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Comparative Study on Effects of some High Protein Content Nutritional Additives on some Hybrids of Mulberry Silkworm, *Bombyx mori* L.

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ABSTRACT

The present study is conducted to investigate the supplementary effect of soy flour and spirulina as protein sources added to mulberry leaves with different concentrations (2.5, 5 & 10% w/v) on the biological, economical and physiological characters of three hybrids of silkworm, *Bombyx mori*; two imported hybrids (Bulgarian and Chinese) and the local Egyptian one (was used as a control or reference value). The schedule of application was applied since the beginning of the fifth larval instar till end up to spinning (one diet/day). Most of the studied treatments concentrations revealed a significant enhancement in the silkworm characters under investigation; a significant increase was exhibited in larval and pupal weight, cocooning percentage and the effective rate of rearing of the imported hybrids compared with the local one. Especially, larvae reared on the concentration 5% of soy flour then spirulina with the concentration 2.5%. Furthermore, the same concentrations of protein sources increased significantly the cocoon and silk filament characters. Moreover, the total protein content and protease activity of larval haemolymph exhibited a significant increase owing to rearing on mulberry leaves fortified with the same concentrations of protein additives respectively. Conclusively, soy flour as a high protein content nutritional additive with the tested concentrations is superior and better than spirulina. As well, silkworm larvae of the imported hybrids (Bulgarian and Chinese) are manifested more susceptible positively to protein nutritional additives than local hybrid. So, it could be prescribed to the breeding programs to achieve high quantity and quality of cocoon yield for silk production.

Keywords: *Bombyx mori*, hybrids, spirulina, soy flour, protein.

INTRODUCTION

The silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) is an important beneficial economic insect owing to its ability to convert mulberry leaf protein to silk. Silk production has a direct relationship with larval growth. Economically, nutritional study of silkworm is an essential factor for obtaining a high silk crop, because it is the only factor that controls the efficiency and quantity of silk production. Hence, the enrichment of mulberry leaves with supplementary nutrients such as proteins, carbohydrates, amino acids, vitamins, sterols, hormones, antibiotics etc. affects primarily on the improvement of larval growth and cocoon production (Laskar and Datta 2000; Sannappa *et al.*, 2002; Jeyapaul *et al.*, 2003; Sheeba *et al.*, 2006 & Thangapandiyam and Dharanipriya, 2019).

Quantitative and qualitative supplementation of protein plays a critical role in influencing the growth and development of *Bombyx mori*. Spirulina is a natural source rich in protein and essential amino acids derived from algae and commonly used by humans as a healthy diet. It contains 18 amino acids such as glycine, glutamine, histidine, lysine, methionine, cysteine, creatine, phenylalanine, serine, proline, tryptophan, asparagine, pyruvic acid and vital vitamins like tocopherol, thiamine, riboflavin, biotin, niacin, folic acid, pyrodozoic acid, vitamin B12 and beta- carotene etc. (Venkataramana *et al.*, 2003; Kumar *et al.*, 2009 and Venkatesh *et al.*, 2009). By the same way, soy milk contains appreciable levels of

vitamins (Vit. A, Vit. B and Vit.D) in a range of 10 to 45 per cent, with calcium and magnesium also in significant content (Shruti *et al.*, 2019). Furthermore, soy protein is a rich source of dietary protein and daily supplementation known to enhance larval growth and to elevate the economic characters of *Bombyx mori* by increasing the quality and quantity of the silk cocoon production (Ito, 1980 and Kamaraj *et al.*, 2017). Almost 70% of the silk filament protein is found to be derived from the mulberry leave source which is rich in protein content. As well, protease enzyme present in the mid gut of the silkworm, *Bombyx mori* L. is the most important enzyme responsible for the conversion of mulberry protein to silk protein and its activity varies from breed to breed and between hybrids (Narayanan *et al.*, 1967 & Kumar and Kalpana, 2009).

Therefore, the present experiment aims to evaluate spirulina and soy flour as a protein sources added to mulberry leaves as nutritional additives with different concentrations and study their effects on larval growth, larval physiology and cocoon commercial characters of imported and local silkworm hybrids.

MATERIALS AND METHODS

Silkworm *Bombyx mori* L.

Mulberry silkworm *Bombyx mori* L. eggs of three hybrids; two imported hybrids (Bulgarian and Chinese) and the local Egyptian hybrid (was used as a control or

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reference value) were procured from the Sericulture Research Department, Plant Protection Research Institute (PPRI), Agricultural Research Center, Giza, Egypt.

Protein sources

Spirulina (100% natural blue green algae powder) produced by Imtenan Health Shop. Soy flour produced by Food Technology Research Institute, Agricultural Research Center.

Preparation for rearing

Before commencement of rearing, the rearing room, boxes and tools were thoroughly washed and treated with a disinfective solution (formalin 3%) and left to dry before using.

