The effect of mulberry leaves enrichment with different nutritional supplements on biological aspects and economic traits of silkworm, *Bombyx mori* (L.)

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Abstract

The presented study was carried out during spring season of 2016 at the laboratory of Department of Plant Protection, Faculty of Agriculture AL-Azhar University. It was planned to evaluate the certain biological and economic parameters of (HoxKKxG2xV2) hybrid of silkworm, *Bombyx mori* (L.) fed on mulberry leaves *Morus alba* (Rose variety), supplemented with four types of protein sources (pollen grains, royal jelly, an amino acid and bee honey) during fourth and fifth instars of *B.mori* larvae, and to study the impact of these supplements on the larval duration, and cocoon, shell and pupal weight and shell ratio. Many studies were accomplished on the effects of mulberry leaves enrichment with protein supplements on certain biological and economic parameters. One of the alternative ways to improve the larval feeding is the enrichment of mulberry leaves with supplementary pollen grains, royal jelly, amino acid and bee honey. Obtained results showed that feeding of larvae on leaves supplemented by pollen grains, royal jelly, amino acid and bee honey increased fresh cocoon weight, cocoon shell weight; shell/ cocoon ratio. Also such feeding led to shorter larval duration and decreased larval mortality percentages.

Key words: mulberry leaves, nutritional supplements, biological aspects, Bombyx mori (L.)

Introduction

Food additions produced from different sources of protein, raise the protein content for the larvae of silkworm which affect the natural silk stored in the silk glands, which in turn leads to the higher production of silk thread and reduces breeder effort for providing larvae with mulberry leaves. Mulberry Mours alba (L.) (Fam: Moraceae) is the sole host plant for feeding the silkworm *Bombyx mori* Linn. to produce silk. Nutrition plays an important role in improving the growth and development of the silkworm, Bombyx mori (L.), like other organisms. Seki and Oshikane (1959) observed better growth and development of silkworm larvae as well as good quality of cocoons when fed on nutritionally enriched leaves. Enriching mulberry leaves by nutrient supplementation is one of the ways to improve growth rate in B.mori.

The effect of mulberry leaves enriched with amino acids on the growth of *B. mori* has been studied by (Khan and Saha, 1995; Nirwana and Kaliwal, 1998 and Radjabi, 2010). The protein content of the silk gland, fat body, and muscles was found to increase significantly when larval food was supplemented with ascorbic acid (Quraiza et *al.*2008). Different combinations of mineral nutrients were found to improve larval growth and silk production (Ahmad 1993). Mulberry leaves enriched with nickel chloride and/or potassium iodide has increased cocoons weight at low concentrations (Islam et al. 2004). The growth and development of larvae, and subsequent cocoons production, are greatly influenced by the nutritional quality of mulberry leaves (**Masthan** *et al.***2011**). The aim of this study is to evaluate the effect of different nutritional substances added to the mulberry leaves on silkworm, *Bombyx mori.* L.

Material and Methods

The experiments were conducted in the laboratories of the Department of Plant Protection, Faculty of Agriculture, AL-Azhar Univ., Nasr City, Cairo, Egypt.

Mulberry leaves and silkworm hybrid resources

Mulberry variety rose was planted in experimental field in the Faculty of Agriculture, AL-Azhar Univ, Nasr City, Cairo, Egypt. Eggs of the silkworm hybrid (HoxKKxG2xV2), [Produced by sericulture and agriculture experiment station, Vra Tza, Bulgaria silkworm, *B.mori*, L.], were obtained from the Sericulture Research Department of Plant Protection Research Institute, Agricultural Research Centre, (ARC.) at Giza-Egypt.

Source of protein.

Four sources of protein supplements were used: (pollen, royal jelly, amino acid (amino meg) and bee honey).

Silkworm rearing technique:

Preparation for rearing: Before beginning of rearing, the rearing room and appliances were thoroughly cleaned and the floor was washed with 5% bleaching powder solution. The whole room was disinfected by spraying with 2.5% sanitech in 0.5% slacked lime solutions (Dandin *et al.*, 2003). **Incubation of eggs:**

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

Brushing and rearing:

The newly hatched larvae of the hybrid (HoxKKxG2xV2), were placed separately, in the disinfected rearing trays supplied with tender mulberry leaves from M.alba var. (Rose) cut into small squares of 0.25cm² each and sprinkled over the egg cards. The neonate larvae crawled over the tender leaves and started feeding. Later, cards were tapped onto the other rearing bed, whereas the remaining worms were brushed out. Bed cleaning was done once during the first instar and twice during second one. Two beds each containing 3000 larvae for young instar (from 1st till 3rd instar) reared only on mulberry leaves and from the beginning of 4th instar four treatments with three mixing rates were replicated three times each replicate contains 100 larvae per tray and in addition to the untreated. This experiment was conducted in completely randomized design with three replications.

