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Effect of Mulberry Leaves Enrichment with Amino Acid Supplementary Nutrients on Silkworm, *Bombyx mori* L. at North of Iran

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Abstract: Effects of mulberry leaves enrichment with aspargine (0.01, 0.1, 0.2 and 0.5% concentrations) and alanine (0.1, 0.2, 0.5 and 1% concentrations) on growth and characteristics of cocoon silkworm *Bombyx mori* L. were studied. Silkworm larvae were fed on fresh mulberry leaves of shin-ichinose enriched with supplementary nutrients from beginning of 1st instar until end of 5th instar, once a day. Fresh mulberry leaves also used for control treatment. All biological and economic parameters were determined by using standard technique in sericulture. Results showed that aspargine and alanine amino acids could not improve silk production in sericulture significantly, then they can not recommend for enrichment of mulberry leaves for increase of farmer income.

Key words: Silkworm · Amino acid · Enrichment · Economic traits

INTRODUCTION

Nutrition plays an important role in improving the growth and development of the silkworm, *Bombyx mori* L. like other organisms. It is stated that silk production is dependent on the larval nutrition and nutritive value of mulberry leaves plays a very effective role in producing good quality cocoons [1]. 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 [2]. 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. Sengupta *et al.* [3] showed that *B. mori* requires specific essential sugars, amino acids, proteins and vitamins for its normal growth, survival and also for the silkgland growth. Nutritional supplements can include minerals, vitamins, proteins, amino acids and sugars [4-12]. It was observed that better growth and development of silkworm larvae as well as good quality cocoons can obtained when silk worms fed on nutritionally enriched leaves [13]. In silkworms, silk fibroin is derived mainly from four amino acids: alanine, serine, glycine and tyrosine [14] which come from their dietary source of protein and amino acids [15]. Silkworms obtain 72-86% of their amino acids from mulberry leaves and more than 60% of the absorbed amino acids are used for silk production [16].

In general, silkworms do not encounter aspargine and alanine deficiency, adding it to the silkworm diet has no positive effect [17]. Alanine is 1-group non-essential amino acid for silkworm [18]. It is present at a level of 1.23% in dry mulberry leaves which is what a silkworm requires for its normal growth [2]. 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. Aspargine used as aspartic acids by silkworm and it is regarded as one of 2-group essential amino acids which their deficiencies lead to growth retardation [18]. Although quite few studies have been conducted on amino acids supplementation, their results vary [6, 7, 9, 10, 12, 19]. Thus, in the present study a comprehensive effort was made to determine whether mentioned amino acids supplementation influences the growth and the economic traits of the silkworm, *B. mori.*

MATERIALS AND METHODS

Silkworm Rearing: The eggs of bivoltine Chinese-Japanese hybrid silkworms (103×104) were reared in the Iran Silkworm Research Center (Rasht, Iran) under

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standard conditions of 25°C with a RH of 75±5% and a photoperiod of 16L: 8D as described by Harizanis [20]. Fresh leaves of the shin-ichinose variety of mulberry (*Morus alba*) were used for feeding the silkworms in autumn.

Treatments: Amino acids was dissolved in distilled water and diluted to different concentrations (0.01, 0.1, 0.2 and 0.5% for L-aspargine and in 0.1, 0.2, 0.5 and 1% for L-alanine). Silkworm larvae fed on leaves from first until end of 3rd instar. 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. One batch (150 larvae) of silkworms was fed with normal leaves (No treatment control) in three replications. 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 and L-aspargine 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.

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:

$$\text{Cocoon shell percentage} = (\text{shell weight}/\text{cocoon weight}) \times 100$$

Effective Rate of Rearing Percentage: The effective rate of rearing (ERR %) was calculated by following the formula: ERR % = (No of cocoons harvested/No. of larvae retained) × 100

Egg Number and its Hatchability: Mated adults, female transferred to paper including amylase and covered with plastic container. After 24 hours female was removed and egg number was calculated and weight of 50 eggs was measured by digital balance (±0.001g). This experiment including 5 replication for each treatment. Egg hatchability was obtained by storaging of eggs until next year.

Effective Rate of Rearing Percentage: The effective rate of rearing (ERR%) was calculated by following the formula of Joshi [21]:

$$\text{ERR percentage} = (\text{No. of cocoons harvested}/\text{No. of larvae retained}) \times 100$$

Statistical Analysis: The data were subjected to analysis of variance (ANOVA) to determine if the differences found among treatments and the differences between treatments and the controls were significant. For analysis of variance, Duncan's multiple range function in SAS was used [22].

RESULTS

The effect of mulberry leaves enrichment with amino acids on larval weight is shown in Tables 1 and 2. Maximum of larval weight recorded in 0.1% for aspargine and control for alanine amino acid in the end of larval growth. It seems positive effects of aspargine were noticeable as compare with alanine amino acid. The feeding of larvae with mulberry leaves enriched with alanine showed that the larval weight increased with the concentration mainly on the 7th day of the 5th instar and in a gradual fashion which is not significant.

