



RESEARCH ARTICLE

IMPACT OF PLANT AND ANIMAL PROTEINS ON BIOCHEMICAL CONTENTS AND ECONOMIC TRAITS OF BOMBYX MORI L.

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ARTICLE INFO

Article History:

Received 18th September, 2016

Received in revised form

15th October, 2016

Accepted 22nd November, 2016

Published online 30th December, 2016

Key words:

Silkworm,
Supplementary feeding,
Protein
and Economic traits.

ABSTRACT

Bombyx mori L. is a commercial exploited variety used large scale production of silk. One of the most important characteristics of the silkworm, *B.mori* is its ability to switch plant protein from feeding material to silk protein. The current study revealed that the impact of feeding *B.mori* with plant and animal protein enriched mulberry leaves has been examined with reference to biochemical content and economic parameters of sericulture. Fortification of the mulberry leaves by nutrient supplementation can be increased the quality and productivity of silk. The feeding of mulberry leaves soaked in 3, 5 and 7 per cent plant protein (soya flour) and animal proteins (*T. castaneum* larvae) to *B.mori* larvae from the first day of third, fourth and fifth instar. Biochemical constituents such as protein, carbohydrate and lipid content of silk gland, haemolymph, fat body and muscles were noted. *B.mori* proteins are stored in the middle silk gland and they are discharged through the anterior duct and spinneret, at the end of the fifth instar. The highest silk gland protein (92.01 per cent) was noted in the anterior silk gland at the 5 per cent soya flour treated groups. During the feeding period of the last larval instar, all the major proteins are synthesized by the fat body and secreted in to the haemolymph for temporary accumulation. The results of present study clearly demonstrated that the protein content of haemolymph and fat body were 55.68 and 36.25 per cent significantly increase when the larvae fed with 7 and 3 per cent soya treated groups. Among the treatments 5 % animal protein showed a significant increase in shell ratio (19.44 per cent) and filament length (81.26 per cent) and 7 % plant protein showed significant increase above parameters (18.66 and 80.43 per cent) when compared to control.

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Citation: Thilsath Fatima Quraiza, M., Kumari Sethu Lakshmi Bai P. K. and Ramani Bai, M. 2016. "Impact of plant and animal proteins on biochemical contents and economic traits of Bombyx mori L.", *International Journal of Current Research*, 8, (12), 42752-42757.

INTRODUCTION

B.mori feeds on the leaves of the mulberry tree *Morus* and serves as an excellent model system because of its life cycle, it is inexpensive and there are no ethical issues involved. Nutrition plays an important role in improving the growth and development of the silkworm, *B.mori* and the silk production is dependent on the larval nutrition and nutritive value of mulberry leaves and finally in producing good quality cocoons (Etebari, 2002). Nearly 70 % of the silk proteins produced by silkworm are directly derived from the protein of mulberry leaves (Narayanan et al., 1967). Sengupta et al. (1972) showed that *B.mori* require specific essential sugars, amino acids, proteins and vitamins for its normal growth, survival and also

for the silk gland growth. Variations in the quality of the mulberry leaves are many times reflected on the performance of the cocoon production. Supplementary nutrients are when added to normal food increases the nutritional value of the food making it more useful (Krishnaswami et al., 1971). Several attempts has been made to fortify leaves with different beneficial nutrients such as carbohydrate, protein, amino acids and hormones to improve the quality of cocoon crops (Subburathinum and Krishna, 1992). Enriching mulberry leaves by nutrient supplementation is one of the ways to improve growth rate of *B.mori*. Soybean protein possesses a high nutritive value. The dietary levels of soybean meal influence larval growth, larval characters and cocoon yield in *B.mori* (Nalini et al., 1994; Manimegalai et al., 2002). Ito (1980) stated that rich sources of dietary proteins like soy bean protein are known to promote growth and to improve economic characters of the silkworm. Supplementation of flour diets increased the larval weight, silk gland weight and

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commercial cocoon characters (Ganga and Gowri, 1990). Defatted soybean meal is commonly added to the artificial diet for the *B.mori* as a main source of protein (Xiaofeng et al., 1996). The SCP, *Spirulina* (blue green algae) had also been used as feed supplements to enhance the energy parameters and yield of *B.mori* (Kamalakaran et al., 2004 and Vijayalekshmi et al., 2004).