Silkworm rearing technique

After egg hatching, larvae were reared in the laboratory at $25 \pm 2^\circ \text{C}$ and $75 \pm 5\%$ relative humidity according to the technique of Krishnaswami (1978). The newly hatched larvae were fed on fresh clean mulberry leaves until the 5th instar, which is the stage that was used in the present bioassays.

Schedule of application

After the fourth molting, the fifth larval instar were divided into three groups represented the three hybrids under investigation; each group divided into two subgroup were feed with mulberry leaves supplemented with natural high protein content additives. Three different concentrations (2.5%, 5% & 10% w/v) were prepared by dissolving protein additives in distilled water and fresh mulberry leaves were dipped in each concentration, allowed to stand as such for few minutes for water evaporation and fed to experimental larvae as the first feed from day one of the fifth larval instar till end up to spinning. Three replicates (100 larva/replicate) were used for each concentration. The Biological, economical characters and silk production related parameters were measured.

Biological aspects

Some biological aspects of the larvae reared on mulberry leaves fortified with different concentrations of the high content protein additives under investigation were studied as; grown larval body weight, Cocooning percentage and effective rate of rearing (ERR %) which calculated by the following formula (Joshi,1985):

$$\text{ERR \%} = \frac{\text{No. of cocoons harvested} \times 100}{\text{No. of larvae retained}}$$

Economic aspects

After spinning of cocoons by all the groups of silkworm, some economic traits such as cocoon parameters like cocoon weight, shell weight, shell ratio, filament weight, filament length and denier respectively were measured.

Cocoon indices

It represented fresh cocoon weight, cocoon shell weight and cocoon shell ratio was calculated according to Tanaka's formula (1964):

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

Silk Filament characters

It represented silk filament length, silk filament weight and silk filament size was calculated according to the following equation (Tanaka's, 1964):

$$\text{Silk filament size (dn.)} = \frac{\text{Weight of silk filament (g)} \times 9000}{\text{Length of silk filament (m)}}$$

Cocoons used for determining the filament characters were dried at 80°C in oven before used.

Physiological aspects

Larval haemolymph samples were collected at the 7th day of the 5th larval instar by removing one of the thoracic legs of the larvae and bending the body to expose the sternum at the position of the removed leg. This ensured proper drainage of the haemolymph avoided any risk of internal organs to be destructed. The haemolymph of each treatment was collected in 1.5 ml Eppendorf tubes with small crystal of phenyl thiourea (PTU) to prevent melanization of sample and to be prepared for physiological analysis according to Mahmoud (1988) protocol.

Total protein content

The protein content of the haemolymph samples were determined using folin phenol reagent according to the method of Lowry *et al.*(1951) as follows: The haemolymph samples were added to 1 ml of 5% trichloroacetic acid, and the precipitated protein was dissolved by boiling for 5 min in 2 ml of 1 N NaOH solution. Then 0.2 ml from this alkaline protein solution was placed in clean test tubes, each contained 1 ml of buffer copper sulphate solution. After 10 minutes, 0.2 ml from folin reagent was added to the mixture and the contents were heated for 2.5 minutes at 50°C (to dissolve the precipitate). The tubes were allowed to stand for 10 minutes to cool at room temperature. The blanks were similarly run using 0.2 ml NaOH instead of the haemolymph sample. Reading was measured spectrophotometrically at 750 nm.

Protease activity

The proteolytic enzyme activity was determined by the casein digestion method described by Ishaaya *et al.* (1971). The reaction mixture consisted of 0.2 ml (0.2 M) glycine buffer (pH 10), 0.4 ml 1.5% casein solution, and 0.2 ml haemolymph sample. Enzymatic activity was terminated after 60 min. incubation at 37°C and adding 1.2 ml of 5% trichloroacetic acid solution. The reaction mixture was centrifuged at 6000 rpm for 15 min. and the supernatant was taken for enzymatic activity evaluation. The proteolytic activity was determined at 280 nm as O.D. unit $\times 10^3$.

Statistical Analysis

Statistical analysis was conducted using Proc ANOVA in SAS (SAS Institute, 1988). Obtained data was subjected to one way test to find the differences among different treatments, P values < 0.05 were observed as statistically significant. Means separation was conducted using Duncan Multiple Range Test (Winer, 1971) using the same program.

RESULTS AND DISCUSSION

Biological aspects

The mean values of biological characters of Bulgarian, Chinese and local Egyptian hybrids of *Bombyx mori* reared on mulberry leaves supplemented with different concentrations of natural high protein content additives showed that, there are a significant differences

between the treatments, as well as the concentrations, as illustrated in Table (1).

