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Study of the Influence of *Aloe vera* Juice at varied Concentrations on Biomolecules in the Silkworm Hybrid FC2

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ABSTRACT: This research explores the impact of different concentrations of *Aloe vera* juice on the biochemical profiles of the silkworm hybrid FC2 (*Bombyx mori* L.), with a particular focus on protein and carbohydrate levels in the fat body and midgut tissues during the fifth instar stage. The silkworms were fed mulberry leaves enhanced with *Aloe vera* juice at concentrations of 0.2%, 0.4%, and 0.6%. The findings showed a notable increase in both protein and carbohydrate content in the fat body and midgut, especially at the highest concentration (0.6%). The study suggests that *Aloe vera* supplementation can boost the nutritional quality of mulberry leaves, thereby promoting better growth and development in silkworms, which is essential for maximizing silk yield. These results highlight the potential of using botanical additives to enhance sericulture productivity.

Keywords: *Aloe vera*, Silkworm hybrid FC2, *Bombyx mori*, Mulberry leaves, Protein content.

INTRODUCTION

The silkworm, *Bombyx mori* L is the specialist feeder (monophagous), depending exclusively on the mulberry leaf (*Morus* spp.) as its food. Needless to say, it has to derive its nutritional requirements from mulberry leaves for its growth and development. Any variation in the nutritional components of mulberry leaves may have some influence on the growth and development of silkworm. Though the availability of silkworm nutrients in mulberry leaf is ensured on most occasions, some time they may not be available in adequate quantities for the larval growth. The quality of the leaf can have profound influence on the superiority of silk produced by *B. mori*. Therefore, the production of good cocoon crop is largely dependent on the quality of leaves. In other words, leaves of superior quality enhance the chances of realizing good cocoon crop (Ravikumar, 1988). It has also been demonstrated that the dietary nutrition management has a direct influence on quality and quantity of silk production in *B. mori* (Murugan *et al.,* 1998). The nutritional parameters of mulberry leaves and silk production are related to each other. The nutritional composition of mulberry leaves varies depending on the nature of the genotype, soil and environmental conditions, precipitation (rainfall/irrigation), agronomical practices employed, plant protection measures adopted, *etc.*

Cocoon production by *B. mori* depends on its rearing on mulberry leaves. For this reason, the quality of its food (mulberry leaf) needs to be high for realizing the full potential of the silkworm in terms of cocoon production. Nevertheless, the quantity of leaf fed by the larva also assumes great significance for accumulating considerable biomass that should culminate in the improvement of cocoon yield. Understandably, whenever the leaf quality if inferior, there arises a need for fortifying the leaf using supplementary nutrients such as vitamins, proteins, amino acids, carbohydrates, minerals, *etc.* so that the nutritional status of the leaf would be ultimately matching with the nutritional requirement of the silkworm. In addition, there is also a necessity to enhance the appetite in the silkworm so that it would grow better and to improve the disease tolerance that should facilitate higher rate of survival of the larvae.

Like in other organisms, in the silkworm, *B. mori* too, nutrition plays an important role in improving the growth and development. The intake of nutrient by the silkworm larvae is influenced by the availability of feed. Therefore, silkworm nutrition is considered as a major area of research in sericulture (Legay, 1958). Nutrition study in silkworm is an essential pre requisite for its proper commercial exploitation. *B. mori* requires specific essential sugars, amino acids, proteins and vitamins for its normal growth, survival and also for the silk gland growth. Akhtar and Asghar (1972) found that

vitamins and mineral salts played an important role in the nutrition of silkworm. Karaksy (1990) showed that silkworms fed on mulberry leaves enriched with ascorbic acid showed better growth and produced heavier cocoons. Similar observations were made by Madhu Babu (1992).

