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Influence of mulberry leaf with soyabean flour supplemention on the economic traits and aminotransferases activity in *Bombyx mori* L.

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Abstract
The silkworm, *Bombyx mori* L. has been regarded as one of the most striking examples of monophagy as its food selection is very narrow and almost limited to mulberry leaves alone. *B.mori* obtain nutrients from the mulberry leaves for its sustained growth and development including cocoon production. However, in recent year, more efforts are made by researchers to enhance cocoon quality parameters by supplementing exogenous protein through diet. Such, an attempt has been made to record the influence of soyabean flour supplemented to mulberry leaf with different concentration *viz*., 2, 4 and 6% on the economic traits and aminotransferase activity in bivoltine CSR2 and CSR4 breeds. Moreover, the data revealed, significant increase in cocoon weight, shell weight, pupal weight and shell ratio at 4% concentration in CSR2 and 6 % concentration in CSR4. The aminotransferase enzyme activity level was higher at 4 and 6 % concentrations in CSR2 and CSR4, respectively.

Keywords: aminotransferases activity, bivoltine breeds, *bombyx mori*, economic traits, soyabean flour

1. Introduction

The silkworm, *Bombyx mori* L. is a monophagous insect and consumes only on mulberry leaves during its larval stages of its life cycle. Mulberry silk contributes 70% of total raw silk production. India trails behind China for silk production and occupies second position in the world and there is a need to enhance the raw silk production and it is possible if farmer rear high yielding bivoltine silkworm breeds. The quality of cocoon production not only depends upon the silkworm breeds and congenial environment maintained during rearing, but also quality of mulberry leaf provided to them.

Legay (1958) [9] opined that the silkworm nutrition is a major area of research in sericulture and Pant (1978) [14] has envisaged great scope of utilizing data for proper exploitation of beneficial insects like silkworms. They have stressed the qualitative and quantitative aspect of cocoon yield that be directly increased through proper dietary management. Hence, proper care of silkworms through dietary management is an essential requisite to maximize sericultural output and to stabilize and augment the economy of farmers in sericulture.

Nutrition involves chemical and physiological activities in which food is digested with the aid of digestive enzymes and get assimilated into body elements. The both essential and non-essential amino acids categories play a pivotal role in determining quality of cocoon production. These amino acids are bio-synthesized in silkworm only when precursors are provided in the diet. If the diet lacks any one of these amino acids, the growth and development of silkworm gets hampered.

The alanine aminotransferase (ALT, EC 2.6.1.2) and aspartate aminotransferase (AST, EC 2.6.1.2) are the enzymes which catalyses the transaminase reaction in which transfer amino group from one amino acid to another keto acid and help to form another amino acid. These enzymes serve as a strategic link between carbohydrate and protein metabolism (Martin *et al.*, 1981) [10]. They are known to be altered during various physiological and pathological conditions (Nath *et al.*, 1997) [13]. The higher levels of enzyme activity indicate the occurrence of greater energy demands which are normally associated with synthetic activities of the cell (Meister, 1965) [12]. In insects, there is a close relationship between high transaminase activity, growth and development (Mc Allan and Chefruka, 1961) [11].

In *B. mori*, the activity level of ALT is comparatively high in the silkglands than the midgut and fat body tissue (Horie and Nakamura, 1986) [6]. Further, they are responsible for regulating the supply of amino acids during protein synthesis in different tissue. It is well documented that, elevation in the activity of these enzymes also indicates the toxicosis, pathogenesis and histolysis of different kinds of tissues in insects, including silkworm. It has been observed that, supplementation of mulberry leaf with *Dolichos lablab* and *Vigna unguiculata* seed flour enhances ALT and AST activity which in turn reflect the synthesis of silk protein (Sarvanan Manjula *et al.*, 2010a and 2010b) [18-19].

