Effect of Egg white on Growth and Cocoon Parameters of the Mulberry Silk worm *Bombyx mori* L.

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Abstract: Effect of egg white on the growth and cocoon parameters of the silkworm was analysed. The larvae of the mulberry silkworm *Bombyx mori* were reared with normal mulberry leaves till the completion of fouth instar in the laboratory maintained with the room temperature of 28ºC and 80% humidity. From the beginning of the fifth instar, the experimental larvae were fed with mulberry leaves smeared with egg white in different concentrations.The data revealed that the albumen showed only insignificant (11.8%) increase in growth. But the albumen exhibited a significant increase of some cocoon parameters such as cocoon weight (40.79%), pupal weight (41.71%) and filament length (32.92%). This indicated that the albumen significantly increased the quality and quantity of the silk. Since cocoon parameters were favourably increased by the albumen, egg white can be suggested as a fortification agent after studying the mechanism in detail.

Index Terms: Albumen, Cocoon parameters, Fortification

Introduction

Silk is the most elegant textile in the world with unparalleled grandeur, natural sheen inherent affinity for dyes, high absorbance, light weight, soft touch and high durability. Because of these unique features silk is known as the "Queen of Textiles". It is produced by the larvae of an insect called silkworm (Nasreen *et al.*, 1999). Silkworm, *B. mori* is an oligophagous insect that feeds mainly on the leaves of the mulberry plant. The successful harvest of quality cocoons depends exclusively on the nutrition of the silkworm (Nagesh and Devaiah, 1996). Nutrition of silkworm is the sole factor which almost individually augments the quality and quantity of cocoon production (Laskar and Datta, 2000). Successful cocoon production by mulberry silkworm largely depends on nutritional quality and quantity of mulberry leaves provided to the developing larvae. It has been proved beyond doubt that *B. mori* requires certain carbohydrates, proteins and minerals for normal growth, survival and for increased silk production. The improvement of silk production by enriching silkworm nutrition through fortification of the mulberry leaves yielded promising results (Kumaraj *et al*., 1972; Alagumalai *et al*., 1999). In recent years, many attempts have been made to fortify mulberry leaves with supplementary agents such as antibiotics, juvenoids, botanicals, feed additive flours, etc., so as to improve the growth of silkworms and in-turn the quality and quantity of silk. Proteins form the main constituents of the silk fibre. The proteins have been reported to improve the growth development immunity and thus increase the silk yield (Nasreen *et al.*, 1999). Among the various dietary protein supplements tested, casein, glutan and soya flour are the most attractive sources in terms of cost and quality and have been extensively studied and generally recommended for fortification (Raj *et al.,* 2000). Horie and Watanabe (1983) and Chapman (1982) reported that dietary protein was effective in increasing the economic characters only up to an optimal level.

A raw hen's egg contains around 33 grams of egg white with 3.6 grams of protein, 0.24 grams of carbohydrate and 55 milligrams of sodium. It contains no cholesterol and the energy content is about 17 Calories. Egg white is an alkaline solution containing around 148 proteins (James *et al*., 1998). Egg- white has been reported to increase the rate of growth and development of egg in many organisms. The present study was therefore, undertaken to determine the effect of albumen of hen's egg on growth, development, cocoon traits of the mulberry silkworm, *Bombyx mori L.* The aim of the present work was to increase the quantity and quality the silk produced by the mulberry silkworm *B. mori* by fortification of mulberry leaves with hen's egg white.

Materials and Methods

The silkworm rearing room and appliances were thoroughly washed, cleaned and properly disinfected with 2 per cent formalin at the rate of 800 ml per 10 m^2 area, using a sprayer. The commercial breed of the mulberry silkworm i.e., the double hybrid CSR6 × CSR26 × CSR2 × CSR27 was used for the study. Disease free layings (DFLs) were kept on wax paper in wooden trays and wet foam rubber strip was placed around the trays and covered with another paraffin paper. The eggs were covered with black paper for 48 hours, at pinhead stage followed by exposure to diffused day light during morning to obtain uniform and maximum hatching.