Effects of mulberry leaves enrichment with amino acids on cocoon characteristics is shown in Tables 3 and 4. It was not showed positive effects on economic characteristics of shell percentage except for female in some concentrations with maximum amount of shell percentage recorded in 0.01% of aspargine. Maximum male sell percentage was recorded in control. For alanine amino acid, the maximum shell percentage was recorded in control in both males and females.

Effects of mulberry leave enrichment with amino acids on egg characteristics are shown in Tables 5 and 6. Results showed no clear effects of aspargine on mentioned parameters but alanine amino acid in 1 % treatment could significantly increase egg number and hatchability.

Table 1: The effect of mulberry leaves enrichment with aspargine on larval weight in the 5th instar of the silkworm

	Larval weight during 5th instar (gr)			
Treatments (%)	1st day	3rd day	5th day	7th day
0.01	0.544a	0.892b	1.536b	2.461b
0.1	0.603a	0.986ab	1.666ab	2.910a
0.2	0.577a	0.905ab	1.599ab	2.609ab
0.5	0.575a	0.981ab	1.685ab	2.613ab
Control	0.615a	1.078a	1.820a	2.579ab

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 larval weight in the 5th instar of the silkworm

	Larval weight during 5th instar (gr)			
Treatments (%)	1st day	3rd day	5th day	7th day
0.1	0.511b	0.745c	1.291c	2.245b
0.2	0.572a	0.885b	1.589b	2.485a
0.5	0.590a	0.907b	1.632b	2.495a
1	0592a	0.942b	1.520b	2.519a
Control	0.615a	1.078a	1.828a	2.579a

Means with the same letter in the columns are not significantly different at P>0.05

Table 3: The effect of mulberry leaves enrichment with aspargine on the economic characteristics of the silkworm

	Male				Female			
Treatments (%)	Cocoon weight (g)	Pupa weight (g)	Cocoon shell weight (g)	Shell percentage	Cocoon weight (g)	Pupa weight (g)	Cocoon shell weight (g)	Shell percentage
0.01	1.094bc	0.838b	0.255a	23.33b	1.354b	1.229a	0.339a	19.89ab
0.1	1.139ab	0.874ab	0.264a	23.21b	1.406ab	1.343a	0.271b	19.26b
0.2	1.181a	0.904a	0.277a	23.49b	1.459a	1.173a	0.285ab	19.61ab
0.5	1.163a	0.884a	0.279a	24.01ab	1.467a	1.175a	0.292ab	19.88ab
Control	1.070c	0.793c	0.276a	25.85a	1.351b	1.074a	0.277ab	19.49a

Means with the same letter in the columns are not significantly different at P>0.05

Table 4: The effect of mulberry leaves enrichment with alanine on the economic characteristics of the silkworm

	Male				Female			
Treatments (%)	Cocoon weight (g)	Pupa weight (g)	Cocoon shell weight (g)	Shell percentage	Cocoon weight (g)	Pupa weight (g)	Cocoon shell weight (g)	Shell percentage
0.1	1.174a	0.899a	0.275a	23.42ab	1.492ab	1.189ab	0.302a	20.24a
0.2	1.241a	0.945a	0.296a	23.81ab	1.514ab	1.219ab	0.295a	19.48a
0.5	1.194a	0.923a	0.270a	22.65b	1.477ab	1.194ab	0.286a	19.37a
1	1.162a	0.895a	0.266a	22.39b	1.695a	1.359a	0.335a	19.83a
Control	1.070a	0.793a	0.276a	25.85a	1.351b	1.074b	0.277a	20.49a

Means with the same letter in the columns are not significantly different at P>0.05

Table 5: The effect of mulberry leaves enrichment with aspargine on the egg characters of the silkworm

Treatments (%)	Average of egg no. for each female	Average of weight of 50 eggs from each female (gr)	Hatchability (%)
0.01	483a	0.026a	94.6a
0.1	498a	0.024a	94.6a
0.2	510.3a	0.025a	95.6a
0.5	452.3a	0.025a	97.3a
Control	492.6a	0.025a	95.6a

Means with the same letter in the columns are not significantly different at P>0.05

Table 6: The effect of mulberry leaves enrichment with alanine on the egg characters of the silkworm

Treatments (%)	Average of egg no. for each female	Average of weight of 50 eggs from each female (gr)	Hatchability (%)
0.1	456.3b	0.024b	94.6ab
0.2	476.3b	0.026a	93b
0.5	439.3b	0.025ab	97.6a
1	579.3a	0.025b	98a
Control	491.6b	0.025b	95.6ab

Means with the same letter in the columns are not significantly different at P>0.05