The fortification of amway protein, influence larval growth of silkworm, which is ultimately reflects in the economic traits (Rani et al., 2011). Konala et al. (2013) found out bovine milk contain several components that aid in healthy growth and it is added to feed additives to silkworm, *B.mori* shows maximum growth and economic traits. The impact analysis of nutilite on the larval growth and silk production stems from the fact that the soya protein, which is the chief ingredient of nutilite, has potential to modulate silk gene expression and silk protein synthesis in *B.mori* (Thulasi et al., 2015). Hence the present investigation was therefore undertaken with a view to test the role of this plant and animal protein on the biochemical content of *B.mori* and to adjudicate its potential for the stimulation of silk production and improvement in the economic traits of sericulture.

MATERIALS AND METHODS

The present investigation was carried out on the L x CSR₂ hybrid variety of the silkworm, *B.mori*. Since the experiments required continuous maintenance of the test species, silkworms were reared in the laboratory itself in accordance with the procedure adopted by Krishnaswami (1978). The present study has been aimed at investigating various biochemical contents and economic traits of the silkworm, when fed on mulberry leaves fortified with plant protein such as soya flour which was purchased from local market and dissolved in distilled water to obtain 3, 5 and 7 per cent solutions and animal protein i.e. *Tribolium castaneum* (Herbst) larvae reared in the research laboratory. Required amount of larvae was ground in distilled water to obtain 3, 5 and 7 per cent solutions. The first day of third instar larvae were selected randomly and grouped into seven batches for the experimental and control, each group consisting of 3 replicates with 50 silkworms. The experimental worms were fed with mulberry leaves soaked in the protein solutions i.e. 3, 5, 7 per cent plant protein and animal protein separately in the first day of third, fourth and fifth instar. The control group of silkworm larvae was fed with normal mulberry leaves. The biochemical content such as protein, carbohydrate and lipids present in the silk gland, haemolymph, muscles and fat body of experimental and control groups of fifth instar larvae were estimated by the method of Lowry et al. (1951), Schiefter et al. (1950) and Folch et al. (1957). The cocoons were harvested on the fifth day after spinning and the cocoon characters were recorded in experimental and control groups. Assessment of various cocoon parameters was made as follows (Sonwalker, 1993). All the data were analyzed statistically by t-test (Zar, 1984).

RESULTS

The findings of the present investigation, depicting the positive influence of plant and animal protein on the biochemical content and economic traits of *B.mori* are presented in table 1-5. Protein content of silk gland, haemolymph, muscles and fat body of *B.mori* are presented in Table 1. Protein content of anterior silk gland increased to 58.00±1.22 mg/g with 5 per

cent plant protein when compared to control (30.21±1.48 mg/g). In middle silk gland maximum protein (51.40±3.21mg/g) was observed with animal protein (5 per cent). The maximum (23.80± 1.30 mg/g) amount of posterior silk gland protein was observed, when the larvae fed with plant protein (5 per cent) and was reduced to 11.60±1.14 mg/g with animal protein (7 per cent) compared to control (12.40±1.51 mg/g). The haemolymph protein was increased by 55.68 per cent in the larvae fed with 7 per cent plant protein. The minimum amount of haemolymph protein (43.00±2.23 mg/ml) was observed, when the larvae fed with 3 per cent plant protein compared to control (34.40±1.51 mg/ml). The maximum muscles protein (48.60±3.84 mg/g) was observed in the larvae fed with 3 per cent animal protein. As observed in the present study, the muscle proteins increase by 83.91 per cent under the influence of 3 per cent animal protein. The highest (21.80±1.30 mg/g) amount of fat body protein was observed with plant (3 per cent) and lowest (5.20±2.04 mg/g) with animal protein (7 per cent). The fat body protein was increased by 36.25 per cent, when the larvae fed with 3 per cent plant protein. The carbohydrate content of anterior, middle and posterior silk gland, haemolymph, muscles and fat body of control larvae was 0.84±0.03 mg/g, 10.60±0.54 mg/g, 2.18±0.31 mg/g, 13.51±0.51mg/ml, 3.30±0.39 mg/g and 6.09±0.64mg/g, respectively (Table 2). The maximum (21.21±1.30 mg/g) amount of silk gland carbohydrate was observed in the middle silk gland, when the larvae fed with 5 per cent animal protein. The highest carbohydrate content of haemolymph, muscles and fat body was 22.84±1.30 mg/ml, 6.61±0.36 mg/g and 11.83±1.17 mg/g, respectively, when the caterpillars fed with 5 per cent animal protein.

