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RESEARCH ARTICLE

IMPACT OF SPIRULINA AND ASHWAGANDHA ON BIOCHEMICAL CONTENTS AND THE ECONOMIC TRAITS OF SILKWORM, *BOMBYX MORI* L.

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ABSTRACT

The purpose of this study investigates the impact of spirulina and ashwagandha on the biochemical and economic characteristic of *B.mori*. Nutrition of silkworm is sole factor which almost augment quantity and quality of slide fortification of mulberry leaves by nutrient supplementation can be increased the quality and productivity of silk. Spirulina is blue green algae. It contains 18 amino acids and vitamins. The nutrients are very easy to digests protein and carbohydrates. *W.somnifera* commonly known as ashwagandha, are used in traditional medicine. The main chemical constituents are alkaloids and steroidal lactones. These include trophine and cuscohygrine. In this experiment consists of 2 treatment (spirulina and ashwagandha) with 5 concentrations (1,2,3,4 and 5 per cent). Hence in this investigation an attempt is made to study the impact of fortification of treated leaves on biochemical content (protein, carbohydrate and lipid) of silk gland, haemolymph, muscle and fat body of *B.mori* larvae.During insect metamorphosis, profound biochemical changes occur in the haemolymph, in particular, the concentration of proteins. The silkworm produces massive amount of silk protein during final stage of larval development. Further, same study was conducted to find out the economic traits of *B.mori.* The major real silk fibre is fibroin and the adhesive substance is sericin. The result indicates there is a better silk production.

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INTRODUCTION

Sericulture is the rearing of silkworms for the production of raw silk although there are several commercial species of silkworm. *Bombyx mori* L. is the most widely used and intensively studies. The silkworm *B.mori* is a phytophagous insect and a typical monophagous feeder on mulberry leaves. Various extracts of medicinal plants have been tested by supplementation in the silkworm, *B.mori* and were seen to influence the body weight, silk gland weight and silk length in *B.mori.* The silkworm, *B.mori* produces massive amount of silk proteins during final stage of larval development. There are two kinds of silk proteins have been distinguished as major components of silk cocoons, the first being real silk fibre namely fibroin and the second being adhesive substance namely sericin. Fortification of the mulberry leaves by nutrient supplementation can be increased the quality and productivity of silk (Sengupta *et al.,* 1972). Mulberry leaf supplemented with spirulina as a food to *B.mori* orally found to be effective in enhancing the larval and cocoon characters (Venkataramana 2003). Mulberry leaf supplemented Spirulina as a feed to *B. mori* orally found to be effective in enhancing the larval and cocoon characters (Sangamithirai *et al.,* 2014). *Zingiber officinale, Lantana camera* and *Acorus calamus* fortified mulberry leaves increased the silk quality of silkworm (Manjunatha *et al.,* 2017). The supplementation of *Vigna unguiculata* (Cow pea)an aqueous extract with mulberry leaves at different concentration enhanced the quality and quantity of silk in *B. mori* (Saravanan, 2011). The impact of supplementation of amway protein on the economic characters and energy budget of silkworm, *B.mori* while amway is the best supplementary protein for the production of good qualified cocoon and silk (Rani *et al*., 2011). In all insects including silkworms, *B. mori* require certain essential biochemical components like sugars, amino acids, proteins, fatty acids, sterols, vitamins etc. for their steady growth and survival. These organic constituents are also necessary for the growth of their silk glands as well as in the production of eggs and silk Ito (1978). The studies carried out by these workers clearly indicate that biochemical profile of the different

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developmental stages in silkworms gave the idea about the requirements of sugar, proteins and lipids for their proper growth potentials. The food consumption has a direct relevance on the weight of larvae, cocoon, pupae and shell, the independent parameters of consumption and productivity vary depending upon the type of nutrition (Shivakumar, 1995) and silkworm breeds (Ramadevi *et al.,* 1992). The present study is an attempt to evaluate the impact of spirulina and ashwagandha extract on the biochemical and economic parameters of silkworm*, Bombyx mori.*