Importance of research on effect of different fortification agents in silkworm nutrition can be judged from the principle of co-operating supplements, which states that supplementary or substitutive sources of proteins co-operating with commonly recognized food stuff of the species are needed to fulfill the nutritional requirements in many insects (House, 1966) [7]. In recent years, attempts have been made to fortify mulberry leaves with nutrients such as proteins, carbohydrates, amino acids, vitamins, sterols, hormones, antibiotics, salts and other chemicals in order to improve the quality and quantity of cocoon yield. However, information on mulberry leaves supplemented with soyabean flour on aminotransferase activity is fragmentary. Hence, investigation was undertaken to record the changes in aminotransferase activity in different larval stages during supplementation of soyabean flour with mulberry.

2. Materials and methods

2.1 Preparation of soyabean flour solution

The healthy soyabean seeds were procured from Mannar's market, Mysore. The seeds were dried under shade and made fine powdered flour by milling. The different concentrations of soyabean flour solution *viz.*, 2, 4 and 6% was prepared by using distilled water.

2.2 Supplementation of soyabean flour

The popular bivoltine breeds CSR_2 and CSR_4 were selected and reared by employing standard rearing technique as per by Dandin *et al. (*2010) [4]. The concentrations 2, 4 and 6 % of soyabean flour was sprayed on the ventral surface of the mulberry leaf and surface dried under shade before feeding to silkworms. The silkworm larvae divided into four batches *viz*., batch I (T1), batch II (T2) and batch III (T3) were fed with soyabean flour supplemented leaves along with control batch IV (T4) larvae were reared on mulberry leaves spayed with distilled water. The larvae were fed twice a day (morning and evening) during fourth and fifth in stars. In each treatment fifty larvae were maintained in three replications. A minimum of ten larvae were used from each batch to record the economic traits such as larval weight, cocoon weight, pupal weight and shell weight. The parameters namely shell ratio, filament length, denier, renditta, reelability and raw silk percentage were calculated by using following formulae.

$$\text{Shell ratio (\%)} = \frac{\text{Shell weight (g)}}{\text{Cocoon weight (g)}} \times 100$$

Filament Length (L) = R X 1.125

Where, R is Number of revolutions recorded by an epprouvette. And 1.125= Circumference of epprouvette in meter

$$\text{Denier} = \frac{\text{Weight of the filament}}{\text{Length of the filament}} \times 9000$$

$$\text{Renditta} = \frac{\text{Weight of cocoon reeled}}{\text{Weight of raw silk obtained}}$$

$$\text{Reelability} = \frac{\text{Number of cocoons taken for reeling}}{\text{Number of breakage + Number of cocoons fed}}$$

$$\text{Raw silk percentage} = \frac{\text{Silk weight}}{\text{Green cocoon weight}} \times 100$$

2.3 Estimation of alanine and aspartate aminotransferase enzymes.

The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity were estimated in fifth instar 1^{st} day, 3^{rd} day and 5^{th} day (prior to spinning stage) of CSR2 and CSR4 breeds in treated and control groups. The fat body tissue homogenate of 1% (w/v) was prepared by using distilled water and centrifuged at 3,000 rpm for 10 minutes, the crude extract supernatant was collected and used as an enzyme source.

The ALT and AST enzymes were estimated by the method of Reitman and Frankel (1957) [17]. Aspartate aminotransferase was estimated by using 1ml of tissue extract, incubated with 0.5ml of glutamic oxalo acetate (substrate) at 37^0 C for 1 hour. To this reaction mixture, 0.5ml of 2, 4-D and 5ml of 0.4 N NaoH was added. The colour intensity was measured at 510 nm by using spectrophotometer. For alanine aminotransferase, 0.5 ml glutamate pyurvate was used as a substrate and standard curve was used for calculation. The enzyme activity was expressed in terms of units /g protein/h.

The obtained data was analyzed by employing one-way factorial completely randomized design at 5% level of significance, OPISTAT online statistical package developed by O.P. Sheoran, Programmer, Computer section Chaudhury Charan Singh Hisar Agriculture University, Hisar, Haryana State, India.