Table 3 shows the lipid content of silk gland, haemolymph, muscles and fat body. These results of present study clearly demonstrated that the silk gland lipid was increased (6.13±0.15mg/g) and decreased (3.27±0.07mg/g), when the larvae fed with 5 per cent animal protein in the anterior silk gland and 5 per cent plant protein in the posterior silk gland. The maximum haemolymph (6.90±0.29 mg/ml), muscles (4.10±0.20 mg/g) and fat body (9.10±0.83 mg/g) lipids were observed with 7 per cent plant protein. Minimum haemolymph (3.04±0.10 mg/ml) and fat body lipid (5.20±1.27 mg/g) were observed with 5 per cent plant protein, but muscles (2.89±0.06 mg/g) lipids observed with 3 per cent plant protein. Among the treatments soya flour (7 %) supplemented with mulberry leaves recorded highest cocoon parameters and *T.castaneum* larvae (5%) of leaves recorded the maximum result. The economic parameters of *B.mori* fed with plant protein are presented in table 4. The maximum cocoon length, cocoon width, cocoon length/width ratio, cocoon size uniformity, cocoon weight, pupal weight, shell weight, shell ratio, filament length, fibroin content and denier was 3.50±0.019 cm, 1.80±0.13 cm, 1.94±0.011, 194.44±13.20, 1580.00±83.04 mg, 1290.00±72.04 mg, 290.00±18.37 mg, 18.35±1.51 %, 844.40±53.81 m, 79.30±4.90 %, and 2.67±0.06, respectively, when *B.mori* larvae fed with 7 per cent plant protein. Table 5 shows the economic traits of *B.mori* fed with animal protein. Maximum cocoon length, cocoon length/width ratio, cocoon size uniformity, cocoon weight, pupal weight, shell weight, shell ratio, filament length, fibroin content and denier (3.70±0.013 cm, 2.17±0.013, 217.64±16.39, 1570.00±107.21 mg, 1280.00±82.60 mg, 290.00±10.73 mg, 18.47±0.97 %, 850.27±67.15 m, 78.70±4.93 % and 2.78±0.09, respectively) was observed, when *B.mori* larvae fed with 5 per cent animal protein.

Table 1. Protein content of *B.mori* larvae fed with plant and animal proteins

Treatment	Anterior silk gland (mg/g)	Middle silk gland (mg/g)	Posterior silk gland (mg/g)	Haemolymph (mg/ml)	Muscles (mg/g)	Fat body (mg/g)	
Control	30.21±1.48	32.20±1.78	12.40±1.51	34.40±1.51	26.40±1.14	16.00±1.00	
Plant protein (%)	3	34.00±2.91 (12.57)	49.2±4.49 (52.70)	22.80±1.64 (83.82)	43.00±2.23 (24.94)	27.00±0.70 (2.27)*	21.80±1.30 (36.25)
	5	58.00±1.22 (92.01)	40.40±2.30 (24.18)	23.80±1.30 (91.88)	51.60±2.60 (49.88)	22.40±3.21 (-15.12)*	16.20±1.00 (1.25)*
	7	45.60±2.30 (50.97)	50.40±3.21 (56.42)	20.80±2.16 (67.70)	53.60±3.36 (55.68)	30.20±2.38 (14.36)	20.60±1.67 (28.75)
Animal protein (%)	3	42.80±2.58 (41.70)	36.80±3.11 (14.26)	22.00±1.87 (77.37)	52.00±3.16 (51.04)	48.60±3.84 (83.91)	18.80±0.83 (17.50)
	5	52.40±3.43 (73.48)	51.40±3.21 (59.52)	14.00±1.00 (12.89)	44.80±2.68 (30.16)	32.00±2.54 (21.17)	16.40±1.51 (2.50)*
	7	26.20±2.16 (-13.24)*	24.20±2.38 (-24.80)*	11.60±1.14 (-6.45)*	43.40±3.51 (26.10)	33.00±2.12 (24.95)	5.20±2.04 (-6.56)*

N=50, Per cent deviation over control values in parentheses, *not significant, All other deviations significant at $P \leq 0.05$ (t-test)