MATERIALS AND METHODS

The present investigation was carried on the hybrid (L x CSR_2) of *B.mori.* The eggs were procured from district sericulture office Konam, Nagercoil. Rearing of silkworm larvae under standard environmental conditions (28^oC and 75% H) followed by the method of krishnaswami (1986). After hatching, the worms were fed with MR_2 variety of mulberry leaves. The present study has been aimed to investigate various biochemical contents and economic traits of the silkworm, when fed on mulberry leaves fortified with spirulina and ashwagandha plant extracts was purchased from Vestige Marketing Private Limited., Nagercoil. After the second moult third instar larvae were divided into six groups of 30 worms in each. The first group was given normal feeding 4-5 times a day and treated on the control. The remaining group was considered as the experiment group and fed with the mulberry leaves fortified with spirulina and ashwagandha plant extract separately. Each group consists of different concentration of plant extracts (1, 2, 3, 4 and 5 per cent respectively). Both experiment and control groups are included the following biochemical estimations such as protein, carbohydrate and lipid present in silk gland, haemolymph, muscle, fat body of fifth instar larvae. The estimation is carried out by the method of Lowry *et al.,* (1951) Schiefter *et al.,* 1950 and Folch et al 1957. The cocoon was harvested on the fifth day after spinning and the cocoon characters were recorded in experimental and control groups. All the data was analyzed by Zar, 1984.

RESULTS

Protein content of silk gland, haemolymph, muscles and fat body of *B. mori* are presented in Table 1. Protein content of silk gland increased to 30.15 $\pm$ 0.54 mg/g with 4 per cent spirulina when compared to control (19.40 $\pm$ 0.58). The haemolymph protein was increased 35.36$\pm$0.50 in the larvae fed with 4 per cent spirulina. The minimum amount of haemolymph protein (21.25$\pm$0.86 mg/ml) was observed, when the larvae fed with 5 per cent ashwagandha compared to control (21.74$\pm$0.89 mg/ml). The maximum muscles protein (32.84$\pm$1.02 mg/g) was observed in the larvae fed with 4 per cent spirulina. The minimum muscle protein observed in ashwagandha 27.62$\pm$ 0.62 mg/g. The fat body protein was increase by 14.34$\pm$0.43, when larvae fed with 4 per cent spirulina table. 2 shows the carbohydrate content of protein, haemolymph, muscles and fat body of control larvae was 15.92 $\pm$ 0.88 mg/g, 12.74 $\pm$ 0.51 mg/ml, 8.18 $\pm$ 0.32 mg/g, 12.07 $\pm$ 0.31 mg/g respectively. The maximum (21.34 $\pm$ 0.52 mg/g) amount of silk gland carbohydrate was observed when the larvae fed with 3 per cent spirulina. The highest carbohydrate content of haemolymph, muscle and fat body was (17.66$\pm$ 0.41 mg/ml, 10.82 $\pm$ 0.24 mg/g and 16.34 $\pm$ 0.40 mg/ g respectively) when the larvae fed with 3 per cent, 4 per cent and 3 per cent spirulina respectively.

Table 3 shows the lipid content of silk gland, haemolymph, muscles and fat body. The silk gland, lipid was increased (2.45$\pm$0.19 mg/g) and decreased (1.83 $\pm$ 0.09 mg/g) when the larvae fed with 4 per cent and 1% spirulina. The maximum haemolymph (3.51$\pm$0.6 mg/ml) muscle (3.70 $\pm$ 0.5 mg/g) and fat body (16.34$\pm$0.04 mg/g) observed with 4 per cent spirulina. The larval parameters gets reflects on the quality of the cocoon and silk fibre. The economic traits of *B. mori* larvae fed with spirulina are presented in Table 4. The maximum cocoons weight, pupal weight, shell weight, shell ratio, filament length, fibroin and denier were 1922 $\pm$ 4604 mg, 15.95 $\pm$ 57.81 mg, 3.28 $\pm$ 28.85 mg, 17.06$\pm$ 1.16 %, 950.25$\pm$ 28.38 m, 24.60$\pm$ 0.96%, 18.70$\pm$ 0.96%, 3.30$\pm$ 0.07 respectively when *B.mori* larvae fed with 4 per cent spirulina sericin and denier, 3 per cent spirulina. Table 5 shows the economic traits of *B.mori* fed with ashwagandha maximum cocoon weight, pupal weight, shell weight, shell ratio, filament length, fibroin, sericin and denier were 17.24 $\pm$ 49.42 mg, 14.50 $\pm$ 32.40 mg, 274$\pm$ 18.50 mg, 15.89 $\pm$ 0.65 %, 867.83 $\pm$ 25.11 m, 76.40 $\pm$ 1.00 % and 3.00$\pm$ 0.06 respectively when larvae fed with 1 and 2 per cent ashwagandha.

