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Effect of mulberry leaf fortified with thiamine on economic traits silkworm and cocoon parameters selected bivoltine hybrids

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Abstract

The investigation was carried out at Department of Sericulture, in University of Mysore, Manasagangotri during 2019. The work was undertaken to study on influence of supplementation of Thiamine with mulberry leaf on selected bivoltine hybrids FC_1 and FC_2 . Supplementation is a modern technique in advanced sericulture research and development industry to enhance the cocoon yield through supplementation of amino acid on mulberry leaf. Amino acids play an important role in the growth and development of silkworm, *Bombyx* mori. The selected bivoltine hybrids FC_2 and FC_1 performed better in respect of economic traits such as larval weight (3.73 and 3.71g), cocoon weight (1.62 and 1.48g), shell weight (0.30 and 0.29g), shell ratio (23.54 and 21.10 %), filament length (1678 and 1513m), filament weight (0.23 and 0.22g), denier (1.17 and 1.23) and renditta (5.12 and 5.52kg) in 600ppm of Thiamine supplementation when compared to other concentrations and control, Further FC_2 showed better reading compared to FC_1 .

Keywords: Mulberry leaf fortified, thiamine, cocoon parameters, bivoltine hybrids

Introduction

The silkworm, *Bombyx mori* L. is monophagous insect and consumes only mulberry leaves during larval stage of its life cycle. The mulberry silk contributes over 69.1% of raw silk produced by sercigenous insects in the country. India ranks second in mulberry silk production in the world, accounting 16% of world's raw silk production. In order to achieve higher productivity of mulberry silk, we should have high yielding mulberry varieties and silkworm breeds, besides providing quality nutrition to silkworm. The quality of mulberry leaves is of outmost importance for production of good quality of cocoons.

Legay (1958)^[6] states that silkworm nutrition is a major area of research in sericulture. Pant (1978)^[9] envisaged great scope of utilizing data for proper exploitation of beneficial insects like silkworm and stressed that the qualitative and quantitative aspects of yield 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 to stabilize and augment 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 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 term 'vitamin' is referred as an accessory indispensable food factor, organic in nature, required by an organism in small amount to maintain normal growth and regulation of metabolism. Generally, vitamins are synthesized by plant and are found in animals as a result of food intake or the activity of the micro-organism in the gut. Some vitamins become part of the enzyme system, which is actively, involved in the enzyme action. Lack of any vitamins in the diet of the young animals prevents growth. Vitamin deficiency disease in organisms can be cured or prevented by supplementation of the vitamin rich food.

The essential vitamins are choline, inositol, nicotinic acid, pantothenate, pyridoxine, riboflavin, thiamine, biotin and folic acid. However, the phenomena of vitamin deficiency are varied according to kinds of vitamins required by the silkworm. For instance, deficiency of biotin leads to retardation of growth by altering fatty acid synthesis. It is generally accepted that vitamins are required at a catalytic level because of their function; choline and inositol are, however, required by the silkworm at a far higher level than other vitamins. Thus, choline and inositol are not useful for catalytic function, but for lipogenic precursors. The vitamin pyridoxine is the precursors for co-enzymes needed for aminotraferases enzymes.

The thiamine is water soluble vitamin and is necessary for the proper functioning of many co-enzymes that are participate in amino acid, carbohydrate and fatty acid metabolism and helps in overall growth and development of silkworms.

Materials and Methods

Rearing and maintenance of silkworm larvae

The bivoltine silkworm hybrids namely FC_1 and FC_2 (Plate.1) was taken for the present investigation. The selected silkworm races were reared by employing standard rearing techniques advocated by Dandin and Giridhar (2010)^[4].





FC1 bivoltine silkworm



FC1 bivoltine cocoons



Plate 1: Larvae and cocoons of bivoltine silkworm hybrid fed on mulberry leaves fortified with thiamine

Supplementation of thiamine

Thiamine at varied concentrations *viz.*, 200, 400 and 600ppm concentrations were sprayed on ventral surface of mulberry leaf and surface dried under shade and fed to the silkworms. The treated leaves are fed to silkworm once in a day (morning) during fourth and fifth instar. The batch I (T_1) larvae received 200ppm thiamine sprayed mulberry leaves, the batch II (T_2) larvae received 400ppm thiamine sprayed mulberry leaves, the batch III (T_3) larvae received 600ppm thiamine sprayed mulberry leaves and the batch IV (T_4) larvae reread on mulberry leaves sprayed with distilled water (control). In each treatment three replications were maintained.

