J. Plant Prot. and Path., Mansoura Univ., Vol.9 (4): 273 – 276, 2018

Effect of the Whey Protein on Economic Traits for Mulberry Silkworm, *Bombyx mori* L.

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ABSTRACT

Prosperous silk production as well as quality of silk cocoon depend on the nutritional value of mulberry leaves and larval growth. The present study is carried out to evaluate the efficiency of whey protein on economic traits for silkworm, *Bombyx mori* L. Three concentrations from liquid whey protein (4, 6, and 8 %) were sprayed on mulberry leaves, also five grams from raw whey protein (powder) were used in feeding of 5th instar larvae. Control larvae were feed on untreated mulberry leaves. Result showed that, the highest mean of shell weight were (0.221, 0.216 and 0.203 g) recorded at concentration 4, 6 and 8% for liquid whey protein, respectively. The lowest mean of shell weight were (0.199 and 0.195 g) in raw whey protein and control, respectively. Highest growth index of larval of 5th instar was recorded in liquid whey protein were (5.009, 3.901 and 3.364) at concentrations 8, 4, and 6% respectively.

Keywords: Whey Protein, Growth index, Cocoon characters, Mulberry Silkworm, *Bombyx mori*.

INTRODUCTION

Silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae), is a local insect which produce silk through spinning. Nutritional value of mulberry leaf depends on prosperous silk production, as well as quality of silk cocoon. By nutrient supplementation is one of the ways to ameliorate growth rate in silkworm, *B. mori* . though only mulberry is nutrition for silkworm (Triubhuvan, 1989; Sengupta *et al.*, 1992 and Hossain *et al.,* 2015). The feeding efficiency of *B. mori* in presence of casein in a diet has enhanced the growth rate of *Manduca sexta* caterpillars. Ito (1960) and Woods (1999). For best get production and higher quantity and quality of cocoons through study the fortification of nutrient supplements such as carbohydrates, proteins, vitamins, amino acids, sterols, antibiotics and hormones etc., on silkworm, Sannapa *et al*. (2002). Feeding *B. mori* on mulberry leaves treated with bovine milk containing carbohydrates, protein and fat, which would increase the growth rate and production of silk (Masthan *et al*., 2011 a and 2011 b). Widely used in the food industry uses whey protein products due to excellent functional and nutritional properties and whey proteins are quickly and easily digested, such as whey protein concentrates and whey protein isolate (Jovanović, *et al*., 2005 and Shankar Pancell, 2013). Whey protein contains casein existing in whey protein contains fatty acids, cholesterol, vitamins, minerals, sugars and fatty acids (Vanderzant, 1966). Found a positive effect of cholesterol led to increased food efficiency in *B. mori* (Ito *et al*., 1963). Whey protein contains fat, lactose, vitamins, minerals, immunoglobulin, essential amino acids (EAAs), including three branched chain amino acids (BCAAs) (Ha and Zemel, 2003). The study of dietary supplements such as the effect of enriched amino acids on mulberry leaves led to the growth of *B. Mori*, one of the ways to improve the growth rate in *B. Mori* (Khan and Suha, 1995; Nirwani and Kaliwal, 1998 and Rajabi, 2010). It is known that the rich sources of food proteins improve the economic characteristics and promote the growth of silkworm such as soy protein (Ito, 1980 and Krishnan *et al*., 1995). In recent years, to improve the quality of the cocoon crop has been using beneficial nutrients such as, Hormones (Magadum and Hooli 1988), Proteins (Subburathinum and Krishna, 1992), Amino acids (Kabila, *et al*., 1994), chemicals and salts (Balamani, *et al.*, 1995), carbohydrates Goudar and Kaliwal (1999), and combination of nutrients (Rajegowda, 2002).

The aim of this study was to determine the of effect mulberry leaves fortified with whey protein on the growth index of larval during 5th instar, cocoon characters, gland weight and also percentage of gland body ratio and total soluble solids of mulberry silkworm *B. mori*.