The feeding of nutritionally enriched leaves showed better growth and development of silkworm larvae and, in turn, influenced the quality and quantity of silk produced. Nearly 70% of the silk proteins produced by silkworm are directly derived from the protein of the mulberry leaves. In recent years a number of botanicals have been identified with insect growth regulation (IGR), juvenile hormone (JH) secretion, *etc.* However, silkworm, *B. mori* supplemented with plant exctracts namely *Tribulus terrestris, Pssoraleacoryleifolia, Phyllanthus niruri, Polypodium* Sp*., Parthenium hysterophorus and Tridaxprocumbens* increases larval growth as well as cocoon parameters (Rajashekargouda, 1991; Murugan *et al*., 1994). According to Mamadapur (1994); Santosh Kumar (1997), application of *Lantana camara* and *Clerodendroninermae* at 5% once on 48h old fifth instar larvae increased all the economic traits. A good number of reports are available on fortifying agents, feed additives and botanical extractions which enhances economics parameters of the silk worm. However, study on the botanical extracts on biochemical changes takes place in silk worm is meager.

MATERIALS AND METHODS

Maintenance of mulberry garden. The experiment was conducted using mulberry garden (V-1 variety) maintained at Manasagangothri campus, Mysuru. The tender and medium mulberry leaves were used for young-age silkworm rearing. While, coarse leaves were used for late-age rearing. To maintain good quality leaf for feeding, proper methods were followed during harvesting and storage. Fresh leaves were collected during morning and evening hours of the day and stored in leaf preservation chamber covered with wet gunny cloth to reduce loss of moisture and degradation of nutrients.

Disinfection of rearing house and appliances. To ensure the rearing house and appliances pathogen free, they were thoroughly cleaned with water and 2% bleaching powder solution followed by 2.5% Sanitech solution @1.5 liter per square meter to achieve disinfection (Dandin *et al.,* 2003).

Procurement of disease free layings (DFLs). The disease free layings (eggs) of popular bivoltine silkworm hybrids namely FC2 (CSR6 × CSR26) was procured from the National Silkworm Seed Organization (NSSO), Central Silk Board (CSB), Mysuru. The egg cards were surface sterilized by dipping them in 2% formalin for 8-10 minutes and dried in shade before use.

Incubation of DFLS. The eggs of FC2 were incubated at room temperature (25°C) and 70-80% relative humidity by adopting standard incubation method and were subjected to black boxing for about 48 h at pin head stage (Benchamin and Nagaraj 1987).

Rearing of FC2 silkworm. After brushing the eggs, newly hatched silkworms were carefully shifted on to the rearing bed and fed fresh tender leaves and reared by employing stranded rearing techniques advocated by Dandin and Giridhar (2010).

Selection of plant extract

Aloe vera

Classification

Kingdom-Plantae
Order - Asparagales
Family - Asphodelaceae
Sub family- Asphodeloideae
Genus - *Aloe*
Species - *vera*

Aloe vera is a stem less or very short-stemmed plant growing to 60-100cm tall, spreading by offsets. The leaves are thick and fleshy, green to grey-green with some varieties showing white flecks on their upper and lower stem surfaces. The margin of leaf is serrated and has small white teeth. The flowers are produced in summer on a spike up to 90cm tall. *A. vera* forms arbuscular mycorrhiza, a symbiosis that allows the plant better access to mineral nutrients in soil.

Medicinal properties. *A. vera* used to treat inflammation in tissues, burning injuries, wound healing, gastric ulcers, diabetes, cancer and boosting immune system, etc. It is also used in manufacture of moisturizers and gels.

Preparation of plant extract. The *A. vera* juice (Dabur) procured from Charaka Ayurvedic Medicines and General Stores, Brindavan Extension Mysuru-20. The stock solution was diluted at the rate of 0.2, 0.4 and 0.6% by using distilled water. The prepared solution was preserved in refrigerator at 4°C.

Estimations of biomolecules in silkworm

Collection and preservation of samples. The midgut and fat body tissue was collected by dissecting larva and fat body was taken after removing the digestive track. The collected samples were preserved at -20°C until further use. Biochemical analysis was carried out in the fat body and midgut tissue of silkworm during 5th instar 1st day to 6th day old larvae in respective treatments as well as control batches.