Grown larval body weight (g)

As seen in Table (1), a high significant increase was noticed in the imported hybrids (Bulgarian and Chinese) grown larval weights reared on different concentrations of spirulina and soy flour additives (2.19g & 2.09g) respectively, compared with the local hybrid larval weights (1.76g). Results cleared also, larvae of the imported hybrids which reared on soy flour diet recorded significantly heavier weights (2.32g & 2.19g) respectively, than others reared on spirulina (2.07g & 1.98g) respectively. Especially, those reared on mulberry leaves supplemented with 5% soy flour (2.94g & 2.74g) respectively, followed by those which reared on 2.5% spirulina recorded (2.60g & 2.40g) respectively, compared with larvae of the local hybrid recorded the lightest weights with soy flour (1.95g) and spirulina diets (1.93g) with the same concentrations.

Cocooning %

Data represented in Table (1) revealed a high significant increase in the mean values of cocooning% of the imported hybrids of silkworm; Bulgarian and Chinese hybrids recorded (89.89% & 88.78%) respectively, than those of Egyptian hybrid (80.50%). By the same sequence of hybrids, larvae reared on mulberry leaves fortified with soy flour showed a superior effect significantly (91.33%, 90.11% & 82%) respectively, than those feed with spirulina (88.44%, 87.44% & 79%) respectively. Rearing larvae with 5% soy flour exhibited significantly higher cocooning % especially Bulgarian and Chinese hybrids (98% & 97.33%) respectively, than Egyptian one (88%), and also 2.5% spirulina recorded high cocooning % in the imported hybrids compared with the local one (96%, 96% & 86%) respectively. Followed by the concentration 2.5% of soy flour (95%, 93% & 85%) respectively, then the concentration 5% of spirulina recorded (92.33%, 90% & 83%) respectively.

Pupal weight (g)

According to the results of Table (1), the Bulgarian and Chinese hybrids of *Bombyx mori* revealed significantly heavier pupal weights (0.91g & 0.88g) respectively, compared with the local hybrid (0.76g). Rearing on mulberry leaves supplemented with 5% soy flour resulted significantly increased pupal weights of the investigated hybrids (1.14g, 1.09g & 0.90g) respectively, than other concentrations. Also, the concentration 2.5% spirulina recorded high pupal weights (1.04g, 1.01g & 0.88g) respectively. While no significant difference was noticed among the means of diets.

Effective rate of rearing (ERR %)

As shown in Table (1), a significant increase was noticed in the effective rate of rearing of silkworm larvae of the Bulgarian and Chinese hybrids (94.98% & 94.48%) respectively, while the Egyptian one recorded 91.84%.

Larvae of the imported hybrids reared on 5% soy flour revealed a significant increase in ERR% (97.3% & 97.13%) respectively, compared with the local hybrid recorded 95.65%. As well, 2.5% spirulina increased ERR% for the same hybrids (97% & 96.9%) respectively, while the Egyptian one reared on 2.5% spirulina revealed ERR% (95.35 %). Followed by the concentration 2.5% of

soy flour (96.64%, 96.44% & 94.79%) respectively, then the concentration 5% of spirulina recorded (96.14%, 95.92% & 93.85%) respectively. While, no significant difference was noticed among the means of diets.

Table 1. Influence of spirulina and soy flour additives with different concentrations on some biological aspects of the fifth larval instar of some hybrids of silkworm, *Bombyx mori* L.

Hybrid	Diet	Conc.	Larval weight (g)	Cocooning %	Pupal weight (g)	ERR%
Bulgarian	Soy flour	2.5%	2.25 ^{cd}	95 ^{ab}	0.96 ^{de}	96.64 ^{ab}
		5%	2.94 ^a	98 ^a	1.14 ^a	97.3 ^a
		10%	1.77 ^{ghi}	81 ^{fgh}	0.71 ⁱ	92.37 ^{cde}
	Mean		2.32 ^a	91.33 ^a	0.94 ^a	95.44 ^a
	Spirulina	2.5%	2.60 ^b	96 ^{ab}	1.04 ^{bc}	97 ^a
		5%	2.00 ^{ef}	92.33 ^{abc}	0.91 ^{efg}	96.14 ^{ab}
		10%	1.60 ^{ij}	77 ^{ghi}	0.67 ^{ij}	90.41 ^{ef}
	Mean		2.07 ^{ab}	88.44 ^{ab}	0.88 ^{ab}	94.52 ^{ab}
	Mean		2.19 ^a	89.89 ^a	0.91 ^a	94.98 ^a
	Chinese	Soy flour	2.5%	2.10 ^{de}	93 ^{abc}	0.93 ^{ef}
5%			2.74 ^b	97.33 ^a	1.09 ^{ab}	97.13 ^a
10%			1.74 ^{hi}	80 ^{fgh}	0.71 ⁱ	91.55 ^{def}
Mean			2.19 ^a	90.11 ^{ab}	0.91 ^a	95.04 ^{ab}
Spirulina		2.5%	2.40 ^c	96 ^{ab}	1.01 ^{cd}	96.9 ^a
		5%	1.98 ^{ef}	90 ^{bcd}	0.90 ^{efg}	95.92 ^{ab}
		10%	1.55 ^{jk}	76.33 ^{hi}	0.64 ^j	88.92 ^{fg}
Mean			1.98 ^{ab}	87.44 ^{ab}	0.85 ^{ab}	93.91 ^{ab}
Mean			2.09 ^a	88.78 ^a	0.88 ^a	94.48 ^a
Egyptian		Soy flour	2.5%	1.90 ^{fgh}	85 ^{def}	0.85 ^{gh}
	5%		1.95 ^{efg}	88 ^{cde}	0.90 ^{efg}	95.65 ^{ab}
	10%		1.55 ^{jk}	73 ^{ij}	0.61 ^j	86.75 ^{gh}
	Mean		1.80 ^b	82 ^{bc}	0.79 ^{ab}	92.40 ^{ab}
	Spirulina	2.5%	1.93 ^{efg}	86 ^{def}	0.88 ^{fg}	95.35 ^{abc}
		5%	1.85 ^{fgh}	83 ^{efg}	0.79 ^h	93.85 ^{bcd}
		10%	1.37 ^k	68 ^j	0.54 ^k	84.67 ^h
	Mean		1.72 ^b	79 ^c	0.74 ^b	91.29 ^b
	Mean		1.76 ^b	80.50 ^b	0.76 ^b	91.84 ^b
	LSD _{0.05} (hybrids)			0.265**	5.752**	0.109*
LSD _{0.05} (diets)			0.374*	8.273*	n.s.	n.s.
LSD _{0.05} (concentrations)			0.183***	6.294***	0.066***	3.018***