3. Results and discussion

3.1 Larval weight

The silkworm breeds CSR_2 and CSR_4 nourished with mulberry leaves extrafoliated with soyabean flour at varied concentrations exerted significant influence on larval weight. The 4% concentration of soyabean flour at 4% resulted in significantly higher larval weight (4.167g) in CSR_2, whereas in CSR4, the larval weight was 4.0g at 6% concentration over control batch (Table 1). Perhaps, increase in larval weight in both silkworm breeds may be due to the increased protein synthesis and subsequent accumulation of storage protein in the body on account of additional oral proteins (Vanisree *et al.*, 1996). [27] (Sundar Raj *et al.*, 2000a) [24] have also reported the increased in larval weight (3.78g) in the silkworm hybrid $PM{\times}NB_4D_2$ when fed with protein rich diet. Similar trend was reported by Saravanan Manjula *et al.* (2010a and 2011) [18, 20], Rathinam *et al. (*1994) [15], Ganga and Gowri (1990) [5], Krishnan *et al*, (1995) [8], Chairman *et al.* (2001) [3] and Ravi *et al.* (1994) [16].

3.2 Cocoon weight

Silkworm breeds reared on enriched mulberry leaves with soyabean flour at varied concentrations exhibited significant improvement on cocoon weight. The cocoon weight was 1.820 and 1.800g in CSR2 and CSR4, breeds respectively at 4 and 6% concentrations, against control (Table 1). Significant increase in cocoon weight was might be due to increased proteinaceous source in the diet. These results are on line with the earlier observations of Sundar Raj *et al*. (2000a) [24] who have reported in hybrid $PM{\times}NB_4D_2$ reared on mulberry leaves supplemented with soyabean. Further, silkworms reared on mulberry leaves supplemented with black gram and ground nut seed powder enhanced the cocoon weight (Chairman *et al.*, 2011) [3]. Similar results were observed by Saravanan Manjula *et al.* (2010a and 2011) [19, 20], Rathinam *et al.* (1994) [15], Krishnan *et al.* (1995) [8] and Vanisree *et al.* (1996) [27].

3.3 Shell weight

Supplementation of soyabean flour at varied concentrations on silkworm breeds recorded significant effect on shell weight. The larvae reared on soyabean flour at 4% expressed higher shell weight (0.410g) in CSR_2 and it was 0.390g in CSR4 at 6% over control (Table 1). The increase in shell weight in both breeds might be due to impact of soya protein. These results are in agreement with those of Rathinam *et al.* (1994) [15] and Krishnan *et al.* (1995) [8] who have reported when silkworm were fed with hydrolyzed soya protein (2-2.5%) enhances of shell weight and haemolymph proteins. Similar trend was noticed with cereal flour supplementation by Babu (1994) [2], Sreenivasagaperunal *et al.* (1994) [21], Ganga and Gowri (1990) [5], Saravanan Manjula *et al.* (2010a and 2010b) [18, 19], Sundar Raj *et al.* (2000b and 2001) [25, 26] and Chairman *et al.* (2011) [3].

3.4 Pupal weight

The silkworm breeds supplemented with soyabean flour at 4% exerted higher pupal weight (1.410g) in CSR_2. On the other hand, CSR_4 recorded highest pupal weight (1.403g) over control batch (Table 1). The significant increase in the pupal weight was due to increase in metabolism leads to gain in pupal weight. These results are in agreement with the earlier observations of Sundar Raj *et al.* (2000a and 2001) [24,26] who have noticed the maximum pupal weight (1.498g) in silkworms hybrid $PM\times NB_4D_2$ fed on mulberry leaves supplemented with soyabean flour followed by defatted soyabean (1.475g) over control batch (1.310g). Similar results were observed by Sundar Raj *et al.* (2000b) [25], Saravanan Manjula *et al.* (2010a) [18] and Chairman *et al.* (2011) [3].