Observations recorded

1. Larval weight (g)

Ten larvae were randomly selected in each replication of

every treatment and weighed just before spinning and average single larval weight was computed.

2. Cocoon weight (g)

Ten cocoons were randomly selected from each treatment replication-wise, weighed individually and average single cocoon weight was computed.

3. Shell weight (g)

After removing the pupa and larval exuvium from the cocoons, the individual shell weight was recorded.

4. Shell ratio (%)

The shell ratio was calculated using the formula:

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

5. Filament Length (m)

Ten cocoons were randomly selected from each batch was reeled to find out the single filament length of the cocoon using epprouvette and was determined by adopting the formula:

$L = R \times 1.125$

R = Number of revolutions recorded by an epprouvette. 1.125 = Circumference of epprouvette in meter.

6. Filament Weight (g)

Ten cocoons were randomly selected from each batch was reeled and average filament weight was recorded.

7. Denier

This denotes the thickness of the filament, 9000 meters of the silk filament weighing 1g is considered as 1 denier. It was calculated using following formula:

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

9. Renditta

This is a measure of actual silk available from the cocoons. The renditta was expressed as the quantity of green cocoons required to get a kg of raw silk.

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

Results and Discussion

Influence of mulberry leaves supplemented with thiamine on different economic traits of bivoltine silkworm hybrids are.

Larval weight

Silkworms fed on mulberry leaves supplemented with thiamine expressed difference in respect of larval weight with maximum being in FC₂ and FC₁ at 600ppm (3.73 and 3.71g) followed by 400ppm (3.68 and 3.66g), respectively. While it was minimum at 200ppm in FC₁ and FC₂ (3.55 and 3.58g) when compared to control batches (3.48 and 3.52g), respectively (Table.1). The increase in larval weight might be due to additional supplementation of thiamine along with mulberry leaves. The present finds were in conformity with the findings of Balasundaram *et al.* (2013b) ^[3] who have reported that the supplementation of ascorbic acid at 0.2 %

found to be optimum in which gain in the larval weight of 3.54g was noticed over other concentrations as well as control batch (2.71g) in bivoltine silkworm hybrid (CSR₂ x CSR₄). Similar results were also observed with supplementation of folic acid, para-amino benzoic acid and combination of both in the silkworm larvae NB₄D₂ by Singaravelu *et al.* (2001)^[3].

Cocoon weight

The hybrids FC_2 and FC_1 exhibit highest cocoon weight on extra foliated mulberry leaves with thiamine at 600ppm (1.62 and 1.48g) followed by 400ppm (1.45 and 1.39g), respectively. In contrast, it was lowest at 200ppm in FC₂ (1.35g) and FC₁ (1.34g). However, larval fed on mulberry leaves sprayed with distilled water registered a cocoon weight of 1.22 and 1.21g in FC₂ and FC₁, respectively (Table.1). The increase in cocoon weight in both breeds might be due to increase in absorption of thiamine 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)^[5] 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 were 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; Tantra and Kanika Trivedy 2011; Balasundaram *et al.*, 2013a)^[1, 10, 7, 13, 12, 11, 15, 2].

Shell weight

Shell weight in the indicator of shell yield and it is positively correlated with length of the shell filament. The larvae reared on mulberry leaves fortified with thiamine at varied concentrations registered notable variation with respect to shell weight. The highest shell weight was observed on mulberry leaves supplemented with thiamine at 600ppm in FC_2 and FC_1 (0.30 and 0.29g) followed by 400ppm (0.28 and 0.27g), respectively. While it was least at 200ppm in FC₁ $(0.21 \text{ and } FC_2 (0.23g))$. The minimum shell weight of 0.22 and 0.23g were noticed in control batches of FC_1 and FC_2 , respectively (Table.1). The increase in shell weight might be due to additional supplementation of thiamine which enhances the biosynthesis of silkworm protein. These results corroborate the earlier findings of Balasundaram et al. (2013a)^[2] who have opined that supplementation of mulberry leaves with ascorbic acid at the rate 200ppm enhance shell weight of 0.80g over control batch (0.63g) in the silkworm hybrid CSR₂ x CSR₄. Similar trend also noticed in some other vitamins by (Prasad et al., Nirwani and Kaliwal, 1998; Singaravelu *et al.*, 2001; Rai *et al.*, 2002; Rahmathulla *et al.*, 2007; Tantra and Kanika Trivedy 2011)^[10, 8, 13, 12, 11, 15].

