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EFFECT OF SUPPLEMENTAL FEEDING ON THE WEIGHT OF SILK GLANDS AND LARVAL DEVELOPMENT OF MULBERRY SILKWORM (*Bombyx mori*)

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ABSTRACT

The study aimed to revive the Sericulture in our area after three decades of absence and to investigate the role of supplemental feeding on silk glands, larval growth, and some economic traits of cocoons of mulberry silkworms (*Bombyx mori*). Larvae of 5th instar fed on vitamin C during their 4th and 5th instars showed a significant increase in their length (6.953 ± 0.248 mm). Mature larvae fed on vitamin C showed a significant increase in their weight (3.690±0.129 g). Vitamin C was the most effective supplement for the stimulation of silk gland growth. Larvae fed on vitamin C showed a significant increase in their cocoon weight (2.158 ± 0.131 g) followed by those fed on honey (2.126 ± 0.156 g). Only cocoons fed as larvae on vitamin C showed a significant increase in their length (3.793 ± 0.125 cm). Larvae fed on vitamin C showed a significant increase in their cocoon width (1.873 ± 0.050 cm). Feeding silkworms during their 4th and 5th instars on mulberry leaves sprayed with vitamin C was more effective in stimulating larval growth, silk glands and improving economic characteristics of silk cocoons.

KEYWORDS; Silkworm, Silk gland, Supplements, Vitamins, Probiotics

1. INTRODUCTION

Insects' domestication has received less attention compared to other animals. Insects are the most diverse animals on the earth, they provide valuable services to humans in both man-made and natural ecosystems (Lecocq, 2018).

Sericulture has supplied humans with a natural animal silk since long time. Silkworm are the larvae of the moth *Bombyx mori* (Lepidoptera, Bombycidae). They are the most important insects serving the human economy, because they are the main producer of silk worldwide (Aswartha et al., 2017; Chauhan and Tayal, 2017).

Bombyx mori is an insect of Bombycidae family, Lepidoptera order, and Insecta class. It is a domesticated insect, fully adapted to commercial breeding, which arises after many years of evolution and artificial selection. This species is not present in nature in a free state, as it has lost the ability to fly and survive under extreme environmental conditions. (Torres McCook et al., 2021).

B. mori is a monophagous species, with complete metamorphosis, eating only mulberry leaves during its larval stage (Ravikumar et al., 2019). Nutrition is considered the most important and essential component for the living organisms; it is impossible to survive and attain a healthy disease-free growth without getting nutritious food. Silkworms are very sensitive towards nutritional factors (Panizzi and Parra, 1991).

The worms in the 5th instar ingest more than 88 % of mulberry, and they reach maximum weight one or two days before starting to spin the cocoon. In addition, they rapidly develop the silk gland, which occupies up to 40 % of their weight. When they finish their development and stop eating, larval integument appears transparent. At this stage, they are called mature larvae, and begin to form the cocoon for two or three days (Cifuentes and Sohn, 1998).

The scientific literature indicated that food supplementation of silkworm larvae has resulted in the improvement of commercial and biological aspects of sericulture (Ahsan et al., 2013; Kanafi et al., 2007).

Nutritional supplements include vitamins, amino acids, proteins and probiotics when added to larval feed tend to increase nutritional efficiency and economic traits of silkworm (Etebari and Matindoost, 2005; Amalarani et al., 2011).

Vitamins are essential substances to adjust the physiological function and metabolism in cells. The silkworm mainly requires vitamin B complex and Ascorbic acid (vitamin C) for their growth and development (Shamsuddin, 2009). Ascorbic acid has many important functions in the silkworm body. It is a powerful antioxidant, protecting against oxidative damage to proteins. Ascorbic acid significantly increased the weight of silkworm larvae (Radjabi et al.,2009), and has been regarded as indispensable and the exclusive food for the silkworm (Borah and Praban, 2020).

The commercial traits of silkworm cocoons were enhanced when mulberry leaves were supplemented with vitamin C (Hussain and Javed, 2002; Etebari et al., 2004 a; Chang and Li, 2004).