The significant increase in the biological aspects studied may be due to the increased protein synthesis and subsequent accumulation of storage protein in the body on account of additional oral proteins, leads to better growth, development and gain in larval weight, pupal weight, cocooning % and ERR%. This increase in the biological aspects differed between soy flour and spirulina treatments may be owing to the effect of increased concentrations of spirulina resulted in poor feeding by silkworm larvae. The present results are in concurrence with the work of Ito (1980) stated that rich sources of dietary proteins like soy protein are known to promote larval growth. Results were supported also by Krishnan *et al.* (1995) and Ganga and Gowri, (1990), observed that, soy protein and flour diets supplementation increased the larval weight. Sundar Raj *et al.* (2000a and 2001) have noticed the maximum pupal weight in silkworms hybrid PM×NB4D2 fed on mulberry leaves supplemented with soya bean flour. By the same way, the results of the present study correlate with Venkataramana *et al.* (2003), reported that mulberry leaf treated with spirulina and fed to silkworm found to be augment the larval growth and cocoon characters. Also,

Govindan and Narayanaswamy (1988) stated that growth promoting effect of water soluble proteins and vitamins are found in spirulina enhance the larval growth. The increased ERR% observed in the present study when supplemented with high protein content additives, may be due to lower incidence of diseases in the treated silkworms because of the influence of feed additives containing protein, amino acids, carbohydrates and minerals which act as building blocks of tissues making them more healthy as documented by Rekha and Neelu (2010). Similar trend was reported by Mathavan *et al.* (1984), Etebari and Fazilati, (2003), Ravi *et al.*, (2010) & yadav and Bagdi,(2015).

Economic aspects (Cocoon indices)

The mean values of cocoon indices of Bulgarian, Chinese and local Egyptian hybrids of *Bombyx mori* reared on mulberry leaves supplemented with different concentrations of natural high protein content additives showed that, there are significant differences between the treatments, as well as the concentrations. As recorded in Table (2).

Fresh cocoon weight (g)

Obtained data in Table (2), revealed that the Bulgarian and Chinese hybrids recorded significantly increased weights of cocoons (1.12 & 1.08g) respectively, than the local hybrid (0.90g). Larvae of the imported hybrids reared on mulberry leaves supplemented with 5% soy flour exhibited significantly the highest cocoon weights (1.46g, 1.40g) respectively, compared with the Egyptian one (1.07g), followed by the concentration 2.5% of spirulina recorded (1.35g & 1.29g) respectively. While, the local hybrid recorded 1.05g. Meanwhile, no significant difference was noticed among the means of diets.

Cocoon shell weight (g)

As illustrated in Table (2), the highest recorded weights of cocoon shell were obtained by the Bulgarian and Chinese hybrids of silkworm (0.19, 0.17g) respectively, higher significantly than shell weights of Egyptian hybrid (0.11g). Statistical analysis revealed that, there was a significant increase in produced cocoon shell weights resulting in rearing larvae of the imported hybrids on soy flour diets (0.20g & 0.18g) respectively, than Egyptian one (0.11g). Followed by spirulina diets (0.17g & 0.16g) respectively, compared with the local one recorded 0.10g. Furthermore, highly significant increases were noticed among the diets concentrations; 5% of soy flour caused a significant increase in produced cocoon shell weights of the investigated hybrids recording (0.28g & 0.27g) respectively, and 2.5% of spirulina (0.26g & 0.24g) respectively, except the local one recorded (0.14g & 0.15g) respectively with the two concentrations.