3.5 Shell ratio

Supplementation of soyabean flour at different concentrations on silkworm breeds recorded significant improved of shell ratio. The 4% concentration of soyabean flour resulted in significantly higher shell ratio of 22.52% in CSR_2 whereas (21.66%) in CSR_4 at 6%. For this trait control batch recorded 19.59% and 19.16% in CSR_2 and CSR_4, respectively (Table 1). Increase in the shell ratio might be due to enhanced silk productivity by additional supplementation of soyabean flour. These results are also supported by the observations of Sundar Raj *et al.* (2000a) [24] in the silkworm breed NB4D2 reared on mulberry leaf supplemented with soyabean flour recorded significantly higher shell percentage (21.02%) when compared to control (19.35%). Similar trend was also observed by Sundar Raj *et al.* (2001 and 2000 a) [26, 24], Chairman *et al.* (2011) [3], Manjula Saravanan *et al.* (2010a) [18] and Manjula Saravanan *et al.* (2010b) [19].

3.6 Filament length

The silkworms supplemented with soyabean at 4% exerted longer filament length (1023.33m) in CSR_2. While it was 955.0 m in CSR_4 as against control (Table 2). The increase in the filament length may be due to higher silk protein synthesis by additional supplementation of soya protein. These results are in conformity with the finding of Sundar Raj *et al.* (1999) [23] who have opined that supplementation of mulberry leaf with soyabean and ragi flour enhances filament length 615.84 and 508.12m, respectively over unsupplemented batch (488.87m). Similar results was also observed in some other silkworm breeds by Chairman *et al.* (2011) [3], Saravanan Manjula *et al.* (2010a) [18], Sundar Raj *et al.* (2000a) [24] and Sundar Raj *et al.* (2001) [26].

3.7 Denier

Silkworms nourished with mulberry leaves extra foliated with soyabean flour exerted significant influence on denier being lowest (2.583) at 6% and highest (2.913) in control batch of CSR_4. While the breed CSR2 expressed non-significant variation in all concentrations (Table 2). These results are on par with the findings of Sundar Raj *et al.* (2001) [26] in the silkworm race Pure Mysore reared on mulberry leaves supplemented with soyabean flour exhibit finer denier (1.82) against control (2.14). Further, the silkworm hybrid $PM\times NB_4D_2$ reared on mulberry leaves supplemented with soyabean flour exhibited finer denier (2.70) over control batch (2.96) reported by Sundar Raj *et al.* (2000b) [25]. Similar trend was also noticed in some other silkworm breeds by Sundar Raj *et al.* (1999) [23], Saravanan Manjula *et al.* (2010a) [18] and Chairman *et al.* (2011) [3].

3.8 Renditta

The silkworms reared on enriched mulberry leaves with soyabean flour at different concentrations expressed significant influence on renditta. The lowest renditta (6.850) was recorded in CSR_2 at 4% and it was 7.763 in CSR_4 at 6% over control batch (Table 2). Both the breeds expressed improvement for this trait at 4 and 6% concentrations may be due to effective absorption and utilization of soya protein for the formation of cocoons.

3.9 Reelability

Supplementation of soyabean flour with mulberry leaves has significant influence on reelability percentage. The larvae of CSR_2 fed on soyabean flour with mulberry leaves at 4% exhibited highest reelability percentage (80.24%) and it was 75.90% in CSR_4 over control batch (Table 2). The reelability is directly correlated with non-broken filament length. Longer the filament length higher will be the reelability. The better reelability percentage was recorded in CSR_2 and CSR_4 at 4 and 6%, respectively which in turn reflect on higher reelable cocoons.