Preparation of samples. The tissue homogenate (1%) was prepared by using distilled water. The content was centrifuged at 3000rpm for 10min and supernatant was collected

Estimation of protein. The protein content in fat body and midgut tissue were estimated adopting the method of Lowry *et al.* (1951). 1ml tissue homogenate was taken with 5ml protein reagent then the reaction mixture was incubated in room temperature for 10min and 0.5ml protein reagent was added. The contents were mixed thoroughly and kept for 30min until the development of blue colour 1ml Bovine Serum Albumin (BSA) was used as standard. A blank was prepared using 1ml distilled water in place of sample. The optical density (OD) was measured using spectrophotometer at 660nm against blank. The results were expressed in mg/g of tissue.

$$\text{Amount of protein (mg/g)} = \frac{\text{OD of the sample} \times \text{mg of BSA in standard}}{\text{OD of the standard} \times \text{mg of tissue taken}} \times 100$$

Estimation of carbohydrate. Total carbohydrate was determined by Anthrone method (Dubios *et al*., 1956) using glucose as standard. To 1ml sample, 4ml anthrone reagent was added and contents were boiled for 8 min using water bath, then cooled with running water. The absorbency was measured using spectrophotometer at 630 nm against blank and carbohydrate content was expressed in mg/g of tissue.

$$\text{Amount of carbohydrate (mg/g)} = \frac{\text{OD of the sample} \times \text{mg of glucose in standard}}{\text{OD of the standard} \times \text{mg of tissue taken}} \times 100$$

RESULTS

Influence of mulberry leaves supplemented with *Aloevera* juice on protein level in FC2 silkworm. Silkworm feed on mulberry leaves supplemented with *Aloe vera* juice of different concentrations recorded significant variation on protein level in both midgut and fatbody of matured larvae. In fat body maximum protein level was observed in 5th instars 6th day larvae supplemented with aloe vera at 0.6% concentration (129.741g of protein/mg of tissue) followed by 0.4% (121.362g of protein/mg of tissue) and 0.2% (109.497g of protein/mg of tissue) against to absolute and distilled water control batches (102.479 and 103.39g of protein/mg of tissue) Similar trend was also observed in fifth instar 5th, 4th, 3rd, 2nd and 1st day old larvae. In contrast, the minimum protein content was observed in fifth instar 1stday larvae with absolute control (31.147g of protein /mg of tissue) and distilled water (40.396g of protein/mg of tissue) batches.

Similar difference was observed in midgut tissue of matured silkworms *i.e.* maximum protein content was registered in 5th instars 6th day larvae supplemented with aloe vera at 0.6% concentration (113.296g of protein/mg of tissue) followed by 0.4% (110.289g of protein/mg of tissue) and 0.6% (103.186g of protein/mg of tissue) against to absolute and distilled water control batches (99.019 and 99.984g of protein/mg of tissue). Similar results were also observed in fifth instar 5, 4, 3, 2nd and 1st day old larvae. As against, the minimum protein content was observed in fifth instar 1st day larvae with absolute control (30.842g of protein/mg of tissue) and distilled water (31.773g of protein/mg of tissue) batches. The hybrid FC2 silkworm has scored maximum protein content in fat body when compared to mid gut in *A. vera* treatments and gradually increased protein content in fifth instar 1st day to 6thday

Influence of mulberry leaves supplemented with *Aloe vera* juice on carbohydrate content in FC2 silkworm. Silkworm nourished with mulberry leaf fortified with varied concentration of *A. vera* in fat body maximum carbohydrates content was observed in 5th instars 6th day larvae supplemented with aloe vera at 0.6% concentration (38.588g of carbohydrate/mg of tissue) followed by 0.4% (37.541g of carbohydrate/mg of tissue) and 0.2%(36.021g of carbohydrate/mg of tissue) against to absolute and distilled water control batches (31.639g and 32.236g of carbohydrate /mg of tissue). Similar trend was also observed in fifth instar 5th, 4th, 3rd, 2nd and 1st day old larvae. In contrast, the minimum protein content was observed in fifth instar 1stday larvae with absolute control (3.008g) and distilled water (3.106g of carbohydrate/mg of tissue) batches.