MATERIALS AND METHODS

1- Rearing of silkworm:

This work was conducted in the Laboratory of Silkworm Rearing, Plant Protection Department, Faculty of Agriculture, Assiut University, during 2016. Local hybrid of silkworm was supplied from Sericulture Division, Plant Protection Institute, Ministry of Agriculture, Giza, The larvae were reared in cardboard boxes at 23 ± 1° C and 65–70% RH. They were fed with fresh mulberry leaves until the 5th instar.

2- Composition of whey protein:

Protein 12.5 %, fat 2 %, Lactose 63 % and ash 9.5 %. Whey protein obtained from local market.

3- Treatments:

Three concentrations from liquid whey protein (4, 6, and 8 %) were sprayed on mulberry leaves, and also five grams from raw whey protein (powder) were used in feeding of 5th instar larvae. Eeach treatment was replicated three times in three carton boxes (20.5 x 19.5 x 6.5 cm), each contain twenty five silkworms larvae / treatment. Feeding with treated leaves was once / day. Control was fed with untreated leaves, according to Abdel-Rahman (2013).

- **Weight of larvae, cocoons, shell, pupae and silk glands:**

Weight mean of larvae, cocoons, shell, pupae and silk glands and control of each treatment was calculated in grams

- **Cocoon shell ratio (%):**

Cocoon shell ratio for each treatment was calculated according to Tanaka (1964) as follows:

$$\text{Cocoon shell ratio \%} = \frac{\text{Weight of cocoon shell}}{\text{Weight of cocoon}} \times 100$$

- **Growth index:**

Twenty five 5th instar larvae of one, three, five and seven day old were weighed in gram and the following formula was used for calculation of the Growth index:

$$\text{Growth Index} = \frac{\text{Final weight of the larvae (gms)} - \text{Initial weight of the larvae (gms)}}{\text{Initial weight of the larvae (gms)}}$$

- **Percentage of total soluble solids (T.S.S. %):**

Determination of (T.S.S.%), was conducted by using a hand refractometer was used. Haemolymph samples were obtained by puncturing the larval cuticle with a fine hypodermic needle. The exuded fluid from wound was drawn into a refractometer, then (T.S.S.%), in all treatment was determined 5th instar larvae according to (Arnold and Hinks, 1976)

4- Statistical analysis:

Data were analyzed using a one way analysis of variance by MSTAT-C (1988) software package and means were separated using the least significant differences method only when a significant "F" test was obtained.

RESULTS AND DISCUSSION

Data in (Table 1) show that effect of mulberry leaves fortified with whey protein on economic parameters of mulberry silkworm.

The highest weight mean of larvae (6.088 g) was recorded at concentration 8% in liquid whey protein, while the lowest weight mean of larvae (2.395 g) was recorded in raw whey protein. The highest weight mean of cocoon (1.081 g) was recorded at concentration 4% of liquid whey protein, while the lowest weight mean of cocoon (0.960 g) was in raw whey protein. The highest weight mean of shell (0.221, 0.216 and 0.203 g) were recorded at concentrations 4, 6 and 8% in liquid whey protein, respectively. While the lowest weight mean of shell (0.199 and 0.195 g) were recorded in raw whey protein and control, respectively. The highest weight mean of pupae was (0.860 g) at concentration 4% of liquid whey protein

Generally, statistical analysis revealed that there were significant differences between all treatments, but no significant difference in cocoon shell ratio.

Data in (Table 2) show the effect of treatments on the weight of larvae and silk glands, percentage of gland body ratio, and total soluble solids.

Table 1. Effect of mulberry leaves fortified with whey protein on economic traits of silkworm.

