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Pyridoxine as a nutrient supplement with mulberry leaf and its impact on succinate dehydrogenase activity and economic traits of the silkworm, *Bombyx mori* l

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Abstract
The silkworm, *Bombyx mori* L. is a monophagous insect and consumes only mulberry leaves during larval stage of its life cycle. The performance of silkworm such as growth, reproductive potentiality and quantum and quality of cocoon production depends on nutrient composition of food, which includes both absolute and relative amounts of proteins, amino acids, lipids, carbohydrates, sterols, water, minerals, vitamins, etc., besides its genetic endowment. The silkworm requires several vitamins for their growth and survival. The pyridoxine is necessary for the proper functioning of over 60 enzymes that participate in amino acid metabolism. In the present investigation, the silkworm bivoltine hybrids namely FC_1 and FC_2 were taken and mulberry leaf supplemented with pyridoxine at varied concentrations *viz*., 100, 500 and 1000 ppm. Both hybrids performed well for the most of the economic traits except denier at 1000 ppm. Further, the succinate dehydrogenase activity level was more in FC_2 when compared to FC_1 at 1000 ppm in fifth instar fifth day followed by fifth instar third day and fifth instar first day larvae.

Keywords: *Bombyx mori* L, economic traits, pyridoxine, succinate dehydrogenase

1. Introduction

India is the second largest producer of mulberry silk next only china (Vijayaprakash and Dandin, 2005) [22]. The mulberry silk contributes over 76% of raw silk produced by sercigenous insects in the country. In order to achieve higher productivity of mulberry silk, we should have high yielding mulberry varieties and silkworm breeds. In addition to this, quality of mulberry leaf should be nutritionally rich. The quality of mulberry leaves is of outmost importance for the production of good quality cocoons.

Legay (1958) [10] states that silkworm nutrition is a major area of research in sericulture. Pant (1978) [15] envisaged great scope of utilizing data for proper exploitation of beneficial insects like silkworm and stressed that the qualitative and quantitative aspects of cocoon can be directly increased through proper dietary management. Hence, proper care of silkworm through dietary management is an essential requisite to maximum sericultural output and augments the economy of peasants in sericulture. The knowledge of silkworm nutrition is of great applied value. Nutrition involves chemical and physiological activities which transforms food element into body elements. Insect nutrition primarily possesses biochemical substances that are necessary to activate various metabolic processes resulting in growth and development.

The vitamins are organic compound and which is required by all living organisms including insects in little quantity for the proper growth and development. Generally, vitamins are synthesized by plant and are found in animals as a result of food intake. (Ravi Kumar and Anil Kumar, 2016) [19]. Some of them are synthesized by the living organism and it is also become part of the enzyme system. The vitamin deficiency in insects can be overcome by supplementation of vitamins rich diet.

It is almost certain that dose differences among various vitamins reflect their specificity of metabolic function. Pyridoxine plays a pivotal role in amino acid, carbohydrate and fat metabolism.

In silkworm, growth is quite improved by adding pyridoxine to the diet which inturn reflect on commercial characters. It is well known fact that, the vitamins are required for proper functioning of enzymes systems. One such enzyme selected for the present study is succinate dehydrogenase (SDH) involved in biological oxidation of carbohydrate.

The SDH is found in inner membrane of mitochondria oftenly referred as mitochondrial index enzyme and it is present at lowest concentration. The SDH catalyses the reaction in the Krebs cycle and help to forming fumarate from succinate. The activity level of SDH has been studied in a wide range of insects by many workers who have defined the enzyme in egg, larva, pupa and adult stages.