In midgut tissue of matured silkworms the maximum carbohydrate content was registered in 5th instars 6th day larvae supplemented with aloe vera at 0.6% concentration (33.846g of carbohydrate/mg of tissue) followed by 0.4% (31.970g of carbohydrate/mg of tissue) and 0.2%(28.881g of carbohydrate/mg of tissue) against to absolute and distilled water control batches (27.930g of carbohydrate/mg of tissue and 28.152g of carbohydrate/mg of tissue). Similar results were also observed in fifth instar 5th, 4th, 3rd, 2nd and 1stday old larvae. As against, the minimum carbohydrate content was observed in fifth instar 1stday larvae with absolute control (2.531g of carbohydrate/mg of tissue) and distilled water (2.639g of carbohydrate/mg of tissue) batches. The hybrid FC2 silkworm has scored maximum carbohydrates content in fat body when compared to mid gut in aloe vera treatments and gradually increased carbohydrates content in fifth instar 1stday to 6thday.

Table 1: Effect of fortified mulberry leaf with *Aloe vera* juice on fat body protein in FC2 silkworm hybrid.

Concentrations (%)	Fat body					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
0.2	44.04±1.22	53.43±0.21	73.15±0.20	82.38±0.65	105.48±0.65	109.49±0.16
0.4	47.53±0.27	55.92±0.96	79.23±0.25	95.71±0.34	114.53±0.44	121.36±0.55
0.6	49.36±0.27	58.67±0.34	42.35±0.65	100.42±0.43	118.35±0.45	129.74±0.15
Distilled water	40.39±0.65	50.92±2.06	60.81±0.62	77.32±0.29	97.81±0.48	103.3±0.19
Absolute control	39.14±0.81	48.25±0.37	59.18±0.54	75.13±0.55	96.26±0.14	102.47±0.05

Table 2: Effect of fortified mulberry leaf with *Aloe vera* juice on midgut protein in FC2 silkworm hybrid.

Concentrations (%)	Midgut					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
0.2	35.13±0.44	45.57±0.18	53.82±0.12	76.92±0.08	97.35±0.45	103.19±0.46
0.4	38.78±0.35	48.58±0.15	58.27±0.69	73.19±17.46	103.58±0.66	110.29±0.26
0.6S	41.09±0.72	51.63±0.06	65.63±0.21	88.13±0.08	109.29±0.30	113.3±0.40
Distilled water	31.77±0.75	43.92±0.09	50.81±0.13	73.45±0.19	94.56±0.18	99.98±0.59
Absolute control	30.84±0.43	41.35±0.10	49.21±0.25	72.13±0.38	92.41±0.57	99.01±0.23

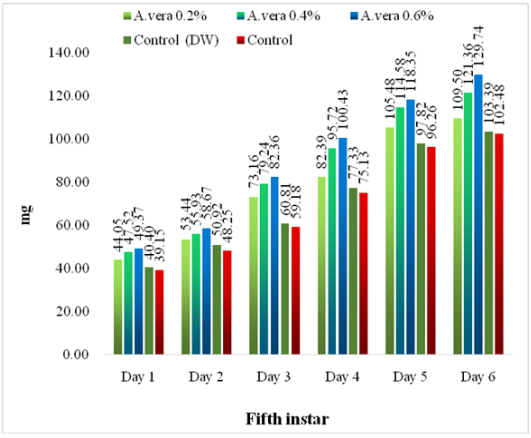

Fig. 1. Effect of fortified mulberry leaf with *Aloe vera* on protein content in fat body of FC2 silkworm hybrid.