Feed utilization studies are generally confined to 4th and 5th instar of silkworm larvae as 80% of total leaves was consumed in these instars. Several researchers have reported about beneficial role played by the use of the probiotics in humans, animals and insects (Brigidi et al., 2000). Studies were investigated the impact of a honey-enriched diet on several parameters of *Bombyx mori*, mainly on the larval and silk gland growth. They confirmed that the optimal concentration was 2 % honey for ideal larval growth. A great improvement was observed by adding honey into the silkworms' diet (Thulasi and Sivaprasad, 2015).

The silk gland is an organ specialized for the synthesis and secretion of silk proteins (Zhang, 2006). It is the major storage organ of the silk. This silk gland represents one of the most active proteins synthesizing system among the entire organ. Silk glands constitute approximately one quarter of the worm's mass and produce liquid silk. This is composed of complex proteins (Wurm, 2003).

This study aimed to revive the Sericulture in our area after three decades of absence and to investigate the role of supplemental feeding on silk glands, larval growth, and some economic traits of cocoons of mulberry silkworms.

2. MATERIALS AND METHODS

The experiments were performed at the Department of plant protection, College of Agricultural Engineering Sciences, University of Duhok, Kurdistan region, Iraq; during March and April 2022. The disease-free eggs of *Bombyx mori* (Bursa White; 'Bursa Beyazi F1 hybrid eggs') were obtained from Sericulture Center in Bursa, Turkey.

Preparation of the stock culture of silkworms

In order to obtain a large number of 4th instar silkworms' larvae for providing the experimental rearing trays, the eggs were subjected to incubation at 27 ± 1°C and 80 ± 5% RH with 16 h light: 8h of darkness (Hussain et al., 2011).

After the incubation period, the freshly hatched larvae were transferred to large plastic trays (50 x 50 x10 cm) with transparent plastic covers to prevent the loss of water from the leaf bed, and to prevent the continuous darkness inside these trays. Three trays were used for this purpose. Early larval instars (1st to 3rd) were reared at 27 ±1 °C temperature and 80 ± 5% RH with 16 h light: 8h of darkness. Early larval instars were supplied with sufficient amounts of mulberry leaves.

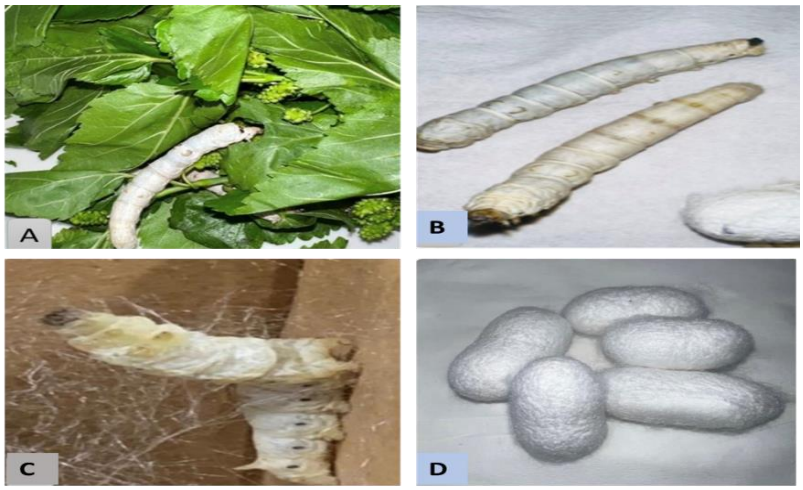

Fig.(1). A; larva of 4th instar, B; larvae of 5th instar, C; mature larva prior to spinning of cocoon and D; completed cocoons.

Providing mulberry leaves with different supplements

The 4th and 5th larval instars were reared at 25 ±1 °C temperature and 70 –80 % RH (Rahmathulla, 2012). At the beginning of the 4th instar four groups of larvae of silkworm were picked up by hand, and transferred to experimental trays prepared in the laboratory and were fed from the beginning of the fourth larval instar on mulberry leaves treated with different supplemental feeding. Three replicates, each with twenty-five larvae were used for each tested supplement; (Figure 1 A and B).