3.10 Raw silk percentage

Silkworms nourished with mulberry leaves fortified with soyabean flour at varied concentrations registered significant influence on raw silk percentage. The mulberry leaf supplemented with soyabean flour at 4% registered highest raw silk percentage (19.22%) in CSR_2, while it was 18.46% in CSR_4 at 6% as against control (Table 2). Increase in the raw silk percentage may be due to additional supplementation of soyabean nutrients. These results are in conformity with those of Sujatha and Purushotham Rao (2001) [22], who have observed that mulberry leaf fortified with phyto-chemicals such as resepine and picrotoxin enhances the raw silk percentage.

3.11 Effect of supplemented mulberry leaves with soyabean flour on alanine and aspartate aminotransferase activity

The silkworm breed CSR_2 fed on mulberry leaves supplemented with soyabean flour recorded highest level of alanine activity (ALT) and aspartate (AST) aminotransferase in the fat body at 4% concentration (518.0 units/g protein /hour) and (185.66 units/g protein/hour) in fifth instar 6th day larvae followed by (459.8 units/g protein /hour) and (158.5 units/g protein /hour) fifth instar 3rd day (423.4 units/g protein /hour) and (121.3 units/g protein /hour) fifth instar 1st day, respectively. Similar trend was noticed in CSR_4 at 6% concentration (units/g protein /hour) (Tables 3 and 4).

The fat body stores glycogen, proteins, carbohydrates etc, and it is a site of various intermediary metabolic pathways. The fat body tissue synthesis various metabolites and it has intimate contact with the haemolymph responsible for interchanging the metabolites. Hence, it is necessary site to study by biochemical changes during supplementation of soyabean flour. The aminotrasferases are the enzymes play a pivotal role in amino acid metabolism. In insects, the higher activity level of aspartate (AST) and alanine (ALT) aminotransferases was reported in the haemolymph and fat body tissue. The increase in the activity levels of ALT and AST in fat body might be due to additional supplementation of soyabean proteins which increases the formation of free amino acids in the haemolymph as well as fat body tissue. These results are in agreement with the earlier observations of Saravanan Manjula *et al.* (2010a) [18] who have reported the elevation of both AST and ALT enzymes activity in the haemolymph of silkworm breeds supplemented with *Dolichos lablab* flour at 7.5% concentration indicating an active transport of amino acids which provide keto acid to serve as precursor in the synthesis of essential constituents for the synthesis of silk. Similar results was also observed by Manjula Sarvanan *et al.* (2010b) [19] in silkworm fed on mulberry leaves fortified with *Vigna unguiculata* at 7.5% concentration.

In both breeds, the ALT and AST being highest in fifth instar 6th day, followed by fifth instar 3rd day and fifth instar 1st day over control batch. It clearly shows that, the effective absorption and utilization of soya protein with the advancement of age. These results are on line with the earlier observations of Anil Kumar (2009) [1], who has opined that increase in protease activity with advancement of age. Further, the activity level of ALT and AST being maximum in the silkworm breed CSR_2 supplemented with soyabean flour at 4% whereas in CSR_4 at 6%. These results showed that absorption of soyabean flour nutrients varies among silkworm breeds due to differing in their genetic constitution.

Table 1: Effect of fortified mulberry leaf with soyabean flour on rearing traits of bivoltine silkworm breeds.