In some selected silkworm breeds, the activity level of SDH has positive correlation with most of the economic characters except renditta was reported by Kasmaei and Mahesha (2012) [8]. Further, gel electrophoresis study revealed that, variations in enzyme activity among silkworm breeds and hybrids was observed by Mahesha *et al*. (2015) [11]. The increase in the activity levels of succinate dehydrogenase and amylase in F_1 progeny raised from EMS treated silkworm batches at varied concentrations was also reported by Mahesha and Honnaiah (2002) [12]. However, not much information available on mulberry leaf supplemented with pyridoxine on succinate dehydrogenase activity and economic traits of silkworm particularly double hybrids. Hence, the present investigation was undertaken.

2. Materials and Methods

The bivoltine silkworm hybrids namely FC_1 and FC_2 were taken for the present experiment and silkworm were reared by employing standard rearing technique advocated by Dandin and Giridhar (2010) [6].

Supplementation of Pyridoxine

Pyridoxine at varied concentrations *viz.*, 100, 500 and 1000 ppm were sprayed on ventral surface of mulberry leaf and surface dried under shade and fed to the silkworms. The silkworm larvae divided into four batches *viz.*, batch I (T_1), batch II (T_2) batch III (T_3) were reared with pyridoxine at 100, 500 and 1000 ppm, respectively along with control batch IV (T_4) reared on mulberry leaves supplemented with distilled water. The treated leaves are fed to silkworm once in a day (morning) during fourth and fifth instar. In each treatment three replications were maintained. A minimum of ten larvae were used from each batch to record the economic traits namely larval weight, cocoon weight, shell weight, shell ratio, filament length, filament weight, denier and renditta. The parameters namely shell percentage, denier and renditta were calculated by using following formulae.

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

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

$$\text{Renditta} = \frac{\text{Weight of the cocoons reeled}}{\text{weight of the silk obtained}}$$

Estimation of succinate dehydrogenase (SDH) activity level in selected bivoltine hybrids.

The succinate dehydrogenase activity was estimated in fifth instar 1^{st} day, 3^{rd} day and prior to spinning stage (5^{th} day) of bivoltine hybrids FC_1 and FC_2 in respective treatments and control batches. 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 SDH activity was estimated by the method of Nachlas *et al.* (1960) [13]. 1ml of tissue extract was incubated with 1ml of sodium succinate, 1ml of phosphate buffer, 1ml of INT and reaction mixture was incubated in water bath at 37^0 C for 1 h. The enzyme activity was stopped by adding 6ml of glacial acetic acid followed by 6ml of toluene. The reaction mixture was kept overnight at refrigerator. The colour intensity was measured at 495nm by using spectrophotometer. The standard curve was used for calculation. The enzyme activity was expressed in terms of µ moles of formazone (product generated) /g protein/h. The mean values were expressed.

The data obtained were analyzed by standard deviation (±) method and mean values were expressed

3. Results and discussion

In general, plant synthesizes large amount of vitamins. A few animals and insects also synthesizes 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. It is well documented that the different nutrients and vitamins required at specific dose to perform various catabolic and anabolic process in *Bombyx mori*.

The Pyridoxine (B_6) oftenly referred to as B complex vitamins and widely distributed in nature. It play pivotal role in amino acid metabolism and also serve as a co-enzyme in non-oxidative decarboxylation of some amino acids. It is essential for the conversion of tryptophan to niacin and interconversion of glycine and serine. It is also help to synthesis neuro secretary hormone. The Pyridoxine also required for synthesis of sphingosine from serine and architonic acid from linoleic acid.

In silkworm, *B. mori* pyridoxine deficiency leads to retard in growth of silk gland, reduction in protein in haemolymph, increase in uric acid excretion and alteration in free amino acid composition of haemolymph was also reported by Horie and Watanabe (1983) [9]. The data pertained to proposed work tabulated in Table 1 and Table 2.

Table 1: Influence of supplemented mulberry leaves with pyridoxine at varied concentrations on succinate dehydrogenase activity level in the fat body of bivoltine hybrids.

