

## Influence of Supplementation of Niacin with Mulberry Leaf on the Dehydrogenase Activity and Economic Traits in Silkworm *Bombyx mori* L.

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### ABSTRACT

Changes in the levels of dehydrogenase activity when supplemented with niacin on mulberry leaves with different concentration were assayed in the fat body of FC<sub>1</sub> and FC<sub>2</sub> bivoltine silkworm hybrids. They performed better with respect to economic traits such as larval weight, cocoon weight, shell weight, filament length, filament weight, renditta except denier at 0.6% of niacin supplementation over other concentrations as well as control batches. Further, FC<sub>2</sub> scored better for these traits compare to FC<sub>1</sub>. The silkworm larvae reared on mulberry leaves fortified with niacin at 0.6% concentration recorded highest activity levels of SDH and LDH activities over other concentration as well as control batch. Both the enzymes activities were relatively higher in V instar 5<sup>th</sup> day, V instar 3<sup>rd</sup> day and V instar 1<sup>st</sup> day in both the hybrids. Further, the activity levels were maximum in FC<sub>2</sub> when compared to FC<sub>1</sub>. The present study divulged that, the mulberry leaf fortified with niacin at 0.6 % intensify economic parameters of selected bivoltine hybrid silkworm *Bombyx mori*.

**Keywords:** Bivoltine hybrids, FC<sub>1</sub> and FC<sub>2</sub>, Niacin, dehydrogenase activity, economic parameters.

### 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 76% of raw silk produced by serigenous 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 utmost importance for production of good quality of cocoons. Legay (1958) states that silkworm nutrition is a major area of research in sericulture.

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Pant (1978) 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 aminotransferases enzymes. The silkworms require several vitamins for their growth and survival. 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 the kinds of vitamin. The higher level of choline and inositol are required by the silkworm and are not useful for catalytic function. It is almost certain that dose differences among various vitamins reflect their specificity of metabolic function. Niacin is necessary for the proper functioning of over 60 enzymes that participate in amino acid metabolism. It is also involved in carbohydrate and fat metabolism. Without niacin or its derivatives no larva reached the third instar under aseptic condition. The silkworm growth was quite improved by adding to niacin the diet, because of their association is involved in commercial characters of silkworm. The niacin (Vitamin B3) also called Nicotinic acid. It is water soluble, heat stable vitamin made up of pyridine-3-carboxylic acid. It occurs in tissue as niacinamide which is component of two coenzymes NAD and NADP. It can be synthesized from tryptophan. In response to physiological demands for energy, glucose or glycogen may be completely converted to carbon dioxide and water via lactate or pentose depending upon the nature of the tissue. Unlike the glycolytic enzymes the complete set of enzymes responsible for the citric acid cycle reactions is found in the mitochondrial function of the cell, in proximity to the enzymes of the respiratory chain. Such an association facilitates quick electron transport chain oxidation. The Succinate dehydrogenase (SDH) is found in inner membrane of mitochondria oftenly referred as mitochondrial index enzyme and is present at lowest

concentration. The SDH catalyses the reaction in the Krebs cycle and help to forming fumarate from succinate. In insects, the activity levels 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.

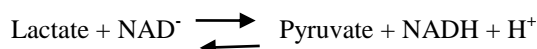
The catalytic activity of this enzyme s represented below:



In some selected silkworm breeds, the activity levels of SDH have positive correlation with most of the economic characters except renditta was reported by Kasmaei and Mahesha (2012). Further, gel electrophoresis study revealed that, the enzyme exhibit variations among selected breeds. In silkworm, *B. mori* the activity levels of SDH varies among different silkworm breeds and hybrids was observed by Mahesha et al. (2015). It has been observed that increase in the activity levels of succinate dehydrogenase and amylase in F<sub>1</sub> progeny raised from EMS treatment at varied concentrations was also reported by

Mahesha and Honnaiah (2002). The lactate dehydrogenase abundantly found in fat bodies of insects. It is a glycolactic enzyme involved in conversion of lactic acid into pyruvic acid and viceversa by the help of co-enzyme NAD. However, not much more information is available on biochemical changes takes place during amino acid supplementation. Keeping this in view, the proposed research work was under taken to study the supplementation of tryptophan by the diet on selected dehydrogenase enzymes in silkworm larvae and economic parameters.

The catalytic activity of this enzyme s represented below:



However, information available on supplementation of niacin on succinate dehydrogenase and lactate dehydrogenase activities and economic traits in silkworm is fragmentary. In this context, the present investigation has been undertaken.

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<sub>1</sub>) larvae received 0.2 % niacin sprayed mulberry leaves, the batch II (T<sub>2</sub>) larvae received 0.4 % niacin sprayed mulberry leaves, the batch III (T<sub>3</sub>) larvae received 0.6 % niacin sprayed mulberry leaves and the batch IV (T<sub>4</sub>) larvae reread on mulberry leaves sprayed with distilled water (control). In each treatment three replications were maintained.