The treatments are as follows.

T 1 –pollen grains 2, 4 and 6 % of mulberry leaves.

T 2 – Royal jelly 2, 4 and 6 % of mulberry leaves.

T 3 – Amino acid 2, 4 and 6 % of mulberry leaves.

T4-Bee honey 2, 4 and 6 % of mulberry leaves.

T 5 – Untreated (without additions).

Required quantities of mulberry leaves and pollen, royal jelly, amino acid and bee honey were weighed and supplemented with mulberry leaves for feeding fourth and fifth instar larvae. The larvae were fed on *M.alba* var. (Rose) mulberry leaves with supplements till pupation. Collapsible frames were used as montages for cocoon spinning After ten days of spinning, cocoons,those were collected.

Silkworm performance:

Biological aspects:

Larval duration: Total larval duration was calculated from egg hatching till cocoon spinning for each treatment.

Larvae weight: At the end of fourth and fifth larval instars, three replicates were taken, each replicate consisted of twenty larvae and were weighed and the average weight was calculated.

Larval mortality: At the beginning of fourth larval instar, 300 larvae were separated from the mass rearing and used for estimating mortality percentage for the grown larvae. The mortality percentages among the fourth and fifth instars were calculated according to the following formula of Megalla (1984).

Percentage of mortality = No. of dead larvae / Total No. of larvae X 100.

Pupal weight: Pupae of the previous cocoons of each treatment after being sexed, were removed from

cocoons and weighed separately, the average weight was calculated.

Economical parameters.

Cocoon indices:

Weight of cocoons: Twenty fresh cocoons were cut and pupae were sexed. The whole cocoons were weighed separately for each sex. Cocoons from each treatment were carefully opened and pupae were sexed, then 30 cocoon of each sex were weighed separately and the average weight for each was calculated.

Fresh of cocoon weight: Twenty fresh cocoons were opened and pupae were sexed. The whole cocoons were weighed separately for each sex.

Cocoons shell weight: After removing the pupae from the previous cocoons as mentioned, and cleaning them from exuviae,those were weighed and the average weight was estimated.

Cocoon shell ratio %: Cocoon shell ratio was calculated for both sexes in each treatment according to the formula of **Krishnaswami** *et al.* (1973) as follows.

Cocoon shell ratio (%) = Cocoon shell weight/ Fresh cocoon weight X100.

Biological and economic parameters data were statistically analyzed (Little & Hills 1975) using ANOVA implemented by the Costat software program.

Results and discussion.

Biological parameters of (*Bombyx mori*, L) hybrid (HoxKKxG2Xv2) fed on mulberry leaves (Rose variety) mixed with different protein sources.

Larval duration.

Data in (Table 1) show that the larval duration of silkworm larvae fed on mulberry leaves treated by pollen grains recorded 26.16, 25, 66 & 25.16 days for the 2, 4 and 6g of mulberry leaves treatments, respectively. The feeding on untreated mulberry leaves lead to slightly prolongation (28.98days) with no significant differences.

It is clear that silkworm feeding on *M.alba* var. (Rose variety) treated with pollen recorded the shortest mean of larval duration (25.66 days) followed by royal jelly, amino acid and bee honey treatments (26.37, 26.99, and 27.70 days). Statistical analysis showed that all the protein supplements caused highly significant decrease in larval duration than that of the untreated, while pollen grains effect was superior to royal jelly, amino acid and bee honey as source of protein for silkworm hybrid.

These results are supported by the findings of **Krishnan** *et al.* (1995) who they showed that the hydrolyzed soyaprotein (P-soyatase) supplementation decreased the larval duration increased the accumulation of haemolymph protein, larval weight and cocoon characters.

Larval weight (Fourth and fifth larval instars.)

The data (Table, 1)showed that the highest mean weights of ten larvae (fourth instar larvae) recorded 1.630, 1.257, 1.071 and 1.001g for the four treatments of pollen grains, royal jelly, amino acid and bee honey, respectively, and when they fed on untreated mulberry leaves those weighed 0.878 g, with significant difference between pollen grains treatment and untreated(P=0.008).