Treatment (ppm)	Hybrid	V Instar 1st day	V Instar 3rd day	V Instar 5th day
100	FC_1	2.650 ± 0.087	2.833 ± 0.093	2.910 ± 0.020
	FC_2	2.857 ± 0.035	2.953 ± 0.025	3.270 ± 0.020
500	FC_1	2.953 ± 0.035	3.030 ± 0.044	3.126 ± 0.025
	FC_2	3.033 ± 0.059	3.257 ± 0.042	3.480 ± 0.030
1000	FC_1	3.042 ± 0.044	3.273 ± 0.021	3.353 ± 0.035

	FC_2	3.130 ± 0.082	3.353 ± 0.015	3.553 ± 0.031
Control	FC_1	2.613 ± 0.032	2.733 ± 0.015	2.820 ± 0.030
	FC_2	2.777 ± 0.015	2.970 ± 0.020	3.033 ± 0.076

Table 2: Influence of supplemented mulberry leaves with pyridoxine at varied concentrations on larval and cocoon characters

Treatment (ppm)	Hybrid	Matured Larval Weight (g)	Cocoon Weight (g)	Shell Weight (g)	Shell Percentage (%)	Filament Length (m)	Filament Weight (g)	Denier	Renditta
100	FC1	3.381±0.09	1.305±0.005	0.242±0.003	18.54±0.273	1015±8.505	0.192±0.000	1.709±0.018	6.775±0.027
	FC2	3.622±0.004	1.314±0.007	0.261±0.001	19.86±0.140	1185±2.646	0.206±0.006	1.565±0.047	6.380±0.167
500	FC1	3.358±0.059	1.333±0.004	0.249±0.001	18.57±0.074	1043±7.572	0.204±0.002	1.759±0.026	6.570±0.035
	FC2	3.630±0.004	1.339±0.006	0.268±0.001	20.08±0.057	1194±1.528	0.215±0.002	1.619±0.014	6.202±0.029
1000	FC1	3.827±0.004	1.372±0.003	0.262±0.001	19.10±0.040	1074±12.66	0.217±0.007	1.815±0.047	6.333±0.180
	FC2	3.903±0.004	1.512±0.009	0.331±0.001	21.90±0.051	1204±3.000	0.246±0.003	1.839±0.017	6.148±0.087
Control	FC1	3.256±0.004	1.285±0.004	0.123±0.001	18.10±0.044	992±2.000	0.185±0.003	1.682±0.034	6.933±0.144
	FC2	3.537±0.004	1.305±0.003	0.237±0.001	18.21±0.101	1174±2.082	0.199±0.002	1.528±0.009	6.548±0.042

Influence of supplemented mulberry leaves with pyridoxine on SDH activity

The fat body has a storage function of reserve materials such as glycogen, proteins, etc., and carries out synthesis and intermediary metabolism of various nutritional substances. The fat body functions not only as a depot for storage materials but also a controller of histolysis of silk gland. In *B. mori* the fat body is the major tissue for the synthesis of various metabolites and the haemolymph which is in intimate contact with the fat body, acts as a medium for the interchange of metabolites. Thus it is reasonable to expect that the disruption of the fat body and correlated with biochemical parameters of the haemolymph. The SDH in vertebrate mitochondria is a ferro-flavo protein, which removes hydrogen from succinate and transfers two electrons to cytochrome-b. The activity level of SDH varies not only in different insect species but also in the same species at different stages of metamorphosis. Hence, in the present investigation was undertaken to record the impact of mulberry leaves supplemented with pyridoxine on succinate dehydrogenase activity in the fat body of selected bivoltine hybrids.

The silkworm hybrids FC_2 and FC_1 fed on mulberry leaves supplemented with pyridoxine recorded highest activity level of SDH in the fat body at 1000 ppm concentration (3.553µM of formazone/g protein/h) and (3.353µM of formazone/g protein/h) respectively, in V-instar 5th day larvae. Similar trend was noticed in V-instar 3rd day and V-instar 1st day in both the hybrids at 500 and 100 ppm (Table1).