For rearing silkworms, shelf/tray method as suggested by Krishnasamy *et al* (1970) was followed. On the day of hatching at 10 AM, fresh tender leaves of MR2 mulberry were chopped into 0.5 cm^2 bits and spread over the newly hatched larvae on the egg cards. After 20 minutes they were brushed on to paraffin paper in the wooden tray. The relative humidity and temperature were maintained at 80% and 28ºC. This procedure was continued up to third instar and mass rearing was done till the end of third instar by self/tray method. The silkworms were reared by feeding three times a day (9.00 AM, 1.00 PM and 5.00PM) with chopped tender leaves to chawki worms and whole leaves to grown up worms. Bed cleaning was done once, twice and thrice a day during

I, II and III instars respectively, whereas once in a day during IVand V instars. After III instar, hundred worms were separated and maintained in selected silkworms trays for different treatments and each treatment was replicated thrice.

2.1 preparation of aqueous solution of egg white

Fresh hen's eggs were collected and brought to the laboratory, washed thoroughly in clean tap water. Manually the white of the egg was separated and dissolved in water. Three different concentrations were prepared. i.e., 5 ml/100ml, 10 ml/100ml and 15ml/100ml, 2g of sucrose was added in all these to increase the palatability.

2.2 Method of supplementation

The mulberry leaves were smeared with the different concentrations of albumen, shade dried and fed to V instar silkworms daily in the morning and in the evening. The control larvae were fed with normal leaves till cocoon spinning. Another set of control larvae were fed with the mulberry leaves smeared with 2% sucrose solution. The matured worms were handpicked and mounted on bamboo mountage and cocoons were harvested manually on 5th day of mounting.

2.3 Recording of silkworm parameters

The parameters such as larval weight, cocoon weight, shell weight, shell ratio, pupal weight, silk filament length, silk filament weight and denier were recorded in all control and experimental sets.

2.4 Stastistical analysis

The data collected were subjected to the stastistical tools such as 't'test and single way ANOVA. Then the data were compared with control set and interpreted.

Result and Discussion

3.1 Growth of the V instar larva: The initial weight of a single fifth instar larva was ranging between 0.56 to 0.60 g (Table-1). In all the cases, the weight was found to be increasing from day to day. On the last day of the fifth instar the weight of the larva ranged between 1.52 g in control and 1.69g in the albumen (15ml/L- A3) fed larvae. Moreover zero mortality was recorded in all sets. These showed that the supplemented albumen was not rejected by the larvae. The larval period lasted for eight days in all the cases. This indicated that albumen had no effect on the metamorphosis. The weight of the larvae at the end of the fifth instar revealed that albumen increased the weight of the larvae. The weight of the larvae in all albumen supplemented larvae was greater than that of the control larvae fed with normal leaves and sucrose supplemented larvae. However the statistical analysis showed that the increase was not significant.

3.2 Cocoon parameters

After the cocoons were completely formed, the economic parameters were analyzed in control and the experimental groups. The results on the effect of albumen on the cocoon parameters have been shown in the table 2 and 3. It was interesting to note that all economic parameters of the cocoon were highest in albumen supplemented larvae (15ml/100ml) and lowest in control larvae. Comparatively all economic parameters were higher in the experimental sets than the control sets. Cocoon weight, Pupal weight, Filament length, filament weight were significantly higher than the control. Insignificant increase was observed in the case of shell weight and Denier was found to be lesser than that of control.