As shown in the same table, one larva of the fifth instar (at the beginning of stadium) recorded the mean weights of 0.567, 0.520, 0.476, 0.458 and 0.445g for (HoxKKxG2xV2) hybrid larvae fed on mulberry leaves treated by pollen grains, royal jelly, amino acid and bee honey and the untreated, respectively. The fifth larval instar (at the end of stadium) fed on mulberry leaves treated with the pollen grains gave the means of larval weights (1.860, 1.888, 1.922g) for 2, 4, 6% in the pollen

grains treatment, while they recorded 1.639g for the untreated larva (Table 1). It is clear that silkworm feeding on *M.alba* var. (Rose variety) mixed with 6% pollen grains recorded the highest means of larval weight followed by royal jelly, amino acid and bee honey.

The presented results are in conformity with those reported by **Horie** and **Watanabe** (**1983**).They showed that the supplementation of soya bean protein increased the protein and amino acid content in the larval haemolymph of the silkworm. **Mahmoud (2000) and Ashour (2005)** reported that the superiority of Kokuso-27 might be explained by its increased amounts of crude protein, soluble sugar and starch content in their leaves, which might improve the health and growth of larvae, perhaps by increasing the rates of the digestive and oxidizing enzymes which help in utilizing food and increasing food consumption.

 Table 1. Effect of feeding mulberry leaves with protein supplements on certain biological parameters in silkworm during spring season, of 2016.

Protein Supplements	Mixing rate %	Larval w	Larval				
		Fourth 4 th	Mortality Fifth 5 th			Mortality	duration
			%	Immature	Mature	%	(days)
Pollen grains	2%	1.498 ^b	4	0.511b	1.860abc	6	26.16bc
	4%	1.677 ^a	3	0.584a	1.888ab	4	25.66bc
	6%	1.714 ^a	2	0.605a	1.922a	2	25.16c
	Mean	1.630 a	3	0.567 a	1.890 a	4	25.66 d
Royal jelly	2%	1.211 ^d	9	0.467de	1.715de	9	26.96abc
	4%	1.224 ^d	7	0.505bc	1.807bcd	6	26.99abc
	6%	1.336 ^c	5	0.590a	1.900a	4	25.16c
Amino acid (amino meg) trade name Bee honey	Mean	1.257 b	7	0.520 b	1.807 a	6.4	26.37 c
	2%	1.005 ^e	11	0.454de	1.744de	10	27.66ab
	4%	1.054 ^e	9	0.478cde	1.776cde	9	27.14abc
	6%	1.153 ^d	8	0.496bc	1.798bcd	7	26.16bc
	Mean	1.071 c	9.4	0.476 c	1.773 b	8.7	26.99 bc
	2%	0.981 ^e	13	0.450e	1.699e	14	28.69a
	4%	1.007 ^e	12	0.443e	1.715de	12	27.66ab
	6%	1.014 ^e	9	0.481cd	1.701e	10	26.76abc
	Mean	1.001 d	11.4	0.458 d	1.705 c	12	27.70 b
Untreated	Mean	0.878 e	14	0.445 d	1.639 d	16	28.98 b
Р		0.0567		0.016	0.053		1.117
L.S.D.V*H		0.0439	_	0.0127	0.041	_	2.043

Larval mortality.

The lowest mean mortality percentages (4, 3 and 2%) in the 4th larval instar were obtained when larvae were fed on mulberry leaves mixed with pollen grains. While the highest one (11% on average) was recorded when larvae fed on bee honey followed by amino acid and royal jelly (9.4 and 7% in average). The fifth instar larvae gave the same trend of

mortality, it is clear that feeding on leaves mixed with pollen grains is suitable for silkworm. These results are supported by the finding of **Zannoon** (1999) who reported that rearing *B. mori* larvae with Kokuso-27 led to shorter larval durations and lower larval mortality rate relative to feeding on it than the native variety.

Economical parameters: Cocoon indices.

Fresh cocoon weight (F.C.W).

Feeding on *M.alba* (Rose) mulberry leaves mixed with pollen, royal jelly, amino acid and bee honey supplements resulted in insignificant increase in fresh cocoon weight. The fresh cocoon mean weights were (0.791, 0.735, 0.745, 0.712g) for female and (0.718, 0.696, 0.680, 0.668 g) for male 2, 4 and 6% protein supplements in the four treatments, respectively, compared to 0.687and 0.667g for the three mixing percentages untreated for female and male Highly significant differences were recorded in all treatments comparing with untreated.