Silkworm breed	Treatment	Mature larval weight (g)	Cocoon weight (g)	Shell weight (g)	Pupal weight (g)	Shell ratio (%)
CSR_2	2%	3.970 ± 0.017	1.760 ± 0.012	0.370 ± 0.006	1.390 ± 0.006	21.01 ± 0.191 (27.27 ± 0.134)
	4%	4.167 ± 0.041	1.820 ± 0.012	0.410 ± 0.006	1.410 ± 0.006	22.52 ± 0.173 (28.32 ± 0.119)
	6%	3.877 ± 0.015	1.700 ± 0.012	0.340 ± 0.006	1.360 ± 0.006	19.99 ± 0.202 (26.55 ± 0.145)
	Control	3.787 ± 0.026	1.633 ± 0.020	0.320 ± 0.006	1.313 ± 0.015	19.59 ± 0.115 (26.26 ± 0.083)
	F-test	*	*	*	*	*
	SE(m)±	0.027	0.014	0.006	0.009	0.173 (0.122)
	SE(d)±	0.038	0.020	0.008	0.012	0.245 (0.173)
	C.D. at 5%	0.088	0.047	0.019	0.029	0.574 (0.405)
	C.V (%)	1.167	1.427	2.778	1.116	1.446 (0.782)
CSR_4	2%	3.673 ± 0.015	1.680 ± 0.006	0.343 ± 0.009	1.337 ± 0.003	20.43 ± 0.456 (26.86 ± 0.323)
	4%	3.760 ± 0.023	1.733 ± 0.018	0.370 ± 0.006	1.363 ± 0.017	21.34 ± 0.335 (27.50 ± 0.235)
	6%	4.000 ± 0.025	1.800 ± 0.012	0.390 ± 0.006	1.403 ± 0.017	21.66 ± 0.277 (27.73 ± 0.193)
	Control	3.590 ± 0.006	1.670 ± 0.006	0.320 ± 0.006	1.350 ± 0.010	19.16 ± 0.383 (25.94 ± 0.278)
	F-test	*	*	*	*	*
	SE(m)±	0.019	0.011	0.007	0.013	0.369 (0.262)
	SE(d)±	0.027	0.016	0.009	0.018	0.521 (0.370)
	C.D. at 5%	0.062	0.037	0.022	0.043	1.221 (0.867)
	C.V (%)	0.866	1.138	3.245	1.640	3.092 (1.679)

*: Significant at 5% level. ( ): Angular transformed values. ±: Standard error values

Table 2: Effect of fortified mulberry leaf with soyabean flour on reeling traits of bivoltine silkworm breeds

Silkworm breed	Treatment	Filament length (m)	Denier	Renditta	Reelability (%)	Raw silk (%)
CSR_2	2%	949.33 ±6.066	2.833 ± 0.024	7.120 ± 0.012	80.11 ± 0.000 (63.49 ± 0.017)	18.17 ± 0.208 (25.22 ± 0.154)
	4%	1023.33 ± 6.643	2.803 ± 0.064	6.850 ± 0.058	80.24 ± 0.025 (63.58 ± 0.023)	19.22 ± 0.196 (25.99 ± 0.143)
	6%	917.33 ± 6.361	2.843 ± 0.049	7.250 ± 0.029	79.92 ± 0.046 (63.35 ± 0.032)	17.06 ± 0.456 (24.38 ± 0.347)

	Control	881.33 ± 10.75	2.887 ± 0.009	7.550 ± 0.029	79.71 ± 0.032 (63.21 ± 0.023)	15.93 ± 0.549 (23.51± 0.430)
	F-test	*	NS	*	*	*
	SE(m)±	7.694	0.042	0.036	0.031	0.384
	SE(d)±	10.881	0.060	0.051	0.044	0.543
	C.D. at 5%	25.480	--	0.119	0.103	1.273
	C.V (%)	1.413	2.570	0.863	0.068	3.783
CSR$_4$	2%	917 ± 4.041	2.800 ± 0.026	8.167 ± 0.120	75.53 ± 0.038 (60.33 ± 0.025)	17.60 ± 0.169 (24.79± 0.127)
	4%	938 ± 3.754	2.680 ± 0.012	7.933 ± 0.024	75.74 ± 0.037 (60.47 ± 0.023)	18.19 ± 0.022 (25.23 ± 0.016)
	6%	955 ± 2.903	2.583 ± 0.018	7.763 ± 0.058	75.90 ± 0.017 (60.58 ± 0.012)	18.46 ± 0.090 (25.43 ± 0.066)
	Control	894 ± 3.464	2.913 ± 0.020	8.503 ± 0.062	75.34 ± 0.035 (60.20 ± 0.021)	16.58 ± 0.263 (24.02 ± 0.203)
	F-test	*	*	*	*	*
	SE(m)±	3.567	0.020	0.075	0.030 (0.021)	0.030 (0.125)
	SE(d)±	5.044	0.028	0.105	0.042 (0.030)	0.042 (0.176)
	C.D. at 5%	11.81	0.065	0.247	0.098 (0.069)	0.098 (0.412)
	C.V (%)	0.667	1.245	1.595	0.068 (0.060)	0.068 (0.867)

*: Significant at 5% level. ( ): Angular transformed values. ±: Standard error values.