Table 2. Carbohydrate content of *B.mori* larvae fed with plant and animal proteins

Treatment	Anterior silk gland (mg/g)	Middle silk gland (mg/g)	Posterior silk gland (mg/g)	Haemolymph (mg/ml)	Muscles (mg/g)	Fat body (mg/g)	
Control	0.84±0.03	10.60±0.54	2.18±0.31	13.51±0.51	3.30±0.39	6.09±0.64	
Plant protein (%)	3	0.99±0.02 (17.85)	12.88±0.69 (21.50)	3.30±0.39 (51.37)	16.11±0.66 (19.24)	5.01±0.57 (51.81)	8.09±0.90 (32.84)
	5	1.06±0.04 (26.18)	14.99±0.67 (41.39)	4.26±0.40 (95.40)	19.01±0.13 (40.70)	5.93±0.33 (79.68)	10.76±0.52 (76.68)
	7	1.30±0.06 (54.75)	19.13±0.58 (80.43)	5.02±0.11 (130.27)	20.82±0.63 (54.09)	6.10±0.57 (84.84)	10.95±0.77 (79.80)
Animal protein (%)	3	1.06±0.12 (26.18)	17.01±0.69 (60.44)	3.06±0.49 (40.36)	18.36±0.70 (35.89)	5.72±0.62 (73.32)	9.09±0.61 (49.26)
	5	1.23±0.22 (46.42)	21.21±1.30 (100.05)	6.19±0.54 (183.93)	22.84±1.30 (69.04)	6.61±0.36 (100.29)	11.83±1.17 (94.25)
	7	1.45±0.16 (72.61)	17.44±0.57 (64.50)	4.13±0.17 (89.44)	19.82±0.92 (46.69)	5.38±0.36 (63.02)	10.72±0.77 (76.02)

N=50, Per cent deviation over control values in parentheses, All deviations significant at $P \leq 0.05$ (t-test)

Table 3. Lipid content of *B.mori* larvae fed with plant and animal proteins

Treatment	Anterior silk gland (mg/g)	Middle silk gland (mg/g)	Posterior silk gland (mg/g)	Haemolymph (mg/ml)	Muscles (mg/g)	Fat body (mg/g)	
Control	3.34±0.18	4.09±0.13	3.02±0.11	3.50±0.14	2.07±0.24	7.20±0.45	
Plant protein (%)	3	4.38±0.09 (31.13)	4.97±0.11 (21.50)	4.00±0.06 (32.44)	3.90±0.09 (11.42)	2.89±0.06 (39.60)	8.21±0.17 (14.01)
	5	3.90±0.02 (16.76)	4.13±0.18 (0.97)*	3.27±0.07 (8.27)*	3.04±0.10 (-13.14)*	3.00±0.10 (44.91)	5.20±1.27 (-27.76)*
	7	6.10±0.29 (82.63)	5.92±0.20 (44.72)	5.03±0.11 (66.55)	6.90±0.29 (97.13)	4.10±0.20 (98.00)	9.10±0.83 (26.37)
Animal protein (%)	3	5.31±0.20 (58.98)	4.60±0.30 (12.46)	4.00±0.17 (32.44)	5.11±0.30 (45.99)	2.94±0.06 (42.02)	7.26±0.21 (0.83)*
	5	6.13±0.15 (83.53)	6.00±0.19 (46.68)	5.31±0.21 (75.82)	6.13±0.21 (75.13)	4.09±0.11 (97.56)	8.27±0.46 (14.85)
	7	4.93±0.19 (47.60)	5.29±0.10 (29.33)	4.53±0.13 (49.99)	4.90±0.30 (39.99)	3.01±0.10 (45.40)	6.10±0.09 (-15.26)*

N=50, Per cent deviation over control values in parentheses, * not significant, All other deviations significant at $P \leq 0.05$ (t-test)