Treatments		Mean Weight (g) ± SE				Shell ratio (%)
		Larvae	Cocoon	Shell	Pupal	
*L.W.P	(4%)	4.995 ± 1.096 AB	1.081 ± 0.024 A	0.221 ± 0.003 A	0.860 ± 0.020 A	20.710 ± 0.087 A
	(6%)	4.342 ± 0.238 BC	1.064 ± 0.005 AB	0.216 ± 0.001 A	0.848 ± 0.004 A	20.450 ± 0.028 A
	(8%)	6.088 ± 0.477 A	1.019 ± 0.023 BC	0.203 ± 0.002 B	0.816 ± 0.023 AB	20.153 ± 0.396 A
**R.W.P (5 g)		2.395 ± 0.042 D	0.960 ± 0.020 C	0.199 ± 0.003 B	0.762 ± 0.017 C	20.783 ± 0.636 A
Control		3.132 ± 0.194 CD	0.960 ± 0.017 C	0.195 ± 0.004 B	0.764 ± 0.014 BC	20.606 ± 0.210 A

Means, in the same column, followed by the same letter are not significantly different at 0.05 level of probability.
***L.W.P: Liquid whey protein. **R.W.P: Raw whey protein.**

Table 2. Effect of mulberry leaves fortified with whey protein on larval weight, silk glands weight, percentage gland body ratio and total soluble solids (T.S.S) of silkworm.

Treatments		Mean ± SE			
		Weight (g)		%	
		Larvae	Silk gland	Gland Body ratio	T.S.S.
*L.W.P	(4%)	2.488 ± 0.037 A	0.593 ± 0.013 AB	23.889 ± 0.372 A	14.580 ± 0.238 B
	(6%)	2.425 ± 0.025 A	0.581 ± 0.007 B	24.025 ± 0.525 A	15.156 ± 0.263 B
	(8%)	2.379 ± 0.099 A	0.582 ± 0.007 B	24.614 ± 1.238 A	15.866 ± 0.662 B
**R.W.P (5 g)		2.542 ± 0.039 A	0.636 ± 0.012 A	25.119 ± 0.892 A	17.880 ± 0.024 A
Control		2.145 ± 0.092 B	0.573 ± 0.024 B	26.875 ± 1.778 A	16.220 ± 0.325 AB

Means, in the same column, followed by the same letter are not significantly different at 0.05 level of probability.
***L.W.P: Liquid whey protein. **R.W.P: Raw whey protein.**

The highest weight mean of larval and silk glands were (2.542 and 0.636 g) in raw whey protein, while the lowest were (2.145 and 0.581 g) in control and at concentration 6% of liquid whey protein, respectively. The highest percentage mean of gland body ratio and total soluble solids were (26.875 and 17.880 %) in control and raw whey protein, while the lowest percentage mean of gland body ratio and total soluble solids were (23.889 and 14.580 %) in liquid whey protein at concentration 4%, respectively.

Data in (Table 3) show the effect of tested treatments on growth index of larval 5th instar. High growth index of larval 5th instar were recorded (5.009, 3.901 and 3.364 g) at concentration 8, 4, and 6% of liquid whey protein respectively, while the lowest growth index of larval 5th instar were (0.199 and 0.195 g) in raw whey protein and control.

Konala *et al*. (2013) found that bovine milk had a positive effect on cocoon and body weight of *B. mori* larvae, when silkworm was fed with protein rich food like SERIPRO higher shell weight and cocoon, was obtained. For better larval weight mulberry leaves should be dipped in cow milk and feed larvae of fifth instar (Hossain *et al.*, 2015). Feeding the larvae on dietary protein supplements increases their weight (Etebari and Fazilati, 2003). also on soy a protein supplementation in the larval period may lead to an increase in the amount and quality of the silk cocoon, as well as increased larval growth (Kamaraj *et al*., 2017). Enzyme betaglucosidase was found in larvae of *B. mori* activates in the presence of lactose is one of the main components of carbohydrates in milk, does not show any problem of digestion and can be fed silkworm larvae with milk (Ito, 1960 and Byeon *et al*., 2005). It was found that feeding silkworm larvae of 5th instar on folic acid has a significant effect on the growth of larvae and silk glands and also affects the economic characteristics such as weight of cocoon, shell, shell ratio and quality of silk (Rahmathulla *et al*., 2007). Larvae feed on dietary protein like soybean, black gram, mushroom, and mixture of them causing increased weight larvae, silk gland and improved