Preparation of supplemental solutions

The tested supplemental feeding treatments were prepared as the following; The 1st treatment; without supplements (control); Feeding all larvae during the larval instars with mulberry leaves. The 2nd treatment; (Probiotics); the solution of 2% was prepared, 20 g of Probiotics (included lactobacillus bacteria) were added to one liter of tap water. The probiotics were obtained from bee-shop in Turkey. The 3rd treatment; (vitamin C); the solution of 4% was prepared, 40 g of pure vitamin C was added to one liter of tap water. The vitamin C was obtained from a pharmacy shop in Duhok city. The 4th treatment; (local honey); the solution of 4% was prepared, 40 g of local honey was added to one liter of tap water. The local honey was obtained from bee-shop in Duhok city.

Feeding the 4th and 5th instar larvae of silkworms on mulberry leaves provided with different supplements

One liter of the previously prepared solutions was used for each replicate. The 4th and 5th instar larvae of silkworms were fed on mulberry leaves provided with different supplements. Twenty-five larvae were used for each replicate and the tested larvae were fed on sprayed leaves five times per day. The mulberry leaves were sprayed with the prepared solutions, and then dried at room temperature before feeding the tested larvae to calculate the following:

1. Biological parameters during larval development

The length, width and weight of 5th larval instar and the mature larvae of the tested silkworms with different supplements were measured and calculated (Figure 1 B and C).

2. Measurement of the weight of silk glands

Similar to the other tested physical parameters, the weight of the silk glands was studied. Five mature larvae from every replication were randomly selected (Reddy et al., 2015), and a longitudinal incision was made dorsally in the abdomen of each mature larva. Few drops of physiological saline were added directly to the abdominal viscera (Ayoub, 2011), and both sides of the silk glands were isolated from the larva body, and then the wet weight of the right and left silk glands were separately weighted using a digital scale. The larvae were dissected with the naked eyes, and the weight of each side was measured and calculated; (Figure 2).

Fig.(2): A; dissected mature larva, B; both sides of silk gland.

3. Economic traits of the cocoon

The economic traits like cocoon parameters (length, width and weight) and cocoon shell weight were measured by using a digital balance. Five fresh cocoons from each replicate were cut and the whole cocoons were weighed separately (Figure 1 D). After removing the pupae from the previous cocoons and cleaning them from exuviae, they were weighed (Kamel et al., 2016).

Analysis of variants (ANOVA) was used to compare biological parameters of groups of silkworms fed on mulberry leaves treated with different supplements. Duncan's multiple

comparison tests were used to detect significant differences (at $p < 0.05$) among groups of silkworms fed differently during 4th and 5th larval instars.

3. RESULTS

Effect of supplemental feeding on the characteristics of 5th instar larvae of silkworms.

Larvae of 5th instar fed on vitamin C showed a significant increase in their weight (4.716 ± 0.076 g) followed by those fed on honey (4.686 ± 0.055 g), while larvae fed on probiotics were not differed significantly with those fed on untreated leaves.

Larvae of 5th instar fed on vitamin C showed a significant increase in their length (6.953 ± 0.248 cm) followed by those fed on honey (6.480 ± 0.153 mm), while larvae fed on probiotics were not differed significantly in their length with those fed on untreated leaves.

Only larvae of 5th instar fed on vitamin C showed a significant increase in their width (9.180 ± 0.041 mm). Larvae fed on mulberry leaves sprayed with probiotics and also those fed on mulberry leaves sprayed with honey were not differed significantly in their width with larvae fed on untreated leaves; (Table 1).

Table(1): Effect of supplemental feeding on the weight, length and width of 5th instar larvae of silkworms.

Treatments	Parameters		
	Weight (g)	Length (cm)	Width (mm)
Control	4.300 ± 0.086 b	6.040 ± 0.185 b	7.200 ± 0.056 b
Vitamin C	4.716 ± 0.076 a	6.953 ± 0.248 a	9.180 ± 0.041 a
Probiotic	4.256 ± 0.040 b	5.960 ± 0.640 b	7.280 ± 0.033 b
Honey	4.686 ± 0.055 a	6.480 ± 0.153 ab	7. 690 ± 0.083 b

Table (2): Effect of supplemental feeding on the weight, length, width and the weight of silk glands of mature larvae.

