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Walaa Megahed Mohamed Youssif Helaly
Zagazig University, Faculty of Agriculture, Department, Plant Protection, Zagazig -Egypt

Correspondence
Walaa Megahed Mohamed Youssif Helaly
Zagazig University, Faculty of Agriculture, Department, Plant Protection, Zagazig -Egypt

Evaluation of two food additives on *Bombyx mori* L. characters

Walaa Megahed Mohamed Youssif Helaly

Abstract
The present investigation was carried out to study the influence of bovine milk and white hen's eggs on some biological, biochemical and technological characters of the mulberry silkworm, *Bombyx mori* L at the laboratory of Sericulture, Plant Production Department, Faculty of Agriculture, Zagazig University during spring season of 2016. Rearing silkworm larvae on mulberry leaves enriched with white hen's eggs induced significant increase in the weight of full-grown larva, silk gland, female pupa as well as cocooning percentage. Bovine milk occupied the second rank in this respect, whereas control larvae gave the least values. Feeding mulberry silkworms on leaves treated with white hen's eggs caused the highest positive significant effects on weights of fresh cocoon (1.100 gm for male and 1.456 gm for female) and shell (0.248 gm for male and 0.290 gm for female) as well as silk filament length (1069.67 m) weight (0.203 gm) and size (1.706 dn). Regarding the biochemical traits white hen's eggs resulted in the highest total protein content in the haemolymph of the 5th larval instar, recording (3.8 g /dI) while bovine milk caused the highest total lipid haemolyph (265 mg /dI).

Keywords: *Bombyx mori* L**,** bovine milk, white hen's eggs, biological, biochemical and technological characters

1. Introduction
The mulberry silkworm, *Bombyx mori* L. is a monophagous insect reared solely on mulberry leaves (*Morus* spp.), that plays a major role in the progress of sericulture industry and in turn cocoon and silk production Nagaraju [1] and Sevidav *et al*. [2]. Therefore, much effort has been paid to improve both quality and quantity of mulberry leaves for rearing silkworm larvae. Like other organisms, nutrition plays an effective role in growth and development of the silkworm, *Bombyx mori* L. In addition, one of the possible cost effective methods is to enrich mulberry leaves with food additives. Many researches used nutrients as a food supplement to gain increases in both silk yield and moth fecundity Sengupta *et al*. [3]. From this stand point, the present work was carried out to evaluate the effects of bovine milk and white hen's eggs on some biological, biochemical and technological characters of *B. mori* . Milk is a complex liquid that simultaneously provides bioactive compounds in the form of protein, carbohydrates, fatty acids, minerals and other nutrients that facilitate growth and development. Many bioactive proteins and peptides derived from milk are potential modulators of various regulatory processes. Hughes *et al*. [4] stated that bovine milk is tranformity growth factor that enhances development and immune response. Also, Konala *et al*. [5] mentioned that bovine milk has a positive effect on body and cocoon weights of *B. mori* larvae. In addition, egg white is a good source of riboflavin and selenium. It rich in amino acids and essential vitamins which are required for optimal growth of an insect Szalay [6]. The present work aimed to study the effect of bovine milk and white hen's eggs on some biological characters *viz.*, weights of full-grown larvae, silk gland, pupa, moth, fecundity and hatchability, as well as, biochemical and technological characters to improve the current state of Sericulture is of immense importance.

2. Material and Methods
The present investigation was conducted to the laboratory of Sericulture, Plant Protection Department, Faculty of Agriculture, Zagazig University during spring season of 2016 under the durnal hygrothermic conditions of 23-28 °C and 60-70% R.H. However, the technological and biochemical studies were performed at sericulture Department, Plant Protection Research Institute, Sharkia Branch.

2.1 Experimental silkworm
The eggs of silkworm (Chinese hybrid 9E7X) imported annually by the Sericulture Division, Plant Protection Research, Institute, Ministry of Agriculture and Land Reclamination, Dokki, Giza were used in the present study.

2.2 Rearing method
The eggs of the mulberry silkworm (Chinese hybrid) imported in small boxes covered with muslin cloth, were kept in a cooling incubator running at 3 °C till the beginning of March. Therefore, the eggs were taken out and placed in rearing plastic trays (42 X 30 X 10cm) under laboratory conditions. Hatching lasted approximately for 10 days. Mulberry leaves (Balady variety) were harvested twice daily i.e. at 8 a.m. and 4. p.m.