Cocoon shell weight (C.S.W).

Cocoon shell weights marked variation between male and female and also between different treatments. Results of cocoon shell weights followed the same trend of the fresh cocoon weights previously pointed out. Supplementation with pollen led to the heaviest cocoon shell of females and males of hybrid. The means of cocoon shell weights were (0.159, 0.149, 0.140, 0.130g) for female and (0.129, 0.117, 0.117, 0.114g) for male for the three mixing rates 2, 4 and 6% protein supplements in the four treatments, respectively, in comprising 0.131 and 0.111g of female and male (untreated). With highly significant differences among all treatments (Table 2).

Cocoon shell ratio (C.S.R):

Feeding on *M.alba var*. (rose) mulberry leaves mixed with pollen, royal jelly, bee honey and amino acid supplements (Table 2) resulted in insignificant increase in cocoon shell ratio. The cocoon shell ratio were 19.77, 18.66, 17.69, 17.70g) for female and (17.97, 16.83, 17.28, 17.09g) for male of three mixing rates 2,4 and 6% protein supplements in four treatments, respectively, in comparison to 17.80 and 16.80% for female and male in untreated. With significant differences were found in the first three treatments for female and male compared to untreated. It is clear that feeding on pollen was suitable for silkworm *Bombyx mori*, (L) rearing.

Table 2. Effect of feeding mulberry silkworm on mulberry leaves mixed with protein supplements (pollen grains, royal jelly, bee honey and amino acid) on certain economical parameters of (Female and Male).

		Male and female cocoon parameters							
Protein Supplements	Mixing rate %	Mean of fresh cocoon weight (g).		Mean of cocoon shell weight (g).		Mean of pupae weights (g).		Mean of cocoon shell ratio (C.S.R%)	
		Female	male	Female	male	Female	male	Female	male
Pollen grains	2%	0.757abc	0.698a	0.156ab	0.128a	0.618ab	0.579a	20.54a	17.79ab
	4%	0.780abc	0.725a	0.159a	0.129a	0.605ab	0.585a	19.66ab	18.34a
	6%	0.838a	0.731a	0.164a	0.131a	0.673a	0.567a	19.11bc	17.92ab
	Mean	0.791 a	0.718 a	0.159 a	0.129 a	0.632 a	0.577 a	19.77 a	17.97 a
Royal	2%	0.662d	0.681a	0.135abc	0.111a	0.615ab	0.627a	19.39ab	17.38bc
	4%	0.713bc	0.702a	0.151ab	0.119a	0.636ab	0.586a	18.60bcd	16.90cd
	6%	0.830ab	0.704a	0.162a	0.122a	0.665ab	0.568a	18.00cd	16.30d
	Mean	0.735 ab	0.696 a	0.149 ab	0.117 ab	0.639 a	0.594 a	18.66 b	16.83 cd
Amino acid (amino meg) trade name	2%	0.697cd	0.660a	0.132bc	0.112a	0.559b	0.536a	17.89cd	17.69a
	4%	0.748bc	0.687a	0.140abc	0.120a	0.589b	0.551a	17.71cd	17.47bc
	6%	0.791bc	0.694a	0.149abc	0.121a	0.624b	0.553a	17.49d	16.97cd
	Mean	0.745 ab	0.680 a	0.140 bc	0.117 ab	0.590 a	0.547 a	17.69 c	17.28 b
Bee honey	2%	0.667d	0.654a	0.122c	0.113a	0.570b	0.522a	17.55d	17.13cd
	4%	0.703cd	0.666a	0.130bc	0.111a	0.593b	0.535a	17.83cd	16.97cd
	6%	0.767abc	0.683a	0.140abc	0.117a	0.624b	0.563a	17.73d	16.97cd
	Mean	0.712 b	0.668 a	0.130 cb	0.114 b	0.596 a	0.540 a	17.70 c	17.09 b
untreated		0.687 b	0.667 a	0.131 d	0.111 b	0.589a	0.549 a	17.80 c	16.80 b
L.S.D.V*H	Treatments	0.068	0.017	0.015	0.012	0.064	0.064	0.767	0.573
	Mixing rate %	0.053	0.052	0.012	0.010	0.049	0.0469	0.594	0.444

It was observed higher mean weight of the pupae in case of feeding on royal jelly 0.639g for female followed by pollen grains 0.632g. While the lowest mean weight of pupae was in the case of amino acid 0.590g compared to the untreated 0.605g (Table 2). The higher mean weight of the male pupae was in the case of feeding royal jelly 0.594g; followed by pollen grins (0.577g.), while the lowest mean weight of pupae was in the case of bee honey 0.540g compared to the untreated male 0.549g, and female (0.589g) (Table 2).