## MATERIALS AND METHODS

The methods followed and materials used in the current study are presented under the following headings:

### Materials

The bivoltine silkworm hybrids namely FC<sub>1</sub> and FC<sub>2</sub> were taken for the present investigation.

### Rearing and maintenance of silkworm larvae

The selected silkworm races were reared by employing standard rearing techniques advocated by Dandin and Giridhar (2010).

### Supplementation of niacin

Niacin at varied concentrations viz., 0.2, 0.4 and 0.6 % concentrations were sprayed on

### Observations recorded

#### Estimation of succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) activities in selected bivoltine hybrids.

The succinate dehydrogenase activity was estimated in fifth instar 1<sup>st</sup> day, 3<sup>rd</sup> day and prior to spinning stage (5<sup>th</sup> day) of FC<sub>1</sub> and FC<sub>2</sub> in respective treatments and control batches. The 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). 1ml of tissue extract was incubated with 1ml of sodium succinate, 1ml of phosphate buffer and 1ml of INT at 37<sup>o</sup> C for 1 h followed by addition 6ml of glacial acetic acid was added in order to stop the enzyme activity. Finally, 6ml of toluene was added. 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  $\mu$  moles of formazone (product generated) /g protein/h. For LDH lithium lactate was used as a substrate instead of sodium succinate. The colour intensity was measured at 450nm by using spectrophotometer. The standard curve was used for calculation. The enzyme activity was expressed in terms of  $\mu$  moles of formazone/g protein/h

**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.

**Cocoon weight (g):** Ten cocoons were randomly selected from each treatment replication-wise, weighed individually and average single cocoon weight was computed.

**Shell weight (g):** After removing the pupa and larval exuvium from the cocoons, the individual shell weight was recorded.

**Shell ratio (%):** The shell ratio was calculated using the formula:

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

**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.

**Filament Weight (g):** Ten cocoons were randomly selected from each batch was reeled and average filament weight was recorded.

**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:

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

**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.

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

### Analysis of data

The data obtained on the fortified mulberry leaves with pyridoxine on the SDH activity and economic parameters of the selected bivoltine hybrids are analyzed by adopting standard deviation ( $\pm$ ) method and mean values were expressed.

## RESULTS AND DISCUSSION

Results of the investigation on the “Influence of supplementation of niacin with mulberry leaf on the dehydrogenase activity and economic traits in *Bombyx mori* L” are presented below:

### Influence of supplementation of niacin with mulberry leaf on dehydrogenase activities in the fat body of bivoltine silkworm hybrids.

Silkworm fed on mulberry leaves supplemented with niacin recorded notable difference in respect of SDH level with maximum being in FC<sub>2</sub> at 0.6% (2.93 $\mu$  moles of formazone/g protein/h) followed by 0.4 % (2.75 $\mu$ M formazone/g protein/h). Minimum was recorded at 0.2 % (2.66  $\mu$ M formazoe/g protein/h) as against to control (2.60 $\mu$ M formazone/g protein/h) in V-instar 5<sup>th</sup> day

larvae. Marked difference in enzyme activity level was observed on mulberry leaves fortified with niacin at 0.6 % (2.86  $\mu\text{M}$  of formazone /g protein/h) followed by 0.4 % (2.66 $\mu\text{M}$  of formazone/g protein/h) and 0.2 % (2.57 $\mu\text{M}$  of formazone/g protein/h) over control (2.53  $\mu\text{M}$  of formazone/g protein/h) in V-instar 3<sup>rd</sup> day larvae. Similar trend was also observed at 0.6 % (2.77  $\mu\text{M}$  of formazone/g protein/h) followed by 0.4 % (2.61  $\mu\text{M}$  of formazone/g protein/h) and 0.2 % (2.51  $\mu\text{M}$  of formazone/g protein/h) when compared to control batches (2.47  $\mu\text{M}$  of formazone/g protein/h) in V-instar 1<sup>st</sup> day larvae (Fig.1).