DISCUSSION

The growth and development of larvae, subsequent cocoon production greatly influenced by nutritional quality of mulberry leaves. Protein content in the silk gland, haemolymph, fat body and muscles of larvae fed with spirulina and ashwagandha plant products were analyzed. They observed that the supplementation of *S. platensis* protein to *B. mori* produced the better digestion and absorption and covert into body protein. The report was agreed with Bose et al. (1989), who stated that the food is important factor for growth and silk production. In this study, maximum haemolymph protein (62.64 per cent) was observed, when the larvae fed with 4 per cent spirulina. This work was in agreement with Krishnan et al. (1995) and they reported that the level of storage protein in the haemolymph was increased by increasing concentration of hydrolyzed soy protein supplementation in *B.mori*. Protein concentration in fat body larvae was analyzed in this study. The maximum protein content in the fat body of larvae was (63.69 per cent) was observed when the larvae fed with 4 per cent spirulina. This work was agreed by Wigglesworth, 1972 and who stated that the fat body insect in the main site of protein synthesis as well as intermediating metabolism of amino acids, maximum protein content in the muscles of larvae was 33.22 per cent was observed when the larvae fed with 4 per cent spirulina. According to Horie and Nakane (1968) carbohydrate are utilized by the silkworm as energy sources and for the synthesis of lipids and amino acids. In the present study, spirulina and ashwagandha on the biochemical content of *B. mori* was analyzed maximum increase 34.04 per cent was observed, when larvae fed with 3 per cent spirulina. This report agreed with Prasad and Upadhyay (2011) studied the influence of magnetization on the glucose content in the tissues of silkworm larvae, variation in the static magnetic strength significantly influenced the glucose content in the silk gland, fat body and haemolymph of *B. mori* larvae. This study was also carried out by Hiremath *et al.,* 2009, who described on accumulation of lipid content in different stage of silkworm growth on the mulberry leaves raised from organic based nutrient management. In the present study, lipid content was estimated in the silk gland, haemolymph, muscle, fat body were 2.45$\pm$0.19, 3.51$\pm$ 0.6 mg/ml, 3.70 $\pm$ 0.5 mg/g and 16.34 $\pm$ 0.40 mg/g respectively.

Table 1. Protein content of *B.mori* larvae fed with *S. platensis* and *W.sominifera*

Treatment		Silk Gland mg/g	Haemolymph mg/ml	Muscle mg/g	Fat body mg/g
Control		19.40 ± 0.58	21.74 ±0.89	24.65±0.55	8.76 ±0.43
S. platensis	1	21.22 ± 0.57 (9.38)	25.12 ± 0.69 (15.54)	25.24 ± 0.58 (2.39)*	9.33 ±0.33 (6.50)*
	2	25.52 ± 0.52 (31.54)	27.25 ± 0.64 (25.48)	29.32 ± 0.64 (59.51)	12.92±0.42 (47.48)
	3	28.95 ± 0.45 (49.22)	31.25 ± 0.55 (43.74)	32.42 ± 0.82 (31.52)	11.78 ±0.34 (34.47)
	4	30.15±0.54 (55.41)	35.36 ± 0.50 (62.64)	32.84 ± 1.02 (33.22)	14.34 ± 0.43 (63.69)
	5	25.32 ± 0.49 (30.51)	32.61 ± 0.49 (50.00)	30.61 ± 0.79 (24.17)	12.35 ±0.31 (40.98)
W.sominifera	1	22.56 ± 0.64 (16.28)	24.74 ±0.89 (13.79)	25.72 ± 0.87 ((4.34)	9.92 ±0.27 (13.24)
	2	21.12±0.61 (8.86)	24.15 ± 0.55 (11.08)	24.67 ± 1.02 (0.08)*	9.13 ±0.34 (4.22)*
	3	19.71± 0.59 (1.54)	22.84±0.56 (5.05)	24.03±0.72 (-2.51)*	7.32 ± 0.44 (-16.43)*
	4	17.95 ±0.46 (-7.47)	21.89 ±0.68 (0.68)	22.32 ± 0.81 (-9.45)*	6.83 ± 0.40 (-22.03)*
	5	17.38±0.40 (-10.41)	21.25±0.86 (-2.25)	21.62 ± 0.62 (-12.29)*	6.03±0.51 (-31.16)*

Per cent deviation over control values in parentheses, *not significant, All other deviations significant at P ≤ 0.05 (t-test), N = 30

Table 2. Carbohydrate content of *B.mori* larvae fed with *S. platensis* and *W.sominifera*