Shell ratio

The hybrids FC_2 and FC_1 fed on mulberry leaves fortified with thiamine at 600ppm recorded highest shell ratio (23.54 and 21.10%) followed by 400ppm (20.88 and 19.07%), respectively. On the other hand, it was lowest for said trait at 200ppm in FC_1 (18.85%) and FC_2 (20.38%) over control batches of FC_1 (16.93%) and FC_2 (17.46%), respectively (Table.1). Increase in the shell ratio might be due to enhanced silk productivity by additional supplementation of thiamine. These results are supported by the observations of Rahmathulla *et al.* (2007) ^[11] who have observed that the bivoltine hybrid $CSR_2 \times CSR_4$ administrated with folic acid at concentrations of 100 and 150 ppm exhibit significantly higher shell ratio of 24.08 and 25.25% over control batch (22.85%). Similarly, administration of ascorbic acid at 0.2 % concentration increases shell ratio in bivoltine hybrid (CSR₂ x CSR₄) (Balasundaram *et al.*, 2013a)^[2].

Filament length

Filament length is one of the major contributing quantitative traits in silkworms. The silkworm reared on mulberry leaves supplemented with thiamine noticed marked differences in respect of filament length with maximum being in FC₂ and FC₁ at 600ppm (1678 and 1513m) followed by 400ppm (1398 and 1299m), respectively. However, minimum filament length of 1105m and 1107m were recorded at 200ppm in FC₁ and FC_2 when compared to control batches of FC_1 (862m) and FC₂ (949m), respectively (Table.1). The increase in filament length might be due to higher rate of silk protein synthesis by additional supplementation of thiamine. These results are in conformity in the finding of Rai et al. (2002)^[22] who have reported that administration of folic acid through mulberry leaves facilitated nucleic acid synthesis in silk gland cells in turn improve the absolute silk content in the shell. Similar results are also reported in bivoltine hybrid (CSR₂ x CSR₄) supplemented with folic acid with 200ppm enhanced filament length 965m over control batch 919m (Balasundaram et al., 2013a)^[2].

Filament weight

The hybrids FC₂ and FC₁ expressed higher filament weight on fortified mulberry leaves with thiamine at 600ppm (0.23 and 0.22g) followed by 400ppm (0.22 and 0.21g), respectively. As against to this, it was lowest at 200ppm in FC1 (0.21g) and FC₂ (0.20g) whereas control batches recorded filament weight of 0.17g in FC₁ and 0.18g in FC₂ (Fig.6). Silkworm breeds reared on fortified mulberry leaves with thiamine at varied concentration exhibited notable impact on filament weight. In the current study, FC_2 and FC_1 expressed gain in the filament weight of 0.23 and 0.2g, respectively with thiamine supplementation at 600ppm. The increase in filament weight in both the hybrids might be due to higher rate of bio synthesis of silk protein by additional supplementation of thiamine. These results are agreement in the findings of Singaravelu *et al.* (2001) ^[13] who have observed that supplementation of mulberry leaves with combination of folic acid and para amino benzoic acid increase filament weight.

Denier

It denotes the size of the filament obtained from cocoons of silkworm breeds. Marginal variation was noticed with respect to denier among hybrids provided with mulberry leaves fortified with thiamine at different concentrations. The hybrids FC_2 and FC_1 exhibited lowest denier at 600ppm (1.17 and 1.23) and followed by 400ppm (1.27 and 1.40). Similarly, highest denier was recorded at 200ppm in FC_2 (1.59) and FC_1 (1.72). Whereas lowest denier was recorded in control batches of FC₁ (1.88) and FC₂ (1.12), respectively (Table.1). The larvae reared on thiamine supplementation at 200ppm expressed lower denier of 1.59 in FC₂ and 1.72 in FC₁. These results are in conformity in the findings of Balasundaram et al. (2013a)^[2] Who have observed that lower denier of 2.30 was recorded at 0.8% supplemented ascorbic acid over 0.1% (2.64). Similarly, mulberry leaves supplemented with folic acid at 1.5% concentration to the silkworm yields finer denier Babu *et al.* (1992)^[1].