Treatments	Parameters				
	Body measurements			Weight of silk glands (g)	
	Weight (g)	Length (cm)	Width (mm)	Right side	Left side
Control	3.098±0.035 c	5.866±0.070 b	6.800 ± 0.360 a	0.650 ± 0.036 b	0.642 ± 0.033 b
Vitamin C	3.690 ± 0.129 a	6.376 ± 0.237 a	8.316 ±1.396 a	0.7927 ± 0.006 a	0.807 ± 0.045 a
Probiotic	3.277 ± 0.316 bc	6.156 ± 0.286 ab	7.583 ± 0.401 a	0.648 ± 0.096 b	0.640 ± 0.053 b
Honey	3.490 ± 0.114 ab	6.430 ± 0.355 a	8.500 ± 0.888 a	0.6730 ± 0.077 b	0.679 ± 0.036 b

Effect of supplemental feeding on the characteristics of mature larvae of silkworms.

Mature larvae fed during their 4^{th} and 5^{th} instars on vitamin C showed a significant increase in their weight (3.690 ± 0.129 g) followed by those fed on honey (3.490 ± 0.114 g), while larvae fed on probiotics were not differed significantly in their weight with those fed on untreated leaves; **(**Table 2). Mature larvae fed during their 4^{th} and 5^{th} instars on honey showed a significant increase in their length (6.430 ± 0.355 cm) followed by those fed on vitamin C (6.376 ± 0.237 cm), while larvae fed on probiotics were not differed significantly in their length with those fed on untreated leaves. Significant differences in the weight of silk glands were found among 5^{th} instar larvae fed on mulberry leaves sprayed with supplements compared to those fed on untreated leaves. Vitamin C was superior in stimulating the development of silk glands, larvae of 5th instar fed on vitamin C showed significant increase in

the weight of silk glands of both sides; right side (0.7927 ± 0.006 g) and left side (0.807 ± 0.045 g); (Table 2).

Effect of supplemental feeding on the characteristics of cocoons of silkworms.

Feeding of silkworm larvae during their 4th and 5th instars on vitamin C, and honey as supplements resulted in heavier cocoons. Significant differences in the weight of cocoons were found among groups of silkworms fed on mulberry leaves sprayed with supplements compared to those fed on untreated leaves. Larvae fed during their 4th and 5th instars on vitamin C showed a significant increase in their cocoon weight (2.158 ± 0.131 g) followed by those fed on honey (2.126 ± 0.156 g), while larvae fed on probiotics were not differed significantly in their weight of cocoons with those fed on untreated leaves. Only cocoons fed as larvae on vitamin C during their 4th and 5th instars showed a significant increase in their length (3.793 ± 0.125 cm).

Larvae fed on mulberry leaves sprayed with probiotics and also those fed on mulberry leaves sprayed with honey were not differed significantly in their length of cocoons with larvae fed on untreated leaves.

Feeding of silkworm larvae during their 4th and 5th instars on vitamin C, and honey as supplements resulted in more width of cocoons. Larvae fed during their 4th and 5th instars on vitamin C showed a significant increase in their cocoon width (1.873 ± 0.050 cm), followed by those fed on honey (1.770 ± 0.101 cm), while larvae fed on probiotics were not differed significantly in their width of cocoons with those fed on untreated leaves.

Feeding of silkworm larvae during their 4th and 5th instars on vitamin C as a supplement resulted in heavier cocoon shells. Only cocoons fed as larvae on vitamin C showed a significant increase in their shell (0.513± 0.093 g) compared to those fed on untreated leaves. While cocoons resulted from larvae fed on honey, and probiotics were not differed significantly from those fed on untreated leaves; **(**Table 3).

Table (3): Effect of supplemental feeding on the weight, length, width of cocoons, and cocoon shell weights of silkworms.