Treatment		Silk Gland mg/g	Haemolymph mg/ml	Muscle mg/g	Fat body mg/g
Control		15.92 ± 0.88	12.74 ±0.51	8.18 ± 0.32	12.07 ± 0.31
S. platensis	1	16.71 ± 0.51 (4.96)*	12.92 ± 0.41 (1.88)*	8.52 ± 0.29 (4.15)*	14.37 ± 0.36 (19.05)
	2	16.92 ± 0.32 (6.28)*	14.23 ± 0.55 (15.07)	8.93 ± 0.17 (9.16)	13.76 ±0.24 (14.00)
	3	21.34 ± 0.52 (34.04)	17.66 ± 0.41 (38.61)	10.17 ± 0.35 (24.32)	16.34±0.40 (35.37)
	4	20.64±0.49 (29.64)	17.75 ± 0.33 (39.32)	10.82 ± 0.24 (32.27)	15.78 ± 0.27 (30.73)
	5	18.63 ± 0.35 (16.89)	16.76 ± 0.37 (31.55)	8.46 ± 0.32 (3.42)*	14.04 ±0.43 (16.32)
W.sominifera	1	17.20 ± 0.37 (8.04)*	14.43 ±0.41 (13.26)	9.25 ± 0.42 (13.08)	7.47 ±0.26 (9.05)
	2	16.00 ±0.64 (0.50)*	13.98 ± 0.66 (9.73)	8.38 ± 0.32 (2.44)*	6.83 ±0.48 (9.05)
	3	15.96 ± 0.32 (0.25)*	14.46±0.39 (13.50)	7.83±0.37 (-4.27)*	7.72 ± 0.36 (12.70)
	4	14.32 ±0.47 (-10.05)*	13.72 ±0.41 (7.62)*	7.48 ± 0.35 (-8.55)*	7.85 ± 0.42 (14.59)
	5	13.14 ±0.78 (-17.46)*	13.14 ± 0.41 (3.13)*	7.67 ± 0.47 (-6.23)*	6.25 ±0.29 (-8.75)*

Per cent deviation over control values in parentheses, *not significant, All other deviations significant at P ≤ 0.05 (t-test), N = 30

Table 3. Lipid content of *B.mori* larvae fed with *S. platensis* and *W.sominifera*

Treatment		Silk Gland mg/g	Haemolymph mg/ml	Muscle mg/g	Fat body mg/g
Control		1.76 ± 0.05	2.92 ±0.11	3.04 ± 0.09	12.07 ± 0.31
S. platensis	1	1.83 ± 0.09 (3.97)*	2.85 ± 0.10 (-2.37)*	3.64 ± 0.08 (19.73)	14.37 ± 0.36 (19.05)
	2	1.98 ± 0.09 (12.50)	3.31 ± 0.08 (13.35)	3.99 ± 0.08 (31.25)	13.76 ±0.24 (14.00)
	3	2.21 ± 0.11 (25.56)	3.51 ± 0.06 (20.20)	3.70 ± 0.15 (21.71)	16.34 ±0.40 (35.37)
	4	2.48 ±0.19 (39.20)	3.45 ± 0.05 (18.55)	3.64 ± 0.07 (19.73)	15.78 ± 0.27 (30.73)
	5	2.23 ± 0.13 (26.70)	3.12 ± 0.05 (6.84)*	3.09 ± 0.08 (1.64)*	14.04 ±0.43 (16.32)
W.sominifera	1	1.78 ± 0.06 (1.13)*	3.28 ±0.07 (12.32)	2.92 ± 0.13 (-3.94)*	12.46 ±0.47 (3.23)*
	2	1.92 ±0.05 (9.09)	3.12 ± 0.07 (6.84)*	3.31 ± 0.07 (8.88)*	12.21 ±0.42 (1.15)*
	3	1.71 ± 0.06 (-3.40)	2.71 ±0.04 (-7.19)*	3.02±0.05 (-0.65)*	10.42 ± 0.25 (-3.67)*
	4	1.59 ±0.14 (-9.65)	2.16 ±0.04 (-26.22)*	2.48 ± 0.13 (-18.42)*	11.21 ± 0.49 (-7.12)*
	5	1.57 ±0.09 (-10.79)	1.84 ± 0.08 (-36.98)*	2.71 ± 0.07 (-10.85)*	11.02 ±0.35 (-8.69)*

Per cent deviation over control values in parentheses, *not significant, All other deviations significant at P ≤ 0.05 (t-test), N = 30