2.3 Experimental design
Silkworm rearing was carried out as usuall till the beginning of the 5th larval instar that used in the experiment. Mulberry leaves were dipped in each bovine or white hen's eggs for 30 seconds, and then left to dry under laboratory conditions till needed. Larvae of each treatment were fed on treated mulberry leaves on the odd days (days 1,3,5,7 and 9) of fifth instar. On the other- hand, larvae were fed on untreated mulberry leaves on the even days (days 2,4,6,8 and 10) (Konala *et al*. [5]. Control larvae were offered fresh untreated mulberry leaves. Each treatment was replicated 3 times, and each replicate contained 30 larvae.
Treated mulberry leaves were offered to larvae 4 times daily. The larval bed was cleaned by changing net. For mounting process, carton papers (that used for warpping table eggs) were placed along the trays for providing larvae with suitable place for spinning their cocoons.

2.4 Biological parameters
Fresh weights of each of larva, silk gland, pupa and moth were weighed for each treatment. Ratios of cocooning, pupation and emergence were determined Saad [7].

2.5 Technological parameters
For measuring technological traits, weights of fresh cocoon and cocoon shell were weighed, then cocoons were dried at oven at 60 °C for 8 hours. Dry cocoons were reeled by reeling machine, the length of reeled silk filament was measured and weighed for every cocoon. The size of the reeled filament was estimated according to Tanaka's [8] formula:

$$\text{Size (dn)} = \frac{\text{weight of silk filament (g)}}{\text{Length of filament (m)}} \times 9000$$

2.6 Biochemistry parameters
To evaluate total protein and total lipid, larvae rinsing in sterile water, then bled by cutting a proleg and collecting haemolymph into a 1-5 ml appendorf tab on ice Kevin [9].

2.7 Statistical analysis
Results were statistically analysis according to complete randomized design. The apporiate methods were used for the analysis of data according to Little and Hills [10]. The proper (F) value was calculated to described by Fisher [11].

3. Results and Discussion
Data of the effect of bovine milk and white hen's eggs used to enrich mulberry leaves on biological, technological and biochemical characters of imported Chinese hybrid 9F7X of silkworm are summarized in Tables 1 and 2.

3.1 Biological measurements
3.1.1 Weight o larvae
Data given in Table 1 cleared that the mean weight of full-grown larva was significantly affected by the food additives. The highest weight of 5th instar larva (2.590 gm) was observed with larvae fed on leaves treated with white hen's eggs, while the lowest one (2.311 gm) was recorded with check treatment. For bovine milk treatment, the mean weight of 5th instar larva recorded in an intermediate value (2.471 gm). The same trend was also reported by Konala *et al*. [5] who stated that bovine milk has a positive effect on body weight of *B. mori* larva. Also, Kanafi [12] found that the vitamins showed a significant effects on *B. mori* growth and development.

Table 1: Effect of food additives on some biological characters of mulberry silkworm, *Bombyx mori* L. during 2016 season.

Food additives \ Biological characters		Weight of full-grown larva (gm)	Weight of silk gland (gm)	Weight of pupa (gm)		Weight of moth (gm)		% cocooning	% pupation	Fecundity	Hatchability
				♂	♀	♂	♀				
Bovine milk		2.47 ab	0.997 a	0.790	0.998 b	0.418	0.610	88.00 a	98.38	366.00	98.06
White hen's eggs		2.50 a	0.897 b	0.842	1.148 a	0.452	0.637	90.28 a	90.43	416.67	97.48
Control		2.31 b	0.920 a	0.752	0.962 b	0.376	0.603	72.29 b	73.89	342.33	97.56
L.S.D.	0.05	0.090 *	0.099 *	N.S.	0.104 **	N.S.	N.S.	6.518 **	N.S.	N.S.	N.S.
	0.01	0.125	0.189		0.151			10.810			

- NS indicates that the differences between treatments are not significant.
-*, ** indicates that the differences between treatments are significant and highly significant at 0.05 and 0.01 level of probability.
- Means followed by similar letters are not significantly different at 0.05 level of probability.

