的幼蟲及有效的飼育比率都出現在 1000 ppm 的丙氨酸處理組，但是在其他處理組並沒有相同的趨勢。在對照組方面，桑蠶進食的狀況比實驗組良好。此實驗指出額外添加丙氨酸對於桑蠶幼蟲以及經濟效益沒有正向的提升效果。

關鍵詞：丙氨酸、經濟性狀、增強食品營養、絲腺、桑蠶。

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