The oxidation of carbohydrates like pyruvate is achieved by a series of dehydrogenation and decarboxylation after condensation of the active two carbon fragment with oxaloacetic acid and is almost certainly the main metabolic pathway for the complete oxidation to carbon dioxide and water. Increased in levels of SDH activity might be due to addition supplementation of riboflavin helps to convert flavo protein which act as an important hydrogen carriers in biological oxidation. The data pertaining to the present study revealed that FC_2 expresses higher activity levels of SDH when compare to FC_1. It clearly indicates that the utilization of riboflavin differed genetically among the hybrids.

In both the hybrids, the activity levels of SDH was highest in V-instar 6th day, followed by V-instar 3rd day and V-instar 1st day over control batches. It clearly depicts that effective utilization of additional supplementation of pyridoxine with advancement of age. These results are in line with the earlier observations of Anil Kumar (2009) [1] who opined that increase in protease activity with advancement of age.

Larval weight

Silkworm hybrids reared on fortified mulberry leaves with pyridoxine at different concentrations exhibited notable influence on larval weight with maximum being in FC_2 (3.903 g) and FC_1 (3.827 g) at 1000ppm concentration over control batch (Table 2). The increase in larval weight might be due to additional supplementation of pyridoxine along with mulberry leaves. The present findings are in conformity with the findings of Balasundaram *et al.* (2013a) [4] who reported that the supplementation of 0.2% ascorbic acid found to be optimum in which maximum larval weight noticed among different concentrations in bivoltine silkworm hybrid (CSR_2 x CSR_4). Similar results were also observed with supplementation of folic acid, para-amino benzoic acid and combination of both in the silkworm larvae NB_4D_2 by Singaravelu *et al.* (2001) [20].

Cocoon weight

Supplementation of pyridoxine at varied concentrations on silkworm hybrids registered encouraging results on cocoon weight. The larvae reared on pyridoxine at 1000 ppm expressed higher cocoon weight of 1.512 and 1.372g in FC_2 and FC_1, respectively (Table 2). The increase in cocoon weight in both breeds might be due to increase in absorption of pyridoxine by midgut epithelial cells followed by absorption by different body cells and transformation to cellular structure. These results are in agreement with those of EL-Karaksy and Idriss (1990) [7] who noticed that silkworm hybrid (155 x 156) reared on mulberry leaf supplemented with folic acid at 2% recorded significantly higher cocoon weight over other concentrations as well as control batch. Similar results was also observed on some other vitamins by (Babu *et al*., 1992; Prasad *et al*., 1994; Nirwani and Kaliwal 1995; Singaravelu *et al*., 2001; Rai *et al*., 2002; Rahmathulla *et al*., 2007; Tantray and Kanika Trivedy 2011; Balasundaram *et al*., 2013) [2, 16, 14, 20, 4, 18, 23, 4].

Shell weight

Silkworms nourished with mulberry fortified with pyridoxine at different concentrations registered notable influence on shell weight. The worms supplemented with pyridoxine at 1000 ppm exerted higher shell weight (0.331g) in FC_2. On the other hand, FC_1 recorded highest shell weight of 0.262g over control batch (Table 2). The increase in shell weight might be due to additional supplementation of pyridoxine which enhances the biosynthesis of silkworm protein. These results corroborate the earlier findings of Balasundaram *et al*. (2013) [4] who opined that supplementation of mulberry leaves with folic acid at the rate 0.2% enhance shell weight in the silkworm hybrid (CSR_2 x CSR_4). Similar trend also noticed in some other vitamins by (Prasad *et al.*, Nirwani and Kaliwal, 1995; Singaravelu *et al.*, 2001; Rai *et al*., 2002; Rahmathulla *et al.*, 2007; Tantra and Kanika Trivedy 2011) [16, 14, 20, 18, 17, 23].