3.1.2 Weight of silk gland
Treating mulberry leaves with bovine milk increased the mean weight of silk gland showing (0.997 gm), while feeding larvae with mulberry leaves inriched with white hen's eggs resulted in the lowest value (0.897 gm). Analysis of data revealed significant differences, in this parameter, between the tested treatments. In this respect, Ito [13] mentioned that the major protein component of milk is casein, which was shown to be beneficial for *B. mori* development. In addition, Saad *et al*. [14] stated that the highest weights of silk gland were noticed by treating mulberry leaves by other treatments.

3.1.3 Weight of pupa
The mean weight of pupa recorded 0.790, 0.842 and 0.752 gm for male pupa ; 0.998, 1.148 and 0.962 gm for female one of bovine milk, white hen's eggs and control treatments,

respectively Table 1. It is obvious that the tested nutritional additives (bovine milk and white hen's eggs) caused positive effect on this parameter. However, the differences between means were insignificant for male pupa and highly significant for female one. In this respect, Radjabi [15] mentioned that the heaviest male or female pupa resulted in feeding larvae on leaves treated with amino acids. Also, Gad [16] stated that the highest mean weight of pupa was observed with treating leaves by other food additives.

3.1.4 Weight of moth

Data compiled in Table 1 revealed that larvae fed on mulberry leaves enriched with white hen's eggs developed into male and female moths heavier than that of control, recording 0.452 and 0.637 gm, respectively compared to 0.376 and 0.603 gm for larvae fed on untreated leaves. Bovine milk treatment showed moderate values, recording 0.418 and 0.610 gm for male and female moth, successively. The differences between means were insignificant for males and females moths. The same trend was also reported by Karuppasamy *et al*. [17] who stated that there was a positive effect on body weight of *B. mori* moth by using other food additives.

3.1.5 Cocooning percentage

Data in Table 1 cleared that the mean percentage of cocooning recorded 88.00, 90.28 and 72.29% for bovine milk, white hen's eggs and control treatments, respectively. Analysis of data revealed that the differences between means were highly significant. Obtained results are in parallel with those of Xu *et al*. [18] who using other food additives.

3.1.6 Pupation percentage

As shown in Table 1 the highest mean percentage of pupation (90.43%) was noticed when larvae were fed on leaves supplemented with white hen's eggs, whereas, the lowest percentage (73.89%) was recorded for control treatment. The differences between means were insignificant. Similar trend was also reported by Saad *et al*. [14]

3.1.7 Fecundity and hatchability

Data in Table 1 revealed that the mean number of deposited eggs / female recorded 366.00, 416.67 and 342.33 eggs/ female arised from larvae fed on leaves enriched with bovine milk, white hen's eggs and control, respectively, with insignificant differences between means. For hatchability percentage the highest mean (98.06%) was noticed by larvae fed on leaves treated by bovine milk, whereas, the lowest one (97.48%) was recorded by control treatment. The differences between means were insignificant. These results were in agreement with those of Sengupta *et al*. [3] and Gad [16] who used nutrients as food supplementary to achieve an increase in fecundity of moth. In conclusion, offering mulberry leaves to 5th instar larvae of *B. mori* L. enriched with bovine milk or white hen's eggs improved some biological characters of *Bombyx mori* L. It may be due to the high nutrients (protein and lipid) content that enhance the growth and development of the growing individuals. In connection, Panizzi and Parra [19] and Vanderzant [20] stated that casein contains fatty acids, cholesterol, sugars, vitamins and minerals. In addition, casein stimulate the feeding efficiency of *B. mori* L. Ito [3]. Also, eggs are all natural and provide one of the highest quality proteins of any food available.

3.2 Technological parameters

3.2.1 Weight of fresh cocoon

Data presented in Table 2 cleared that the highest mean weight of male and female cocoon (1.100 and 1.456 gm) was attained when larvae were fed on mulberry leaves treated with white hen's eggs, respectively. Meanwhile, the least values (0.986 and 1.196gm) were observed with control treatment. Analysis of variance revealed significant differences between means. Similar trend was also reported by Bentea and Sara [21] and Konala *et al*. [5] taken in consideration the varied type of additive.