In the experimental sets, the fifth instar larvae were fed with mulberry leaves smeared with different concentrations of Albumen such as 5ml/100ml, 10ml/100ml and 15ml/100ml. No mortality was recorded in the whole larval period. This shows that the higher compatibility of the supplemented egg white. The larvae of the control sets and experimental sets were found to be increasing in weight as the day passed on. When compared with the control larvae, increase in weight of the larvae was observed in all experimental sets but the increase was found to be only insignificant. When compared with the sucrose supplemented larvae, increase in weight of the larvae was observed in all experimental sets but the increase was found to be only insignificant. Increase in the growth of the larvae might be attributed to the accelerated activity of the enzyme protease as reported by Nirmala*et al* (2001) who showed that protease activity increased in the gut of *B. mori* larvae reared on soy protein. Similar results were obtained by supplementation of different nutrients including proteins (Horie and Watanabe, 1983; Sarkar *et al.*, 1992).The silkworm larvae reared on mulberry leaves supplemented with 15ml/100ml concentrations at two feeding/day were having higher mature larval weight (1.61g) as compared with the control (1.52 g) and sucrose control (1.57 g). The increased mature larval weight might be due to protein content, which supports better digestability. The quality of food consumption has direct relevance on weight of larva. Supplementation with albumen perhaps increased the haemolymph protein and amino acid content in the silkworm larvae resulting in better silkworm growth as observed by Horie and Watanabe (1983). The studies are in line with Narayanaswamy and Ananthanarayana (2006) who studied role of feed supplement 'Serifeed' on nutritional parameters and cocoon yield in silkworm and found that the mean body weight of 5th instar larvae was maximum (1.61g) as compared to control (1.52 g). Further, Manimegalai*et al* (2002) reported that higher larval weights of 1.61 g and 1.52 g were recorded in NB4D2 and PM x NB4D2, respectively on supplementation of soy flour at 10 g/kg of mulberry shoot.

The silkworms fed on mulberry leaves supplemented with albumen exhibited better cocoon weight as compared to control. Among the three treatments, maximum cocoon weight was found in larvae fed with mulberry leaves supplemented with 15ml/100ml concentration (1.058g) and minimum in control (0.762 g). The increased cocoon weight was attributed to daily feeding of supplemented leaf during the fifth instar. Feeding silkworms with leaf supplemented with a proteinaceous source can enhance the cocoon weight has been reckoned in earlier works of Rathinam*et al* (1994), Krishnan *et al* (1995) and Vanishree*et al* (1996). Pallavi and Muthuswami (2012) showed that the cocoon weight was maximum when the larval feeding was supplemented with soya flour, amway protein etc compared to control.

The shell weight was significantly influenced by application of different concentrations of albumen. Among the three treatments, mulberry leaves supplemented with 15ml/100ml per cent albumen concentration recorded maximum shell weight (0.199 g) and minimum in control (0.145 g). Shell weight was maximum at 15ml/100ml concentration as compared to control (Fig. 2). This was attributed to high protein content besides carbohydrates, minerals and fat provided by the feed additives. The shell as such contains the silk proteins namely fibroin and sericin which are in turn made of polypeptide chain of amino acids, particularly serine, alanine and glycine. These amino acids might have been assimilated by the worms in the course of supplemented feeding with proteinacious source in the form of albumen supplement can be exploited to enhance shell content and shell of cocoon contains the reelable filament, which is valued by reelers. The present findings are in line with Rekha (2004) who reported that maximum shell weight was recorded with flours of soybean and horsegram (0.43 g and 0.42 g) provided at ratio 1 :10 (flour :

leaf), and minimum was in control (0.145g). Application of aqueous solution of albumen at 15ml/100ml concentration had maximum shell ratio (0.199 %) followed by 10ml/100ml concentration (0.182%). The minimum shell ratio was found in control (0.145 %). The shell ratio was maximum in 15ml/100ml concentration as compared to control (Fig. 3). This may be due to the presence of protein, essential amino acids, minerals and vitamins in the egg white (Lumpkin and Plucknette, 1982). These results are in agreement with that of Sengupta*et al*., (1972) and Babu (1994) who attributed the higher shell ratio to glycine supplementation. Similarly, Rathinam*et al*., (1994) also established the influence of soy protein in maximizing shell content. Murthy *et al*., (1986) indicated the efficiency of 'Seritone', a tonic with beneficial fortificants in increasing the shell content.