Table 4. Economic traits of *B.mori* fed with plant protein

Parameters	Concentrations (%)			
	Control	3	5	7
Cocoon length (cm)	2.70±0.010	3.00±0.016 (57.89)	3.10±0.014 (63.15)	3.50±0.019 (84.21)
Cocoon width (cm)	1.50±0.014	1.60±0.011 (6.67) *	1.70±0.011 (13.33)	1.80±0.13 (20.00)
Cocoon length/width ratio	1.80±0.011	1.87±0.010 (48.41)	1.82±0.012 (44.44)	1.94±0.011 (53.96)
Cocoon size uniformity	180.00±12.30	187.50±15.01 (47.45)	182.35±14.75 (43.44)	194.44±13.20 (52.86)
Cocoon weight (mg)	970.00±71.27	1390.00±80.11 (42.00)	1150.00±70.13 (18.00)	1580.00±83.04 (61.00)
Pupal weight (mg)	820.00±66.31	1150.00±63.14 (39.60)	960.00±67.05 (16.88)	1290.00±72.04 (56.40)
Shell weight (mg)	150.00±14.81	240.00±0.93 (59.40)	190.00±14.53 (26.40)	290.00±18.37 (92.40)
Shell ratio (%)	15.46±1.34	17.26±0.93 (11.65)	16.52±1.02 (6.86) *	18.35±1.51 (18.66)
Filament length (m)	463.31±33.54	602.30±49.05 (29.18)	573.60±54.11 (23.16)	844.40±53.81 (80.43)
Sericin content (%)	28.30±1.29	26.50±2.07 (-6.35) *	29.50±2.11 (4.24) *	20.70±1.73 (-26.83) *
Fibroin content (%)	71.70±4.20	73.50±5.21 (2.50) *	70.50±4.03 (-1.67) *	79.30±4.90 (10.56)
Denier	2.11±0.09	2.33±0.04 (10.42)	2.17±0.02 (2.84)*	2.67±0.06 (26.53)

N=50 Per cent deviation over control values in parentheses * not significant All other deviations significant at P≤0.05 (t-test)

Table 5. Economic traits of *B.mori* fed with animal protein

Parameters	Concentrations (%)			
	Control	3	5	7
Cocoon length (cm)	2.70±0.010	3.10±0.014 (63.15)	3.70±0.013 (94.73)	3.20±0.016 (68.42)
Cocoon width (cm)	1.50±0.014	1.70±0.009 (13.33) *	1.70±0.007 (13.33)	1.60±0.010 (6.67) *
Cocoon length/width ratio	1.80±0.011	1.82±0.014 (44.44)	2.17±0.013 (72.22)	2.00±0.011 (58.73)
Cocoon size uniformity	180.00±12.30	182.35±14.01 (43.44)	217.64±16.39 (70.96)	200.00±15.07 (57.21)
Cocoon weight (mg)	970.00±71.27	1140.00±82.47 (17.00) *	1570.00±107.21 (60.00)	1010.00±87.51 (4.00) *
Pupal weight (mg)	820.00±66.31	940.00±56.03 (14.40)	1280.00±82.60 (55.20)	840.00±64.07 (2.40) *
Shell weight (mg)	150.00±14.81	200.00±12.49 (33.00)	290.00±10.73 (92.40)	170.00±11.72 (13.20)
Shell ratio (%)	15.46±1.34	17.54±1.08 (13.45)	18.47±0.97 (19.44)	16.83±1.16 (8.86) *
Filament length (m)	463.31±33.54	602.17±46.53 (29.16)	850.27±67.15 (81.26)	592.11±36.17 (27.05)
Sericin content (%)	28.30±1.29	26.40±1.75 (-6.71) *	21.30±1.07 (-24.71) *	29.90±1.39 (5.65) *
Fibroin content (%)	71.70±4.20	73.60±5.21 (2.64) *	78.70±4.93 (9.73)	70.10±5.11 (-2.22) *
Denier	2.11±0.09	2.19±0.07 (3.79)*	2.78±0.09 (31.75)	2.46±0.03 (16.58)

N=50 Per cent deviation over control values in parentheses * not significant All other deviations significant at P≤0.05 (t-test)