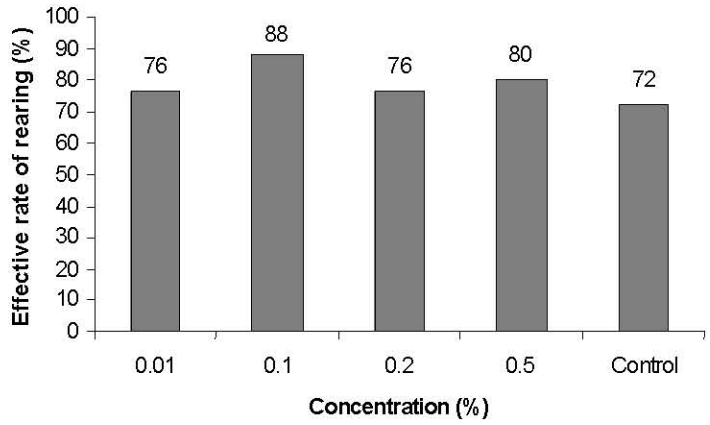

Fig. 1: The effect of mulberry leaves enrichment with aspargine on ERR (%)

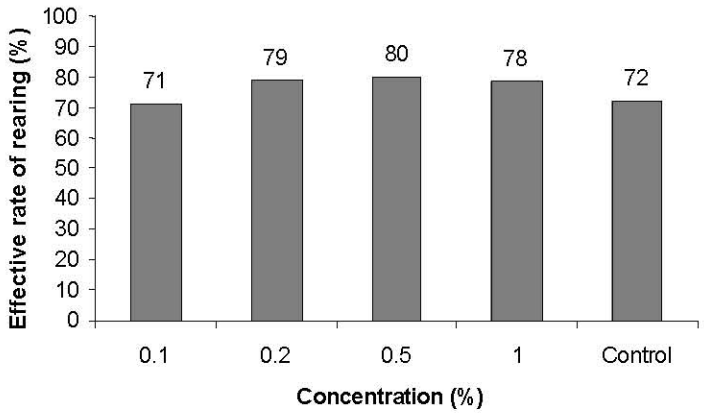

Fig. 2: The effect of mulberry leaves enrichment with alanine on ERR (%)

The effects of mulberry leaves enrichment with amino acids on the effective rate of rearing (ERR %) is shown in Figures 1 and 2. It recorded positive effects for 0.1 % of aspargine and 0.5 % of alanine, but it was negligible and it can not be suitable item for general goal and help in management of sericulture more.

DISCUSSION

Many studies have investigated the improvement in larval weight when feeding silkworms with amino acid enriched mulberry leaves [4, 6, 12]. It has been demonstrated that mulberry leaves enriched with 5% alanine and 5% glycine enhanced the weight of the silkworm by 14% during the 5th instar [6] and 5% glycine contributed substantially to the increase in larval weight if they were fed on enriched mulberry leaves from the first day of the 5th instar. Zaman *et al.* [9] 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 7th 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.

Higher weights lead to higher silk gland weight [1]. 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 5th instar owing to fibroin synthesis [14] and it is obvious that the silk gland weight is one of the important parameters for assessing the silk production potential of the larvae But It is reported that mulberry leaves enrichment with alanine and aspargine could not increase silkgland weight in treated groups compared to control significantly therefore these amino acids could not used for improvement of silk production commercially [23]. Higher larval weight in 0.1% aspargine cause not higher yield positively (Table 3). 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 silkworm [5]. The present study seems to indicate that L-aspargine and L-alanine may not exert the same stimulatory effect in silkworms as in other insects already reported.

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 [5, 24, 25, 26]. Etebari [25] reported that a treatment with 2% ascorbic acid increased the cocoon weight and the shell weight of both male and female silkworms. Kabila *et al.* [5] 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 [9]. Mulberry leaves enriched with 0.2% N and 0.15% Mg increased cocoon shell weight by 94% [9]. However, in the present experiment, amino acids did not improve the economic characteristics. This may be related to the results of Fukuda [27] 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 productive efficacy, it is obvious that such positive effects cannot be guaranteed [25]. 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. According to our results, it is clear that supplementing the diet of silkworms with amino acids does not have any positive impact on the economic traits significantly. No positive impacts of mulberry leaves enrichment with supplementary nutrients reported on larval and economic traits of the silkworm [23]. Supplementation of alanine and aspargine does not seem to stimulate an enhanced synthesis of silk protein. Keep in mind definite negative effects of aspargine on economic parameters, 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 silkworm weight and the shell weight may depend on a proper balance among the required amino acids. The addition of any 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. Therefore, improvement of economic yield did not gain in enriched silkworm larvae for all groups significantly in this investigation. Fecundity of silkworms is related to larval feeding regime directly and larger pupa produce powerful adults for reproduction [1].

Larval feeding has effects on genital development, then fecundity and fertility of adults [28]. Amino acids have effects on fecundity of insects related to allata corpora discharge due to vitellogenesis. This phenomenon will lead to more eggs with high hatchability [1, 28]. It seems that alanine amino acid with this rule positively effects female fecundity and eggs hatchability.

Although ERR% increased for more treatments groups, it means that number of harvested cocoon increased to some extent, but it can not regarded suitable criteria for decision in sericulture because of many factors effects on rearing of larvae which lead to cocoon production [21, 26].

In conclusion, 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. Weather condition is regarded as one of most important factors on enrichment studies indirectly due to influence of time spent for leaves dry after treatments of mulberry leaves which must study in detail.

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