Silk content ratio

Results obtained in Table (2) cleared that a high significant difference was noticed among silk content % means of silkworm hybrids under investigation; the Bulgarian and Chinese hybrids and the local one exhibited (15.88%, 15.04% & 11.83%) respectively. As well, statistical analysis revealed that a significant difference among means of silk content % of protein diets offered to the above silkworm hybrids; soy flour diet recorded (16.24%, 15.64% & 11.85%) respectively, and spirulina diet exhibited (15.52%, 14.43% & 11.80%) respectively. Also, highly significant increases were noticed among the

diets concentrations; 5% of soy flour caused a significant increase in silk content % of the imported hybrids (19.15%, 19.50%) respectively, compared with the Egyptian one (13.06%) and 2.5% of spirulina recorded (19.46%, 18.82% & 13.84%) respectively for the studied hybrids. Followed by the concentration 2.5% of soy flour (17.21%, 16.45% & 12.64%) respectively, then the concentration 5% of spirulina recorded (15%, 14.90% & 12.45%) respectively.

Table 2. Influence of spirulina and soy flour additives with different concentrations on cocoon aspects of some hybrids of silkworm, *Bombyx mori* L.

Hybrid	Diet	Conc.	Cocoon weight (g)	Cocoon shell weight (g)	Silk content ratio
Bulgarian	Soy Flour	2.5%	1.20 ^{de}	0.21 ^{bc}	17.21 ^{abc}
		5%	1.46 ^a	0.28 ^a	19.15 ^a
		10%	0.83 ^{ij}	0.10 ^{hij}	12.36 ^{efgh}
	Mean	1.16 ^a	0.20 ^a	16.24 ^a	
	Spirulina	2.5%	1.35 ^{bc}	0.26 ^a	19.46 ^a
5%		1.11 ^{ef}	0.17 ^{de}	15.00 ^{cd}	
10%		0.79 ^{jk}	0.09 ^{ij}	12.10 ^{fghi}	
Mean	1.08 ^{abc}	0.17 ^{ab}	15.52 ^a		
Mean		1.12 ^a	0.19 ^a	15.88 ^a	
Chinese	Soy Flour	2.5%	1.15 ^{ef}	0.19 ^{cd}	16.45 ^{bc}
		5%	1.40 ^{ab}	0.27 ^a	19.5 ^a
		10%	0.82 ^j	0.08 ^{ijk}	10.98 ^{ghij}
	Mean	1.12 ^{ab}	0.18 ^a	15.64 ^a	
	Spirulina	2.5%	1.29 ^{cd}	0.24 ^{ab}	18.82 ^{ab}
5%		1.09 ^{fg}	0.16 ^{def}	14.90 ^{cde}	
10%		0.73 ^{jk}	0.07 ^{jk}	9.56 ^{ij}	
Mean	1.04 ^{abc}	0.16 ^{abc}	14.43 ^{ab}		
Mean		1.08 ^a	0.17 ^a	15.04 ^a	
Egyptian	Soy Flour	2.5%	1.00 ^{gh}	0.13 ^{fghi}	12.64 ^{defg}
		5%	1.07 ^{fg}	0.14 ^{efgh}	13.06 ^{defg}
		10%	0.70 ^{kl}	0.07 ^{jk}	9.85 ^{hij}
	Mean	0.92 ^{bc}	0.11 ^{bc}	11.85 ^b	
	Spirulina	2.5%	1.05 ^{fg}	0.15 ^{efg}	13.84 ^{def}
5%		0.93 ^{hi}	0.12 ^{ghi}	12.45 ^{defg}	
10%		0.62 ^l	0.06 ^k	9.09 ^j	
Mean	0.87 ^c	0.10 ^c	11.80 ^b		
Mean		0.90 ^b	0.11 ^b	11.83 ^b	
LSD _{0.05} (hybrids)			0.157*	0.045**	2.196**
LSD _{0.05} (diets)			n.s.	0.065*	3.179*
LSD _{0.05} (concentrations)			0.105***	0.040***	2.597***

All the parameters governing yield and quality of cocoons were influenced significantly, and this significant increase was might be due to increased proteinaceous source in the diet leads to increase in metabolism resulted in the production of a higher and better quality cocoon. As well, the absorption of high content protein nutrients varies among silkworm hybrids under study might be due to differing in their genetic constitution.

These results are in conformity with the finding of Anil Kumar and Prashanth (2018), stated that silkworms reared on enriched mulberry leaves with soyabean flour at different concentrations (4 and 6% concentrations) expressed a significant improvement on economic characters may be due to effective absorption and utilization of soya protein for the formation of cocoons. And those of Rathinam *et al.* (1994) and Krishnan *et al.* (1995) have reported that, rearing of silkworm with hydrolyzed soya protein (2-2.5% concentrations) enhances

of shell weights. As observed by Nirwani and Kaliwal, (1996), the results ascribed to the proteineous soya feed supplement ingredients involved in the synthesis of silk proteins and nucleic acids such as RNA and DNA in the silk gland cells there by improves the silk content in the cocoon shell. Similar results obtained by Ravi *et al.*, (2010) and yadav and Bagdi, (2015) observed that, the growth-promoting effect of water-soluble proteins and vitamins are found in Spirulina treated on silkworm leads to enhance the larval and

Silk filament characters

The mean values of silk filament characters of Bulgarian, Chinese and local Egyptian hybrids of *Bombyx mori* reared on mulberry leaves supplemented with different concentrations of natural high protein content additives showed that, there are significant differences between the treatments, as well as the concentrations as recorded in Table (3).