Higher pupal weight was found in both the factors fourth instar to spinning and fifth instar to spinning as compared to control in mulberry leaves supplemented with 15ml/100ml concentration (Fig. 2). Among the six treatments treated the maximum pupal weight was found in 10ml/100ml and 15ml/100ml concentration (0.658 g) and (0.795 g) as compared to water control (0.561 g). These findings are of great significance from the view point of rearing silkworms for production of seed cocoons as fecundity of silk moth is dependent on pupal weight. This necessitates the application of feed additives to silkworm at an optimum level. The present findings are in agreement with the finding of Sridhar and Radha (1986) and Babu (1994), who also reported higher pupal weight in silkworms reared on glycine fortified leaves. Further, Jeyapaul and Padmanatha (2003) observed improved pupal weight (0.630, 0.658, 0.795 g) on leaves supplemented extracts of with *Alternanthera sessilis*, *Eichhornia crassipes* and *Coffea arabica* over the control (0.561 g).

The longest silk filament length was observed in both the instars, fourth instar to spinning and fifth instar to spinning worms in mulberry leaves supplemented with 15ml/100ml concentration. The mulberry leaves supplemented with 15ml/100ml concentration recorded maximum silk filament length (m) followed by 10ml/100ml concentration (518.4 m) and the lowest length was found in absolute control (465.3 m) (Fig. 3). Enhanced filament length is attributed to the application of feed supplement albumen which is rich in proteins, essential amino acids, minerals like Calcium, Potassium, Phosphorus, Fe, Cu, Mg etc. and vitamins and growth promoter intermediaries. The present findings are in confirmation with Manimegalai*et al*. (2002) who studied the effect of fortification of mulberry leaves with soyflour on larval and cocoon characters and found higher filament length (398 m) at 10g/ kg of leaves over the control (868m). Similar findings were observed in the study of Rekha and Neelu (2010) stated that mulberry silkworm hybrids PM x CSR 2 fed on mulberry leaves supplemented with nine flours namely ragi, rice, wheat, sorghum, soyabean, horsegram, greengram, cowpea, bengalgram at (1:10), (1:20) (flour:leaf) showed significantly higher filament length (390,415.4,439,465, 518.4m) respectively over the control (390m).

Maximum silk filament weight was found in mulberry leaves supplemented with 15ml/100ml concentration. The highest silk filament weight was found in leaves supplemented with 15ml/100ml and 10ml/100ml (0.166g and 0.152 g). The minimum filament weight was in control (0.131 g). Higher silk filament weight was increased due to protein supplement through the mulberry at an optimum level which resulted in higher silk filament length in turn resulted in higher silk filament weight. The same trend was found with Rajkumar and Vitthalrao (2013) when supplemented with *Aloe vera* L. herbal formulation at 2.0 per cent concentration.

The maximum denier was recorded in fourth instar to spinning worms (3.704) and minimum was in fifth instar to spinning (2.881). Among the treatments, mulberry leaves treated with 15ml/100ml concentration was having higher denier (2.993) and least was recorded in absolute control (3.023). The significant increase in the denier may be due to fineness of the silk filament. As silk filament length and silk filament weight increased the denier also increased over the control. The similar findings are found in case of Chakrabarty and Kaliwal (2012) who experimented applicaton of Arginine, Histidine and their mixtures to the silkworms and found improved denier at a rate of 150µg/ml over the control.

Thus it is concluded that egg-white supplementation did not accelerate the growth of the larvae whereas it increased the quantity and quality of the silk. Therefore egg white can be recommended as a fortification agent to the sericulture farmers after analyzing the molecular mechanism of the role of egg white in improving the silk yield.

Acknowledgements

We wish to express sincere thanks to the Principal and Head and other staff of the PG and Research Department of Zoology of Government Arts College (Autonomous), Karur, Tamilnadu, for providing the laboratory facilities to carry out this research work.