Table 4. Economic traits of *B.mori* fed with *S. platensis*

Economic Traits	Concentrations (%)					
	Control	1	2	3	4	5
Cocoon Weight (mg)	1650±63.14	1724±53.197 4.48	1792±80.97 (8.60)*	1911±46.95 (15.81)	1922±46.04 (16.48)	1786±61.37 (8.24)*
Pupal Weight (mg)	1391±61.50	1444±38.30 (3.81)*	1498±64.07 (7.69)*	1595±38.72 (14.66)	1595±57.81 (14.66)	1495±45.19 (7.47)*
Shell Weight (mg)	258±25.64	280±26.69 (8.52)*	293±27.06 (13.56)	316±24.84 (22.48)	328±28.85 (27.13)	290±22.07 (12.40)
Shell Ratio (%)	15.63±0.86	16.24±1.22 (3.90)*	16.35±1.08 (4.60)*	16.53±1.14 (5.75)*	17.06±1.61 (9.14)	16.23±0.85 (3.83)*
Filament length (m)	831.55±43.37	865.83±31.48 (4.12)*	890.41± 23.16 (7.07)*	921.74± 23.91 (10.84)	950.24± 28.38 (14.27)	904.40± 29.05 (8.76)*
Fibroin (%)	76.30±1.26	78.20±1.10 (2.49)*	78.90±1.03 (3.40)*	81.30±0.96 (6.55)*	84.60±0.96 (10.87)	79.40±1.31 (4.06)*
Sericin (%)	23.70±1.26	21.80±1.10 (-8.01)*	21.10±1.03 (-10.92)*	18.70±0.96 (-21.09)*	15.40±0.96 (-35.02)*	20.60±1.31 (-13.08)*
Denier	2.50±0.07	2.80±0.07 (12.00)	2.70±0.06 (8.00)*	3.30±0.07 (32.00)	3.15±0.0 (26.00)	3.10±0.07 (24.00)

Per cent deviation over control values in parentheses, *not significant, All other deviations significant at P ≤ 0.05 (t-test), N = 30

Table 5. Economic traits of *B.mori* fed with *W.sominifera*

Economic Traits	Concentrations (%)					
	Control	1	2	3	4	5
Cocoon Weight (mg)	1650±63.14	1724±49.42 (4.48)*	1702±35.81 (3.15)*	1642±34.02 (-0.48)	1578±34.02 (-4.36)	1524±54.12 (-7.63)
Pupal Weight (mg)	1391±61.50	1450±32.40 (4.24)*	1438±28.72 (3.37)*	1390±28.31 (-0.07)*	1342±34.38 (-3.52)*	1301±50.64 (-6.47)*
Shell Weight (mg)	258±25.641	274±18.50 (6.20)*	262±9.08 (1.55)*	251±15.55 (-2.71)*	236±9.61 (-8.52)*	221±7.71 (-14.34)*
Shell Ratio (%)	15.63±0.866	15.89±0.65 (1.66)*	15.39±0.32 (-1.53)*	15.28±0.80 (-2.23)*	14.95±0.65 (-4.35)*	14.50±0.51 (-7.22)*
Filament length (m)	831.55±43.37	867.83± 25.11 (4.63)*	830.47± 19.87 (-0.12)*	815.36± 29.18 (-1.94)*	792.74± 18.54 (-4.66)*	758.24± 18.91 (-8.81)*
Fibroin (%)	76.30±1.26	76.40±1.00 (0.13)*	74.75±1.16 (-2.03)*	73.45±1.11 (-3.73)*	71.72±1.47 (-6.00)*	70.10±1.22 (-8.12)*
Sericin (%)	23.70±1.26	23.60±1.00 (-0.42)*	25.25±1.16 (6.54)*	26.55±1.11 (12.02)	28.28±1.47 (19.32)	29.90±1.22 (26.16)
Denier	2.50±0.07	2.84±0.074 (13.60)	3.00±0.061 (20.00)	2.58±0.090 (3.20)*	2.65±0.041 (6.00)*	2.40±0.070 (-4.00)*

Per cent deviation over control values in parentheses, *not significant, All other deviations significant at P ≤ 0.05 (t-test), N = 30

The present study carried out to when the larvae supplemented with spirulina, ashwagandha plant product on silkworm economic characters. The maximum increase in cocoon weight was 16.48 per cent, when larvae fed with 4 per cent spirulina. Treatment with spirulina showed increase but it was less then reported for ashwagandha. The treatment of spirulina was exhibited significant increase in cocoon weight, pupal weight decrease in ashwagandha. This statement is approved by Kumar et al. (2009) who suggested that silkworm larvae fed with 300 ppm *S. platensis* treated mulberry leaves, gives a significant cocoon weight, pupal weight, shell weight and silk filament length. Govindan and Narayanaswamy (1988) observed that the blue green algae have growth promoting economic traits.

Conclusion

The *S. platensis* and *W.sominifera* plant products orally applied may be utilized by the silkworm larvae for the extra synthesis of silk. The possibility of its use an enriched mulberry diet for silkworm could be explore after judging its economic implication for the sericulture in the actual field condition.

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