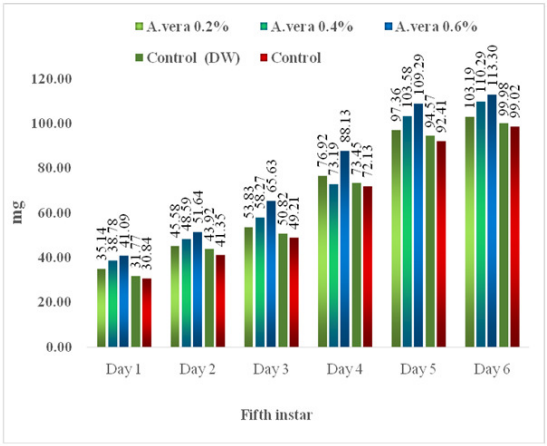

Fig. 2. Effect of fortified mulberry leaf with *Aloe vera* on protein content in midgut of FC2 silkworm hybrid.

Table 3: Effect of fortified mulberry leaf with *Aloe vera* juice carbohydrates content in fat body of FC2 silkworm.

Concentrations (%)	Fat body					
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
0.2	3.22±0.12	5.49±0.51	12.09±0.40	19.05±0.96	25.67±0.19	36.02±0.41
0.4	3.40±0.14	5.50±0.54	12.26±0.19	21.58±0.10	26.51±0.41	37.54±0.28
0.6	3.62±0.24	5.64±0.19	12.47±0.09	22.26±0.61	30.71±0.16	38.58±0.07
Distilled water	3.10±0.15	4.31±0.76	10.75±0.47	18.89±0.40	25.10±0.35	32.23±0.53
Absolute control	3.00±0.18	4.74±0.21	10.31±0.19	18.32±0.45	24.07±0.15	31.63±0.33

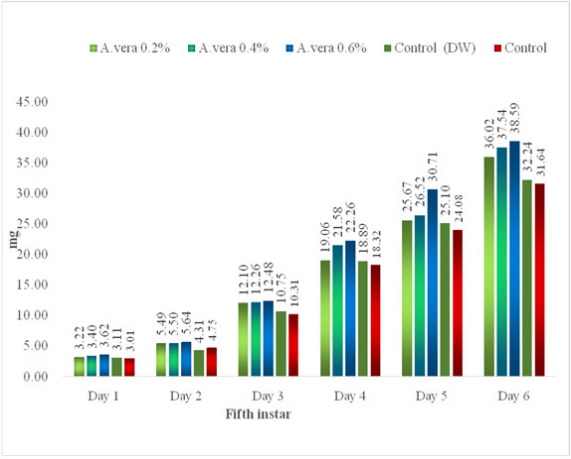

Fig. 3. Effect of fortified mulberry leaf with *Aloe vera* on Carbohydrates content in fat body of FC2 silkworm hybrid.

Table 4: Effect of fortified mulberry leaf with *Aloe vera* on carbohydrates content in midgut of FC2 silkworm hybrid.

Concentrations (%)	Midgut					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
0.2	2.78±0.27	3.95±0.52	9.98±0.39	17.96±0.63	23.78±0.18	28.88±0.86
0.4	3.96±0.27	4.06±0.06	10.97±0.34	18.69±0.50	25.66±0.51	31.97±2.08
0.6	3.02±0.11	4.52±0.38	11.12±0.55	19.56±0.13	28.75±0.27	33.84±0.21
Distilled water	2.63±0.24	3.85±0.55	9.57±0.60	16.69±0.30	23.62±0.52	28.15±0.21
Absolute control	2.53±0.27	3.72±0.23	9.38±0.58	16.27±0.23	23.56±0.38	27.93±0.25