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Table 1- Effect of Albumin on the Growth of the Mulberry Silkworm *Bombyx mori L.*

S. No	**Larval period days**	**LARVAL WEIGHT(g)**				
		Control	**Sucrose**	**A1 (5ml+100ml water)**	**A2(10ml+100ml water)**	**A3 (15ml+ (100ml water)**
1	Day1	0.56	0.56 [0.0%]	0.58 [3.57%]	0.58 [3.57%]	0.60 [7.14%]
2	Day2	0.67 (18.34%)	0.69 (23.21%) [2.98%]	0.70 (20.68%) [4.47%]	0.71 (22.41%) [5.97%]	0.74 (23.33%) [10.44%]
3	Day3	0.83 (25.03%)	0.82 (18.84%) [-1.20%]	0.82 (17.14%) [-1.20%]	0.88 (23.94%) [6.02%]	0.89 (20.27%) [7.22%]
4	Day4	1.03 (23.83%)	0.95 (15.85%) [-7.76%]	0.90 (9.75%) [-12.62%]	1.01 (14.77%) [-1.94%]	0.98 (10.11%) [-4.85%]
5	Day5	1.26 (21.27%)	1.35 (42.42%) [7.14%]	1.33 (47.77%) [5.55%]	1.31 (29.70%) [3.96%]	1.29 (31.63%) [2.38%]
6	Day6	1.41 (11.90%)	1.46 (8.27%) [3.54%]	1.48 (11.27%) [4.96%]	1.41 (7.63%) [0.0%]	1.36 (5.42%) [3.54%]
7	Day7	1.51 (7.09%)	1.55 (5.80%) [2.64%]	1.57 (6.08%) [3.97%]	1.58 (12.05%) [4.63%]	1.57 (15.44%) [9.06%]
8	Day8	1.52 (0.66%)	1.57 (1.29%) [3.28%]	1.62 (3.18%) [6.57%]	1.67 (5.69%) [9.86%]	1.69 (7.64%) [11.18%]
t value	--	--	-0.09*	-0.65*	-0.09*	-0.15*
'F' value			0.48691*			

Note: Values inside the square brackets indicate the percentage of change over the control.

Values inside the parentheses indicate the percentage of change in larval weight over the previous day.

*Significance at the level of $p < 0.05$

Table 2- Effect of Albumin on the Cocoon Parameters of the Mulberry Silkworm *Bombyx mori L.*

S. No	Control and Experimental sets	Cocoon weight(g)	Shell weight(mg)	Pupa weight (mg)
1	Control	0.706	0.145	0.561
2	Sucrose	0.790 [11.89%] t=-2.6*	0.153 [5.51%] t=-0.47**	0.615 [9.62%] t=-3.75*
3	A1	0.768 [8.78%] t=-12.43*	0.161 [11.03%] t=-1.56**	0.630 [12.29%] t=-5.0*
4	A2	0.840 [18.98%] t=-14.96*	0.182 [25.51%] t=-4.44*	0.658 [17.29%] t=-9.41*
5	A3	0.994 [40.79%] t=-31.25*	0.199 [37.24%] t=-1.82**	0.795 [41.71%] t=-26.68*

Note: Values inside the square brackets indicate the percentage of change over the control.

*Significance at the level of $p < 0.05$, **Not significant at the level of $p < 0.05$.

Table 3- Effect the Albumin on the Filament length of the Mulberry Silkworm, *Bombyxmori L.*

S. No.	Control/ Experimental sets	Filament length(m)	Filament weight(g)	Denier
1.	Control	390	0.131	3.023
2.	Sucrose	415.4 [6.51%]	0.171 [30.53%]	3.704 [22.52%]
3.	A1	439 [12.56%]	0.146 [11.45%]	2.993 [-0.99%]
4.	A2	465.3 [19.30%]	0.152 [16.03%]	2.940 [-2.74%]
5.	A2	518.4 [32.92%]	0.166 [26.71%]	2.881 [-4.69%]

Note: Values inside the square brackets indicate the percentage of change over the control.