Silk filament length (m)

The obtained results in Table (3) revealed to a significant increase in filament lengths of silkworm hybrids with different concentrations of treatments; Bulgarian hybrid silkworm larvae reared on protein diets exhibited superiority in filament lengths (1070 m) followed by the Chinese hybrid recorded (1051 m) while the lowest filament length was recorded by the local hybrid (983 m). Rearing larvae of the imported hybrids on mulberry leaves fortified with 5% of soy flour recorded the highest filament lengths (1170 m & 1150 m) respectively, while, the local one recorded (1074 m) with the same concentration. Followed by those reared on 2.5% of spirulina exhibited (1132 m, 1122 m & 1043 m) respectively. Meanwhile, no significant difference was noticed among the means of diets.

Silk filament weight (g)

As shown in Table (3), statistical analysis revealed that, the protein diets increased significantly the silk filament weights of the imported hybrids than the local one recorded (0.256g, 0.251g & 0.229g), respectively. As well, highly significant increases were noticed among the protein diets concentrations. Especially 5% of soy flour increased significantly the filament weights of the Bulgarian and Chinese hybrid recorded (0.288g & 0.280g) respectively, while the Egyptian one recorded 0.254g with the same concentration. Followed by those reared on the concentration 2.5% of spirulina exhibited significantly (0.275g, 0.271g & 0.250g) respectively. Meanwhile, no significant difference was noticed among the means of diets.

Silk filament size (dn)

Data represented in Table (3), cleared that silk filament size of the Bulgarian and Chinese hybrid larvae increased significantly compared with the Egyptian one (2.148dn, 2.144dn & 2.085dn) respectively. Statistical analysis revealed that a highly significant increase was noticed among diets concentrations; larvae of the imported hybrids reared on mulberry leaves supplemented with 5% of soy flour exhibited the highest filament sizes (2.215dn & 2.191dn) respectively, compared with filament sizes of the local one (2.128dn). As well, the concentration 2.5% of spirulina revealed high filament sizes with the above hybrids (2.186dn, 2.174dn & 2.157dn) respectively. Followed by the concentration 2.5% of soy flour (2.157dn, 2.159dn & 2.157dn) respectively, then the concentration 5% of spirulina recorded (2.158dn, 2.144dn & 2.172dn) respectively. While no significant difference was noticed among the means of the protein diets.

Table 3. Influence of spirulina and soy flour additives with different concentrations on silk filament characters of some hybrids of silkworm, *Bombyx mori* L.

Hybrid	Diet	Conc.	Silk filament length (m)	Silk filament weight (g)	Silk filament size (dn)
Bulgarian	Soy Flour	2.5%	1114 ^d	0.267 ^{cde}	2.157 ^{abc}
		5%	1170 ^a	0.288 ^a	2.215 ^a
		10%	990 ^j	0.230 ⁱ	2.091 ^d
	Mean		1091 ^a	0.262 ^a	2.154 ^a
	Spirulina	2.5%	1132 ^c	0.275 ^{bc}	2.186 ^{ab}
5%		1084 ^f	0.260 ^{ef}	2.158 ^{abc}	
10%		930 ^l	0.215 ^{jk}	2.081 ^d	
Mean		1049 ^{abc}	0.250 ^{abc}	2.142 ^{ab}	
Mean		1070 ^a	0.256 ^a	2.148 ^a	
Chinese	Soy Flour	2.5%	1095 ^e	0.263 ^{def}	2.159 ^{abc}
		5%	1150 ^b	0.280 ^{ab}	2.191 ^{ab}
		10%	962 ^k	0.224 ^{ij}	2.096 ^{cd}
	Mean		1069 ^{ab}	0.256 ^{ab}	2.149 ^{ab}
	Spirulina	2.5%	1122 ^d	0.271 ^{bcd}	2.174 ^{ab}
5%		1076 ^{fg}	0.256 ^{fg}	2.144 ^{bcd}	
10%		900 ^m	0.210 ^k	2.100 ^{cd}	
Mean		1033 ^{abc}	0.246 ^{abc}	2.139 ^{ab}	
Mean		1051 ^a	0.251 ^a	2.144 ^a	
Egyptian	Soy Flour	2.5%	1035 ^h	0.248 ^{gh}	2.157 ^{abc}
		5%	1074 ^g	0.254 ^{fgh}	2.128 ^{bcd}
		10%	875 ⁿ	0.190 ^l	1.954 ^e
	Mean		995 ^{bc}	0.231 ^{bc}	2.080 ^b
	Spirulina	2.5%	1043 ^h	0.250 ^{gh}	2.157 ^{abc}
5%		1015 ⁱ	0.245 ^h	2.172 ^{ab}	
10%		857 ^o	0.185 ^l	1.943 ^e	
Mean		972 ^c	0.227 ^c	2.091 ^{ab}	
Mean		983 ^b	0.229 ^b	2.085 ^b	
LSD _{0.05} (hybrids)			59.338*	0.019*	0.051*
LSD _{0.05} (diets)			n.s.	n.s.	n.s.
LSD _{0.05} (concentrations)			9.726***	0.009***	0.065***