of cocoon characters (Mahmoud, 2013). There was an increase in the growth of larvae and improvement of the characteristics of the cocoon through addition extra nutrients such as glycine, glucose, molasses and egg albumin (Sengupta *et al.*, 1972). Feeding on some fatty acids, amino acids, proteins, vitamins and essential sugars due to increase production and silk quality high (Rajabi *et al.*, 2006). Devi and Yellamma (2013) found gradually increase of body weight, silk gland weight and silk gland-body ratio when silkworms feed on Pyridoxine.

Table 3. Effect of mulberry leaves fortified with whey protein on growth index of larval 5th instar of silkworm

Treatments		Mean ± SE					Growth index
		Larval weight during 5th instar (g) at days					
		1	3	5	7	9	
*L.W.P	(4%)	1.019 ± 0.004 A	1.529 ± 0.022B	2.147 ± 0.032A	2.280 ± 0.054A	4.995 ± 1.096AB	3.901
	(6%)	0.994 ± 0.012B	2.080 ± 0.028A	2.080 ± 0.028 AB	2.200 ± 0.025A	4.342 ± 0.238 BC	3.364
	(8%)	1.013 ± 0.005 AB	1.530 ± 0.047 B	1.999 ± 0.095 B	2.161 ± 0.040 A	6.088 ± 0.477 A	5.009
**R.W.P (5 g)		1.024 ± 0.001 A	1.198 ± 0.015 D	1.603 ± 0.013 C	1.907 ± 0.018 B	2.395 ± 0.042 D	1.336
Control		0.994 ± 0.008 B	1.347 ± 0.036 C	1.693 ± 0.058 C	1.995 ± 0.046 B	3.132 ± 0.194 CD	2.147

Means, in the same column, followed by the same letter are not significantly different at 0.05 level of probability.
***L.W.P: Liquid whey protein. **R.W.P: Raw whey protein.**

Studied on the effect of different vitamins on the nutritional enrichment of mulberry leaves and it was found that all the vitamins showed a positive effect on *B. mori* growth and development (Kanafi *et al.*, 2007).

In this study addition of whey protein on mulberry leaves to the larvae of *B. mori*, showed that increase the body weight and cocoon weight, but a higher level of supplementation doesn't have a positive effect on silkworm growth and development.

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تأثير بروتين الشرش على الصفات الاقتصادية لدودة القز ، *Bombyx mori* L.

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يعتمد إنتاج الحرير وكذلك جودة حرير الشرنقة على القيمة الغذائية لأوراق التوت ونمو اليرقات. أجريت هذه الدراسة لتقييم كفاءة بروتين الشرش على الصفات الاقتصادية لدودة القز(*Bombyx mori* L.). تم رش أوراق التوت بثلاثة تركيزات من بروتين الشرش (4 ، 6 ، و 8 ٪) على هيئة سائل ، وخمسة جرامات من بروتين الشرش الخام (مسحوق) لتغذية يرقات العمر الخامس. اما الكنترول فقد تمت تغذيته اليرقات بأوراق التوت غير المعاملة. أظهرت النتائج أن أعلى متوسط لوزن غلاف الشرنقة كان (0.221 ، 0.216 ، 0.203 جم) مسجلاً عند التركيز 4 و 6 و 8٪ لبروتين الشرش السائل، على التوالي. كان أدنى مستوى لوزن غلاف الشرنقة (0.199 و 0.195 جم) في بروتين الشرش الخام والكنترول، على التوالي. كما سجل أعلى معدل نمو ليرقات العمر الخامس في بروتين الشرش السائل (5.009 ، 3.901 و 3.364) عند التركيزات 8 ، 4 ، و 6٪، على التوالي.