Table 3: Influence of fortified mulberry leaf with soyabean flour on alanine aminotransferase activity of bivoltine silkworm breeds (units/g protein/h)

Silkworm breed	Treatment/Larval stage	2%	4%	6%	Control
CSR$_2$	V instar 1^{st} day	359.0 ± 6.8	423.4 ± 10.0	314.5 ± 5.4	294.5 ± 5.3
	V instar 3^{rd} day	390.6 ± 7.1	459.8 ± 6.8	361.5 ± 6.9	325.3 ± 3.6
	V instar 6^{th} day	423.6 ± 1.9	518.0 ± 10.8	387.9 ± 2.4	360.0 ± 6.6
	F-test	*	*	*	*
	SE(m)±	5.752	9.386	5.236	5.290
	SE(d)±	8.134	13.274	7.405	7.482
	C.D. at 5%	20.3	33.1	18.5	18.7
	C.V (%)	2.547	3.481	2.557	2.806
CSR$_4$	V instar 1^{st} day	306.433 ± 3.932	321.6 ± 2.871	366.9 ± 11.641	286.9 ± 5.866
	V instar 3^{rd} day	382.267 ± 1.541	396.233 ± 3.907	419.2 ± 6.428	354.167 ± 4.927
	V instar 6^{th} day	426.533 ± 1.688	470.567 ± 8.011	557.133 ± 7.22	383.033 ± 2.645
	F-test	*	*	*	*
	SE(m)±	2.625	5.406	8.737	4.679
	SE(d)±	3.713	7.645	12.355	6.617
	C.D. at 5%	9.262	19.071	30.82	16.506
	C.V (%)	1.223	2.364	3.38	2.374

*: Significant at 5% level. ±: Standard error values

Table 4: Influence of fortified mulberry leaf with soyabean flour on aspartate aminotransferase activity in the bivoltine Silkworm breeds (units/g protein/h)

Silkworm breed	Treatment/Larval stage	2%	4%	6%	control
CSR$_2$	V instar 1^{st} day	94.157 ± 1.489	121.3 ± 1.747	85.597 ± 1.293	70.923 ± 1.764
	V instar 3^{rd} day	109.067 ± 1.291	158.5 ± 5.701	102 ± 1.513	91.957 ± 2.133
	V instar 6^{th} day	151.367 ± 3.941	185.633 ± 7.137	144.067 ± 2.162	134.5 ± 0.643
	F-test	*	*	*	*
	SE(m)±	2.544	5.369	1.697	1.641
	SE(d)±	3.597	7.593	2.4	2.32
	C.D. at 5%	8.974	18.942	5.986	5.787
	C.V (%)	3.728	5.994	2.658	2.867
CSR$_4$	V instar 1^{st} day	115.2 ± 3.308	149.667 ± 5.943	172.7 ± 3.843	105.2 ± 1.997
	V instar 3^{rd} day	163.667 ± 3.196	190.267 ± 1.298	208.4 ± 3.36	140.367 ± 1.77
	V instar 6^{th} day	201.5 ± 2.589	231.867 ± 2.339	278.567 ± 7.308	164.367 ± 4.463
	F-test	*	*	*	*
	SE(m)±	3.048	3.763	5.147	3.002
	SE(d)±	4.31	5.321	7.279	4.246
	C.D. at 5%	10.751	13.273	18.157	10.591
	C.V (%)	3.297	3.419	4.054	3.805

*: Significant at 5% level. ±: Standard error values

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