From these results it can be concluded that the nutritional supplements added to mulberry for feeding silkworm larvae were effective in increasing the growth rate and development of silkworm larva. The treatment of silkworm larvae with nutrient supplement is good in improving the qualities of silk fibre which can be used for yield enhancement in sericulture industry. The presented results agree with (Nguku et al, 2007) cleared that Royal jelly obtained from Apis mellifera colonies increases moulting and larval weight of silkworm B.mori (L). It contains acetylcholine, which is important requirement in the diet of silkworm larvae for normal moulting as well as growth. The rapid growth and developmentl as well as increasing number of eggs can be obtained by addition royal jelly to the normal mulberry leaves. Effect of mulberry leaves fortified with royal jelly does not show any significant differences on shell weights over normal feeding of silkworm. Radjabi, (2010) stated that the actual silk fibre fibroin is derived from four amino acids viz., Alanine, Serine, Glycine and Tyrosin, which come from the food material of silkworm. The author showed that amino acid Alanine plays an important role in metabolism of glucose, tryptophan and organic acid. Aspergin used as aspartic acid by silkworm is one of the essential amino acids, deficiency of which results growth retardation. The same author added that supplementation of amino acids solutions, dissolved in distilled water by spread in fresh mulberry leaves after being diluted in different concentrations with feeding material at definite level is effective for improvement of growth and development of silkworm, but a high level beyond a certain level does not have a positive effect.

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تأثير الإضافات الغذائية المختلفة لأوراق التوت على النواحى الحياتية والإقتصادية لدودة الحرير التوتية. محمد كامل عبدالصمد حمزة عبيد – جمعة فتح الله محمد أبولبن– مروى مصطفى نبيل. –1قسم وقاية النبات –كلية الزراعة –جامعة الأزهر الشريف –مدينة نصر – القاهرة. –2معهد بحوث وقاية النباتات –مركز البحوث الزراعية –الجيزة –مصر.

تم إنجاز هذا العمل بمعامل كلية الزراعه جامعة الأزهر الشريف بالقاهرة -مدينة نصر .حيث تم تغذية يرقات ديدان الحرير التوتية على أوراق التوت الصنف البلدى المزروع بالكلية والتى تم خلطها بالإضافات الغذائية الأتية)حبوب اللقاح -غذاء الملكات -الحمض الامينى -عسل النحل (والتي أضيفت بثلاث نسب هي 4, 4) و (% 6لأوراق التوت من الصنف البلدى)روز .

وتم إختيار هجين مستورد من بلغاريا (HoxKKxG2xV2)بواسطة قسم بحوث الحرير التابع لمركز البحوث الزراعية بالجيرة .وتم تقسيم اليرقات لأربع معاملات بكل معاملة إضافات كل إضافة ثلاث مكررات كل مكررة 100يرقة بالإضافة للكنترول وتم تغذية اليرقات أربعة وجبات يوميًاً بدايةً من العمر اليرقى الأول حتى دخول اليرقات التعشيش وتكوين الشرنقة.

وأظهرت النتائج تفوق المعاملة المضاف اليها حبوب اللقاح عن المعاملات الأخرى مقارنة بالكنترول يليها غذاء الملكات والحمض الأمينى وكان عسل النحل في المسرتبة الأخيرة.

وكانت الأفضلية لحبوب اللقاح بين المعاملات من حيث التحسين في الصفات البيولوجية والإقتصادية محل الدراسة في زيادة متوسطات الأوزان للشرانق الطازجة (0.718, 0.791)جرام ثم ووزن طبقة الحرير (0.131, 0.159)جرام ، وزيادة نسبة الحرير الناتج من الشرانق , 19.77) (17.79جرام للانثي والذكر على التوالي .كذلك النقص في مدة الطور اليرقي ونسبة الوفيات مقارنة بالإضافات الأخرى والكنترول.