Plate 1. Control and Experimental setup–Rearing of the fifth instar larvae of the mulberry silkworm

Plate 2. Cocoons produced by control and experimental larvae.

Plate 3. Silk thread spun from the cocoons of Control and Experimental silkworm.

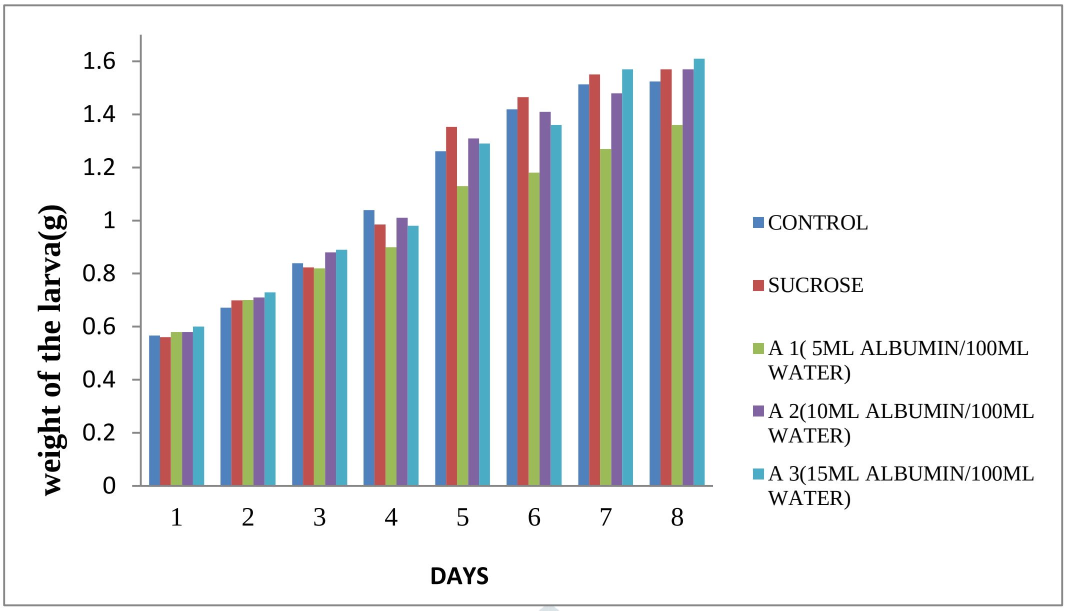

Figure.1- Effect of the Albumin on the Growth of the Mulberry Silk worm *Bombyx mori* L.