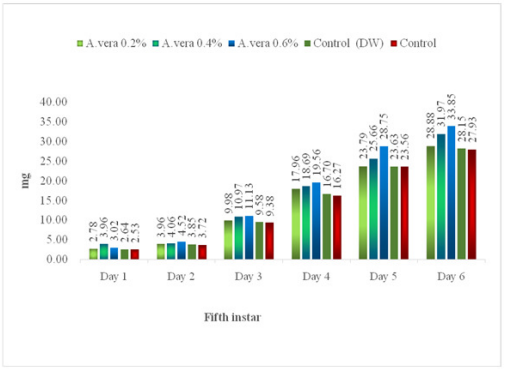

Fig. 4. Effect of fortified mulberry leaf with *Aloe vera* on Carbohydrates content in midgut of FC2 silkworm hybrid.

DISCUSSION

Storage proteins are major haemolymph proteins in the larvae of holometabolous insects, playing an important role as reservoir for various amino acids which are utilized for adult development in metamorphosis. Haemolymph, the only extra cellular fluid in insect circulatory system, perform diverse functions (Pawar and Ramakrishna 1977) such as immunity, transport and storage tissue (Mullins, 1985). Moreover, it acts as a reservoir for the products which are required for every physiological activity. Thus, variation in the composition of haemolymph reflects the morphogenic and biochemical changes in the body of insects. Interestingly, dietary protein influences the haemolymph protein concentration in silkworms. In this regard, Horie *et al*. (1971) have reported the total content of protein increased with amount of protein in artificial diet. Moreover, fat body tissue is the seat of metabolism, active in the synthesis, storage and release of biomolecules to provide clues in relation to energy production, transformation and utilization at cellular and sub-cellular levels during intermediary metabolism of insects. The growth and differentiation are closely related to protein synthesis in fifth instar silkworm. The food intake reaches its peak in accordance with body growth and silk gland development and metabolism (Jolly *et al*., 1974). The level of the total protein in the midgut includes structural proteins, globular proteins like cellular, sub-cellular enzymes and some storage proteins (Bursell, 1970). The fat body synthesizes number of proteins and released into the haemolymph during active larval feeding period (Kiran kumar *et al*., 1998). Some of the midgut proteins, which play an important role as a storage reserve and are mobilized and utilized from the haemolymph during the mid-period of the late instar (Loughton and West 1965).

It is evident from the present study that the silkworm larvae reared on mulberry leaves fortified with *Aloe vera* fruit juice recorded highest activity level of protein in the fat body at 0.2% concentration (118.150 g of protein/mg of tissue) in 5th instar 6thday larvae when compared to control batches. Obviously, protein are high molecular weight biomolecules made up of polymers of alpha amino acids and are the essential components of life process. In insects, protein regulate the growth, development, repairing old tissue and also components of enzymes, antibodies, hormones, etc. (Rama Rao, 2004).

Increase in protein content in the tissues might be due additional supplementation of *Aloe vera* through the diet which might have enhanced the synthesis of soluble and structural protein in the cells. However, protein content was relatively high in the fifth instar 6thday old larvae supplemented with lower concentration of amino acids than the higher. It indicated that the matured larvae require lesser amount of amla and hence progressive increase in protein content in the tissue of fifth instar 1st day to 6thday. Vitamins are organic compounds required in trace amounts in the diet for proper growth and development. In general, plant synthesizes large number of vitamins. A few animals and insects also synthesize in lesser quantities. They resemble hormones in their function and both of them required because of their function as co-factor of enzymes and they are needed in little quantities corresponding to that of the appropriate catalytic activity. The specific dose requirement of different nutrients and vitamins suggests their specificity for various metabolic functions (Horie, 1980).

SUMMARY AND CONCLUSION

Both carbohydrate and protein contents were relatively higher at 0.6% supplementation of *A. vera* when

compared to rest of the treatments as well as controls. The silkworm larvae reared on mulberry leaf fortified with *A. vera* at 0.6% recorded higher protein and carbohydrate contents in **V** instar 6th day followed by 5th day, 4th day, 3rd day, 2nd day and 1st day.

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Conflict of Interest. None.

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