3.2.2 Weight of shell cocoon

As shown in Table 2 data revealed that the mean weight of male and female cocoon shell was 0.238, 0.248 and 0.206 ; 0.232, 0.290 and 0.226 gm for larvae fed on mulberry leaves enriched with bovine milk, white hen's eggs and control, respectively. The differences between means are highly significant with female shell only. In this respect, the same conclusion was also reached by Gad [16] and Saad *et al*. [14] using varied food additives.

Table 2: Effect of food additives on some technological and biochemical characters of mulberry silkworm, *Bombyx mori* L. during 2016 season.

Food additives		Technological characters							Biochemical characters	
		Weight of fresh cocoons (gm)		Weight of shell (gm)		Silk filament				
						Length (gm)	Weight (gm)	Size (dn)	Total protein (g/dl)	Total lipid (mg/dl)
		♂	♀	♂	♀					
Bovine milk		1.056 ab	1.230 b	0.238	0.232 b	456.67 b	0.177 b	1.661	3.7	265
White hen's eggs		1.100 a	1.456 a	0.248	0.290 a	1069.67 a	0.203 a	1.706	3.8	228
Control		0.986 b	1.196 b	0.206	0.226 b	729.23 c	0.123 c	1.575	3.2	203
L.S.D.	0.05	0.081	0.092	N.S.	0.046	221.527	0.036	N.S.		
		*	*		*	*	**			
	0.01	0.117	0.134		0.067	367.403	0.059			

- NS indicates that the differences between treatments are not significant.
-*, ** indicates that the differences between treatments are significant and highly significant at 0.05 and 0.01 level of probability.
- Means followed by similar letters are not significantly different at 0.05 level of probability.

3.2.3 Silk filament length

Data compiled in Table 2 cleared that enriching mulberry leaves with white hen's eggs resulted in the longest reelable silk filament (1069.67 m), compared to 729.23 m recorded for control cocoons. Moreover, the differences between means are significant. Similar trend was also reported by Bentea and Sara [21].

3.2.4 Silk filament weight

As shown in Table 2 the mean weight of reeled silk filament reached 0.177, 0.203 and 0.123 for cocoons spun by *B. mori* larvae fed on mulberry leaves enriched with bovine milk,

white hen's eggs and control, respectively. Analysis of data clear that white hen's eggs caused the highest significant mean weight of silk filament. Bovine milk also caused significant increase in this parameter over the control.

3.2.5 Silk filament size

Data presented in Table 2 cleared that the mean size of reeled silk filament recorded 1.661, 1.706 and 1.575 deneir for the cocoons spun by silkworm larvae fed on mulberry leaves treated with bovine milk, white hen's eggs and the control ones, respectively. The differences between the means are insignificant. These results were in agreement with those of Helaly [22] who used nutrients as food supplementary to achieve an increase in silk filament size.

3.3 Biochemical parameters

As shown in Table 2 data revealed that the total protein content in the haemolymph of the 5th instar larvae recorded 3.7, 3.8 and 3.2 g/dl for larvae fed on mulberry leaves treated with bovine milk, white hen's eggs and control, respectively. For total lipid content in *B. mori* haemolymph it recorded 265, 228 and 203 mg/dl for the aforementioned additives, successively. It is obvious that the two additives caused marked increase in the total protein and lipid contents of the haemolymph of the treated larvae over the control. These increases in total protein or total lipid can be due to that the milk is a suitable liquid that provides body with proteins, carbohydrates, fatty acids and minerals which useful for healthy growth and development . In addition, egg whites are good source of riboflavin and selenium. In this respect, the same conclusion was also reached by Battacharya and Kaliwal [23] using potassium chloride as food additive.

4. Conclusion

The present study has evaluated the effect of bovine milk and egg white on biological, biochemical changes in the haemolymph and technological parameters of silkworm, *Bombyx mori* during its final instar development. Results revealed that, larvae fed on mulberry leaves enriched with white hen's eggs induced significant increase in weights of fresh cocoon and cocoon shell, as well as silk filament length, weight and size. Regarding the biochemical traits white hen's eggs resulted in the highest total protein content in the haemolymph of the 5th larval instar, while bovine milk caused the highest total lipid haemolymph. These results suggest that *B. mori* larvae can be fed with bovine milk and white hen's eggs treated mulberry leaves for increased growth rate and silk production.

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