Shell percentage

Silkworms reared on mulberry leaves extra foliated with pyridoxine exerted marked influence on shell ratio. A concentration of pyridoxine at 1000 ppm resulted in higher shell ratio of 21.90 and 19.10% in FC_2 and FC_1, respectively when compared to control batch (Table 2). Increase in the shell ratio might be due to enhanced silk productivity by additional supplementation of pyridoxine. These results are supported by the observations of Rahmathulla *et al.* (2007) [17] who opined that the bivoltine hybrid (CSR_2 x CSR_4) administrated with folic acid at a concentrations of 100 and 150 ppm exhibit significantly higher shell ratio over other batches. Similarly, administration of ascorbic acid at 0.2% concentration increases shell ratio in bivoltine hybrid (CSR_2 x CSR_4) (Balasundaram *et al*., 2013b) [5].

Filament length

Filament length has positive correlation with shell weight. The silkworm fed on mulberry leaf fortified with pyridoxine at varied concentrations registered marked influence on filament length. The bivoltine hybrids FC_2 and FC_1 supplemented with pyridoxine at 1000 ppm exerted longer filament length 1204 and 1074m over other concentrations and control batch (Table 2). The increase in filament length might be due to higher rate of silk protein synthesis by additional supplementation of pyridoxine. These results are in conformity in the finding of Rai *et al*. (2012) who reported that administration of folic acid through mulberry leaves facilitated nucleic acid synthesis in silk gland cells inturn improve the absolute silk content in the shell. Similar results was also reported in bivoltine hybrid (CSR_2 x CSR_4) supplemented with ascorbic acid with 0.2% over control batch (*Balasundaram et al*., 2013b) [5].

Filament weight

Silkworm hybrids reared on fortified mulberry leaves with pyridoxine at varied concentrations exhibited considerable impact on filament weight. In the study, FC_2 and FC_1 expressed gain in the filament weight of 0.246 and 0.217g, respectively with pyridoxine supplementation at 1000 ppm (Table 2). The increase in filament weight in both the hybrids might be due to higher rate of biosynthesis of silk protein by additional supplementation of pyridoxine. These results are agreement in the findings of Singaravelu *et al*. (2001) [20] who observed that supplementation of mulberry leaves with combination of folic acid and para amino benzoic acid increase filament weight.

Denier

Silkworm nourished with mulberry leaves extrafoliated with pyridoxine at lower concentration registered encouraging results than the higher. The larvae reared on pyridoxine supplementation at 100 ppm expressed lower denier of 1.56 in FC_2 and 1.70 in FC_1 (Table 2). These results are in conformity in the finding of Babu *et al*. (1992) [2] who opined that fine denier was observed when the silkworm larvae provided with 1.5% folic acid through diet.

Renditta

The silkworms reared on fortified mulberry leaves with pyridoxine at different concentrations expressed encouraging results in respect of renditta. The lowest renditta of 6.148 and 6.333kg were recorded in FC_2 and FC_1, respectively (Table 2). The improvement for this trait in both bivoltine hybrids at 1000 ppm of pyridoxine supplementation might be due to effective utilization of this vitamin in transamination reactions inturn reflect on cocoon shell formation. These results are in agreement with the findings of Sridhar and Radha (1987) who noticed that silkworm reared on mulberry leaf supplemented with 10 ppm concentration of glycine exerted significant reduction in renditta.

4. Conclusion

The results of present study inferred that, administration of pyridoxine through the diet at 1000 ppm concentration enhances all the economic traits such as larval weight, cocoon weight, shell weight, filament length, filament weight and renditta except denier in FC2 than FC1 bivoltine hybrid. Similarly the activity levels of succinate dehydrogenase was relatively high in FC2 when compare to FC1. However, both hybrids performed well in respect to economic traits with the supplementation of pyridoxine. Hence farmer could be prescribed to use to this vitamin to get better cocoon yield.

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