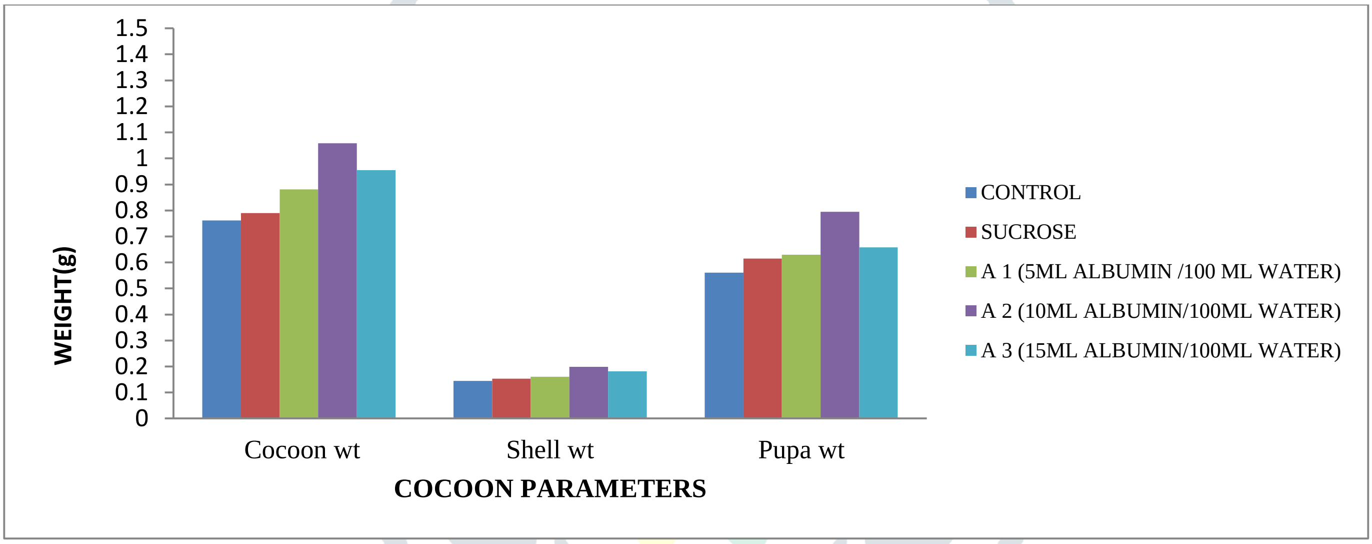

Figure.2- Effect of Albumin on the Cocoon parameters of the Mulberry silkworm *Bombyx mori L.*

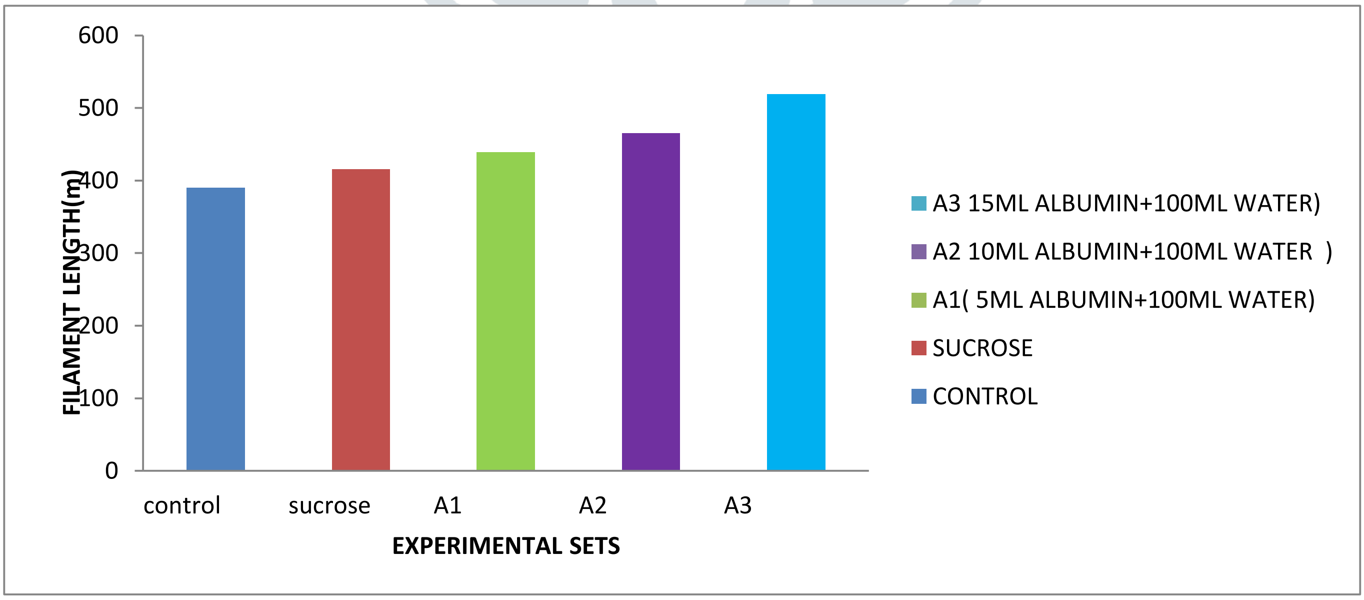

Figure.3-Effect of Albumin on the Filament Length of the silk of the Mulberry Silkworm *Bombyx mori L.*