DISCUSSION

Of the two types of proteins viz., plant protein and animal protein plays significant role in influence of the biochemical content and economic traits of *B.mori*. The silk gland is the major site of silk protein synthesis. It synthesizes and store 93 different proteins that are implicated in larval growth and development including 2 silk proteins i.e., fibroin and sericin (Nirmala et al., 2001). It has been demonstrated that the anterior silk gland, which is relatively inert, shows low protein levels, while the middle and posterior region acts as protein reservoirs in *B.mori* (Shimura, 1993). Silk protein is basically depends on the *B.mori* larval protein metabolism which in turn needs more energy generating events, spinning require more muscular activity and silk is being produced by the silk gland. The current study revealed that the maximum protein content of anterior silk gland was 92.01 per cent, when fed with 5 per cent soya flour. The present findings on the effect of soya flour increasing the silk filament length conforms to the findings of Subburathinam et al. (1992). Sundaramahalingam et al. (1998) noticed that the growth rate and protein utilization of silkworm are high as a results of the supplementation of plant growth regulator. Murugan et al. (1998) suggested a strong correlation and silk production in the silkworm after the treatment with plant extract and attributed the growth promoting effect of the plant extract in the stimulation of biochemical processes leading to protein synthesis. Woods (1999) observed that fifth instar caterpillars reared on essential sugars, amino acids, proteins and vitamins for its normal growth, survival and also for the silk gland growth. It is obvious that the whole body or tissue protein content of an insect could be considered a valuable criterion for the evaluation of the nutritional important of any dietary nutrient (Thomson, 1986). Among the treatment, 7 per cent soya flour protein showed a significant

increase haemolymph protein (55.68 per cent) where as in other treatments there was marginal increase when compared to control. 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 the increasing concentration of hydrolyzed soy protein supplementation in *B.mori*. Watanabe and Horie (1980) suggested that the proteins in the haemolymph of silkworm are known to be influenced by the level of dietary protein. Nagata and Kobayashi (1990) reported that haemolymph storage proteins were specifically sensitive to the dietary protein. The maximum muscles protein (83.91 per cent) was observed in the larvae, when fed with 3 per cent animal protein. In this study, the maximum (36.25 per cent) amount of fat body protein was observed, when fed with 3 per cent plant protein. Wigglesworth (1972) stated that the fat body in insect is the main site of protein synthesis as well as intermediating metabolism of amino acids.

In the present investigation, significant increase of 100.05 per cent carbohydrate was observed in middle silk gland, when the larvae fed with 5 per cent animal protein followed by haemolymph, muscles and fat body (69.04, 100.29 and 94.25 per cent respectively), when the caterpillars fed with 5 per cent animal protein. The results of the present study revealed that the 5 per cent animal protein treated groups significantly elevated carbohydrate contents. This work was agreed by Horie (1978), who stated that carbohydrates are utilized by the silkworm as energy sources and for the synthesis of lipids and amino acids. Lactose is the main carbohydrate component in milk and *B.mori* was shown to have a betaglucosidase enzyme that is active on cellobiose and lactose (Byeon et al., 2005). The present of this enzyme shows a possibility to feed silkworm larvae with milk through lactose does not show any problem for digestion. Ito (1980) found that casein the major

protein component is beneficial for *B.mori* development. Cocoon characters were significantly increased with 7 per cent plant protein and 5 per cent animal protein. The role of soya product in influencing the larval traits was reported by Subburathinam *et al.* (1992). The results of the present experiment agree with the earlier findings. Ashfaq and Sial (2001) reported that the nutritional sources not only use the growth and development of the silkworm larvae, but also final silk produce. Kumar *et al.*(2009) suggested that silkworm larvae fed with 300 ppm spirulina treated mulberry leaves, give 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 effect of water soluble proteins and vitamins and it is treated on silkworm with vitamins and amino acids to enhance the larval weight and economic traits. The present findings were confirmed with Horie and Watanabe (1983), who reported that the protein supplementation along with mulberry leaves enhanced the larval weight, cocoon weight, shell weight and shell ratio. Proline and leucine significantly increased the growth of larvae, pupae and cocoon characters of *B.mori* and the cystine enhanced the cocoon, shell weight and filament length of *B.mori*. According to Matsura (1994), blood meal and blood meal with chicken commercial formula feed found to be a superior source of protein, which increase the weight of cocoon shells in *B.mori*. Konala *et al.* (2013) showed that bovine milk had a positive effect on the body weight and cocoon weight of *B.mori* larvae. Hossain *et al.* (2015) showed that cow milk increase the body weight and cocoon weight of *B.mori*.

Conclusion

The plant and animal proteins has potential to stimulate silk gland protein synthesis in *B.mori*. Similarly, it stimulates silk protein synthesis and also improves the productivity, quality of silk produced by *B.mori*. The possibility of its use as an enriched mulberry diet for silkworm could be explore after judging its economic implication for the sericulture industry in the actual field condition.

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