Treatment	Parameters			
	Weight (g)	Length (cm)	Width (cm)	Shell weight (g)
Control	1.690 ± 0.217 b	3.046 ± 0.085 b	1.6300 ± 0.043 c	0.369 ± 0.015 b
Vitamin C	2.158 ± 0.131 a	3.793 ± 0.125 a	1.873 ± 0.050 a	0.513 ± 0.093 a
Probiotic	1.723 ± 0.153 b	3.126 ± 0.152 b	1.663 ± 0.032 b c	0.38 ± 0.005 b
Honey	2.126 ± 0.156 a	3.406 ± 0.298 b	1.770 ± 0.101 ab	0.410 ± 0.014 b

4. DISCUSSION

Nutritional value of mulberry leaves plays an important role in the silkworm development and the growth of larvae, as well as the economic parameters of the produced cocoons.

The present investigations were carried out to study the influence of supplemental feeding of silkworms during the 4th and 5th larval instars. The results of the supplementation were reflected in many biological aspects of larvae, pupae as well as the development of silk glands. Vitamin C was superior in inducing the growth and development of the 4th and 5th larval instars, and the silk glands. In this investigation maximum weight of silk gland of mature larvae was 0.8 g when mulberry leaves were supplemented with 4 % vitamin C, followed by those fed on supplemented leaves with 4 % local honey (Table 2). These results were consistent with those of (Helaly, 2018); (Saad et al., 2014). Rearing silkworm larvae on mulberry leaves that were enriched with supplements induced a significant increase in the weight of the full-grown larva, silk gland, female pupa as well as the cocooning percentage (Helaly, 2018).

Local honey occupied the second rank respect, while silk worm larvae fed on untreated mulberry leaves gave the least values of most biological parameters tested during these investigations.

Data given in Table 2, indicate that the mean weight of mature larvae was significantly affected by the food additives. In addition to that

the highest values of body dimensions (length and width) of mature larvae were also found when the 5th instar larvae fed on supplemented mulberry leaves. Probiotic was not effective in increasing the weights of 5th larval instars, (Table 1).

Larvae of 5th instar fed on vitamin C showed a maximum increase in their body weight (4.716 ± 0.076 g), body length (6.953 ±0.248 cm) and body width (0.918 ± 0.041 cm) compared to other treatments (Table 1). Generally, in the fifth instar, larvae increase in their body weight until they reach a stationary state when they are ready for spinning (Reddy et al, 2015). While more reduction occurred in the dimensions of mature larvae when supplemented with vitamin C (Table 2), this may be indicating to that, using vitamin C as additive was strongly stimulated silk glands, consequently resulted in smaller mature larvae of this group**.** Reddy et al, (2015) revealed that the reduction in size increases the pressure on silk glands to eject silk from the glands. Hence there is a sudden decrease in length of larvae on 8th day. This decrease in length continues until pupal stage. And on the other had the maximum weight in both sides of silk glands (0.7927 ± 0.006 g for the right side and 0.807 ± 0.045 g for the left side); (Table 2) was found in silk worms supplemented with vitamin C during their 4th and 5th instars. These results confirm the positive effects of supplementation with vitamin C as superuser additive for improvement of silk worms. The silk gland represents one of the most active proteins synthesizing system among the entire organs. Silk glands constitute approximately one quarter of the worm's mass and produce liquid silk. This is composed of complex proteins (Wurm, 2003).

The fresh cocoon weight and shell weight were considered the most important economic features of mulberry silkworms. Bigger cocoons and higher values of cocoon weight were observed in those fed on mulberry leaves supplemented with vitamin C (2.158 ± 0.131); (Table 3).

Saad et al., (2014) obtained fresh cocoons weight of 1.35 g when were fed on supplemented leaves with 4% honey, and cocoon weight of 1.73 g when were supplemented with 5% honey. In the current study heavier cocoon shells were obtained in the case of supplementation with vitamin C (0.513 ± 0.093); (Table 3).

Based on the results of the current study, it can be concluded that the Mulberry leaves supplemented with vitamin C (4%) as well as those supplemented with local honey (4%) were effective in increasing the weight of 5th instar larvae, mature larvae, pupae, cocoons and adult females. Using vitamin C (4%) as an additive food increased the dimensions of 5th instar larvae and the weight of cocoon shell. Vitamin C (4%) was superior in stimulating the development of silk glands of mature larvae. Supplementation with vitamin C (4 %) as well as with local honey (4 %) resulted in the production of heavier cocoons.

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