The significant influence of silk filament characters in the present study may be attributed to the larval enzymatic apparatus used to convert the nutrients to body matter and more specifically to silk proteins. These results are on par with the findings of Rahamathulla *et al.* (2004) and Sundar Raj *et al.* (2000 & 2001) have opined that supplementation of mulberry leaf with soy bean and ragi flour enhanced filament length and exhibit finer denier against control. As well, the results of the present study correlate with Govindan and Narayanaswamy (1988) noticed that supplementation of mulberry leaf with spirulina enhanced silk filament characters. Also, Venkatesh *et al.*, (2009) reported that 300ppm concentration of aqueous solution of spirulina feed to silkworm found to effectively and increases cocoon weight, cocoon shell weight, pupal weight and silk filament length. Similar trend was reported by Kumar *et al.* (2018) and Chairman *et al.* (2011).

Physiological aspects

Proteins constitute represent the most important aspect in silk production; The mean values of the total protein and protease activity of Bulgarian, Chinese and local Egyptian hybrids of *Bombyx mori* reared on mulberry leaves supplemented with different concentrations of natural high protein content additives in this study showed that, there are significant differences between the treatments, as well as the concentrations. As recorded in Table (4).

Total protein content (ug protein /min/ml)

Obtained data in Table (4), revealed that the Bulgarian and Chinese hybrids recorded a high significant increase in total protein values (447.64 & 440.30ug protein /min/ml) respectively, than the local hybrid (401.46ug protein /min/ml). By the same way, larvae of the imported hybrids reared on mulberry leaves supplemented with the concentration 5% of soy flour exhibited the highest total protein values (491.32 & 489.51ug protein /min/ml) respectively. While, the local one recorded (433.35 ug protein /min/ml) with the same concentration. Furthermore, rearing of the same hybrids on 2.5% of spirulina recorded (487.12, 484.49 & 429.17ug protein /min/ml).

Protease enzyme activity (O.D.units x 103/min./ml)

According to the results obtained in Table (4), a highly significant increase was noticed in protease enzyme activity of silkworm larvae of the Bulgarian and Chinese hybrids recorded (738.13 & 721.27O.D.units x103 / min / ml) respectively, while the Egyptian one recorded (615.00O.D. units x 103/min./ml). Larvae of the same hybrids reared on soy flour additive exhibited high significant increase recorded (753.68, 741.52 O.D.units x 103/min./ml) respectively, compared with the local one recorded (622.48O.D.units x 103/min./ml). As well, those reared on spirulina revealed protease activity (722.58, 701.02 & 607.51O.D. units x 103/min./ml) respectively. All protein additives increased the enzyme activity significantly, especially those reared on 5% of soy flour exhibited (884.25, 862.58 & 680.36O.D.units x 103/min./ml) respectively, and 2.5% of spirulina revealed (845.59, 792.42 & 680.09O.D.units x 103/min./ml) respectively.

Table 4. Influence of spirulina and soy flour additives with different concentrations on some physiological characters of the fifth larval instar of some hybrids of silkworm, *Bombyx mori* L.

Hybrid	Diet	Conc.	Total protein content (ug protein/min/ml)	Protease activity (O.D.unitsx103/min./ml)
Bulgarian	Soy Flour	2.5%	474.68 ^c	745.03 ^e
		5%	491.32 ^a	884.25 ^a
		10%	392.29 ^h	631.75 ^{jk}
	Mean		452.76 ^a	753.68 ^a
	Spirulina	2.5%	487.12 ^{ab}	845.59 ^c
		5%	466.89 ^d	710.96 ^g
10%		373.55 ^j	611.20 ^l	
Mean		442.52 ^{ab}	722.58 ^a	
Mean		447.64 ^a	738.13 ^a	
Chinese	Soy Flour	2.5%	472.38 ^{cd}	734.15 ⁱ
		5%	489.51 ^{ab}	862.58 ^b
		10%	384.34 ⁱ	627.82 ^k
	Mean		448.74 ^{ab}	741.52 ^a
	Spirulina	2.5%	484.49 ^b	792.42 ^d
		5%	438.45 ^e	708.61 ^g
10%		372.64 ^j	602.04 ^m	
Mean		431.86 ^{abc}	701.02 ^{ab}	
Mean		440.30 ^a	721.27 ^a	
Egyptian	Soy Flour	2.5%	422.92 ^g	658.58 ⁱ
		5%	433.35 ^{ef}	680.36 ^h
		10%	364.13 ^k	528.50 ⁿ
	Mean		406.8 ^{bc}	622.48 ^{bc}
	Spirulina	2.5%	429.17 ^f	680.09 ^h
		5%	416.72 ^g	638.00 ^j
10%		342.47 ^l	504.45 ^o	
Mean		396.12 ^c	607.51 ^c	
Mean		401.46 ^b	615.00 ^b	
LSD _{0.05} (hybrids)			29.821**	60.781***
LSD _{0.05} (diets)			n.s.	87.384**
LSD _{0.05} (concentrations)			6.223***	6.488***