Table 1: Influence of mulberry leaves supplemented with thiamine at varied concentrations on larval and cocoon characters

Concentration (ppm)	Hybrids	Matured larval	Cocoon weight	Shell weight	Shell percentage	Filament length	Filament weight	Denier	Renditta
	-	weight(g)	(g)	(g)	(%)	(m)	(g)		
200	FC1	2.720±0.1	1.345 ± 0.04	0.217 ± 0.02	18.85±1.6	1105.5 ± 15.8	0.203 ± 0.02	1.728±0.07	6.82±0.3
	FC2	2.744 ± 0.01	1.356 ± 0.01	0.274 ± 0.03	20.38±4.03	1107.4±11.3	0.212 ± 0.01	1.597 ± 0.04	5.89±0.1
400	FC1	2.773 ± 0.01	1.390 ± 0.01	0.278 ± 0.02	19.07 ± 2.6	$1299.4{\pm}12.2$	0.214 ± 0.01	1.405 ± 0.03	5.64 ± 0.2
	FC2	2.812 ± 0.01	1.456 ± 0.03	0.283 ± 0.02	20.88±1.7	1398.3 ± 4.02	0.219 ± 0.01	1.272±0.03	5.550 ± 0.3
600	FC1	2.896 ± 0.03	1.488 ± 0.02	$0.294{\pm}0.02$	$21.10{\pm}1.72$	1513.5 ± 21.4	0.224 ± 0.01	1.232 ± 0.01	5.520 ± 0.1
	FC2	2.944 ± 0.01	1.624 ± 0.03	0.305 ± 0.02	23.54±1.1	1678.5 ± 6.8	0.236 ± 0.01	1.175 ± 0.01	5.120±0.5
Absolute control	FC1	2.486 ± 0.06	1.104 ± 0.02	0.220 ± 0.01	16.93±0.8	862.7±6.7	0.172 ± 0.03	2.123±0.01	8.093 ± 0.4
	FC2	2.630 ± 0.06	1.146 ± 0.03	0225±0.05	17.47±0.4	9490±11.7	0.184 ± 0.02	1.880 ± 0.1	7.576±0.2
Distilled water (Control)	FC1	2.619 ± 0.08	1.216±0.1	0.203±0.1	18.11±0.6	997.2±9.8	0.191±0.01	1.797±0.02	7.136±0.1
	FC2	2.683 ± 0.02	1.221±0.1	0.212 ± 0.02	$18.34{\pm}1.1$	1072.1±14.4	0.196 ± 0.02	1.746 ± 0.04	7.052 ± 0.01

Renditta

This trait indicates as total silk available from the cocoon. Notable variations were noticed with respect to renditta among hybrids provided with mulberry leaves fortified with thiamine at varied concentrations. The hybrids FC_2 and FC_1 expressed lowest renditta at 600ppm (5.12 and 5.52kg) followed by 400ppm (5.55 and 5.64kg), respectively. In contrast, highest renditta was observed at 200ppm in FC₂ (5.88 kg) and FC_1 (6.81 kg). However, control batches recorded renditta of 7.57kg and 8.09kg in FC_1 and FC_2 , respectively (Table.1). The silkworms reared on fortified mulberry leaves with thiamine at different concentrations expressed encouraging results in respect of renditta. The lowest renditta of 5.12 and 5.52kg were recorded in FC₂ and FC_1 , respectively. The improvement for this trait in both bivoltine hybrids at 600ppm of thiamine supplementation might be due to effective utilization of this vitamin which enhances the activity of coenzymes which in turn reflects on cocoon shell formation. These results are in agreement with the findings of Sridhar and Radha (1987)^[14] who have noticed that silkworm reared on mulberry leaf supplemented with glycine at 10 ppm concentration significantly reduces the renditta.

Summary and Conclusion

The results on the "Influence of supplementation of thiamine with mulberry leaf on the dehydrogenase activity and economic traits in *Bombyx mori* L" are summarised as the bivoltine hybrids FC1 and FC2 performed better in respect of economic traits such as larval weight, cocoon weight, shell weight, filament length, filament weight, renditta except denier at 600ppm of thiamine supplementation over other concentrations as well as control batches. Further, FC2 scored better for these traits compare to FC1. The results of the present study revealed that, fortification of mulberry leaf with thiamine at 600ppm enhances economic parameters along with biomolecules of mulberry silkworms.

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