The current study exhibited a significant increase in total protein content and protease enzyme activity of some silkworm hybrids owing to food supplementation with different concentrations of soy flour and spirulina, which may be explained by increase in structural and dynamic levels of organization in the body of larvae reared on high content protein diets, as documented by Venkataramana *et al.* (2003). Moreover, he stated that, the markedly elevated protein was indicating the building up of positive nitrogen balance that may be the characteristic feature of growth phase. Also, the present results are in agreement with those of Krishnan *et al.* (1995) reported that, when silkworm were fed with hydrolyzed soya protein (2-2.5%) enhances the haemolymph proteins. These results are on line also with the earlier observations of Anil Kumar (2009), who has opined that increase in protease activity with advancement of age. Similar trend was reported by Horie and Watanabe (1983), Rathinam *et al.* (1994) and Nagata and Kobayashi (1990).

Consequently, it can be concluded that, the supplementation of high protein content additives as soy flour and spirulina fortified mulberry leaves to the silkworm larvae of the imported hybrids under study, had beneficial effects on the larval growth, and exhibited good quality and quantity of the cocoon yield and silk production owing to the improvement in protein synthesis and protease enzyme activity.

Moreover, the present study cleared the superiority of soy flour nutritional additive more than spirulina, especially with the middle concentration (5%) owing to better palatability to silkworm larvae. While Spirulina can be used with the lesser concentration (2.5%). So, these supplementations could be prescribed to the silk rearers to achieve more quantity of silk yield.

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دراسة مقارنة لتأثير بعض الإضافات الغذائية عالية المحتوى البروتيني على بعض هجن دودة الحرير التوتية
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أجريت هذه الدراسة لتقييم ومقارنة نوعين من الإضافات الغذائية عالية المحتوى البروتيني: دقيق الصويا و سبيروولينا من حيث تأثيرهما على بعض الصفات البيولوجية لليرقات و صفات الشرنقة وكذلك صفات الخيط الحريري. كما تم قياس المحتوى الكلي للبروتين ومستوى نشاط أنزيم البروتياز ليرقات الحرير التوتية. وقد تم استخدام ثلاث تركيزات مذابة في محلول مائي لكل منهما (2.5، 5، 10%)، وذلك لتغذية يرقات العمر الخامس للهجن الثلاث بهذه التركيزات بمعدل وجبة واحدة يوميا طوال العمر الخامس. وقد سجلت الصفات البيولوجية قيد الدراسة زيادة معنوية في وزن اليرقات في العمر الخامس ووزن العذراء ونسبة التشرنق وكذلك معدل التربية الفعال. وذلك بالنسبة ليرقات الهجين البلغاري والصيني عندما تغذت على تركيزين 5% من دقيق الصويا ثم 2.5% من السبيروولينا مقارنة بالهجين المحلي. وعند دراسة صفات الشرنقة والخيط الحريري لوحظت زيادة معنوية في هذه الصفات مع نفس التركيزات السابقة للهجن المستوردة مقارنة بالهجين المصري. كذلك سجلت الدراسة زيادة معنوية في المحتوى الكلي للبروتين ونشاط انزيم البروتياز عند قياسه في دم يرقات الهجين البلغاري والصيني مقارنة بالهجين المحلي عندما تغذت على ورق التوت المدعم بدقيق الصويا ثم السبيروولينا بنفس التركيزات السابقة. التوصية: نستخلص من النتائج السابقة لهذه الدراسة افضلية دقيق الصويا كإضافة غذائية عالية البروتين وخاصة باستخدام التركيز السابق ذكره وتفوقه في إيجابية التأثير عن السبيروولينا. كذلك أتضح أيضا من الدراسة أن الهجينين البلغاري والصيني كانا أكثر تأثير إيجابي بالإضافات الغذائية حيث أدت لزيادة الصفات المختلفة ليرقات الحرير مقارنة بالهجين المحلي، لذا يوصى باستخدامهما في التربية للحصول على إنتاج عالي الجودة من الشرائق وبالتالي إنتاجية الحرير.