The larvae reared on mulberry leaves fortified with niacin registered marked variation in respect of SDH activity level with highest being in FC<sub>1</sub> at 0.6 % (2.82  $\mu\text{M}$  of formazone/g protein/h) followed by 0.4 % (2.71  $\mu\text{M}$  of formazone/g protein/h) and lowest was recorded at 0.2 % (2.62  $\mu\text{M}$  formazone/g protein/h) as against control (2.58  $\mu\text{M}$  of formazone /g protein/h) in V-instar 5<sup>th</sup> day larvae. Notable difference in enzyme activity level was noticed when extra foliated mulberry leaves with niacin at 0.6 (2.78  $\mu\text{M}$  of formazone /g protein/h) followed by 0.4 % (2.65  $\mu\text{M}$  of formazone/g protein/h) and 0.2 % (2.55  $\mu\text{M}$  of formazone/g protein/h) over control batches (2.52  $\mu\text{M}$  of formazone/g protein/h) in V instar 3<sup>rd</sup> day. Similar results were also registered at 0.6 % (2.61  $\mu\text{M}$  of formazone/g protein/h) followed by 0.4 % (2.58 $\mu\text{M}$  of formazone/g protein/h) and 0.2 % (2.42  $\mu\text{M}$  of formazone/g protein/h) when compared to control (2.37  $\mu\text{M}$  of formazone/g protein/h) in V instar 1<sup>st</sup> day larvae (Fig.1).

#### **Influence of mulberry leaves supplemented with niacin on lactate dehydrogenase activity in the fat body of bivoltine silkworm hybrids.**

Silkworm fed on mulberry leaves supplemented with niacin recorded no marked difference in respect of lactate dehydrogenase activity level with maximum being in FC<sub>2</sub> at 0.6 % (3.21 $\mu$  moles of formazone/g protein/h) followed by 0.4 % (3.17 $\mu$  moles of

formazone/g protein/h). The minimum activity was recorded at 0.2 % (3.15  $\mu$  moles of formazone/g protein/h) against to control batches (3.15 $\mu$  moles of formazone /g protein/h) in 5<sup>th</sup> instar 5<sup>th</sup> day larvae. Notable difference in enzyme activity level was observed on mulberry leaves fortified with niacin at 0.6 % (3.14 $\mu$  moles of formazone /g protein/h) followed by 0.4 % (2.99  $\mu$  moles of formazone /g protein/h) and 0.2% (2.98  $\mu$  moles of formazone /g protein/h) over control batches (2.96  $\mu$  moles of formazone /g protein/h) in 5<sup>th</sup> instar 3<sup>rd</sup> day larvae. Similar trend was also observed at 0.6% (3.05  $\mu$  moles of formazone /g protein/h) followed by 0.4% (2.98  $\mu$  moles of formazone /g protein/h) and 0.2% (2.97  $\mu$  moles of formazone /g protein/h) when compared to control batches (2.94  $\mu$  moles of formazone /g protein/h) in 5<sup>th</sup> instar 1<sup>st</sup> day larvae.

The larvae reared on mulberry leaves fortified with niacin registered marked variations in respect of LDH activity level with highest being in FC<sub>1</sub> at 0.6 % (3.18  $\mu$  moles of formazone/g protein/h) followed by 0.4% (3.11 $\mu$  moles of formazone/g protein/h) and lowest was recorded at 0.2% (3.10  $\mu$  moles of formazon /g protein/h) against control (3.06  $\mu$  moles of formazone /g protein/h) in 5<sup>th</sup> instar 5<sup>th</sup> day larvae. Similarly difference in enzyme activity level was also noticed when extrafoliated mulberry leaves with niacin at 0.6 % (3.12  $\mu$  moles of formazone/g protein/h) followed by 0.4 % (2.90  $\mu$  moles of formazone/g protein/h) and 0.2% (2.89  $\mu$  moles of formazone /g protein/h) over control batches (2.88  $\mu$  moles of formazone/g protein/h) in 5<sup>th</sup> instar 3<sup>rd</sup> day. Similar results were also registered at 0.6 % (2.91  $\mu$  moles of formazone/g protein/h) followed by 0.4 % (2.88 $\mu$  moles of formazone/g protein/h) and 0.2 % (2.87 $\mu$  moles of formazone/g protein/h) when compared to control (2.86  $\mu$  moles of formazone /g protein/h) in 5<sup>th</sup> instar 1<sup>st</sup> day larvae (Fig.2).

**Larval weight**

Silkworms fed on mulberry leaves supplemented with niacin expressed difference in respect of larval weight with maximum being in FC<sub>2</sub> and FC<sub>1</sub> at 0.6 % (3.70 and 3.68g) followed by 0.4 % (3.68 and 3.66g), respectively. While it was minimum at 0.2 % in FC<sub>1</sub> and FC<sub>2</sub> (3.52 and 3.54g) when compared to control batches (3.51 and 3.58g), respectively (Fig.3).

**Cocoon weight**

The hybrids FC<sub>2</sub> and FC<sub>1</sub> exhibit highest cocoon weight on extra foliated mulberry leaves with niacin at 0.6% (1.58 and 1.56g) followed by 0.4% (1.55 and 1.54g), respectively. In contrast, it was lowest at 0.2% in FC<sub>1</sub> (1.48g) and FC<sub>2</sub> (1.53g). However, larval fed on mulberry leaves sprayed with distilled water registered a cocoon weight of 1.51 and 1.45g in FC<sub>2</sub> and FC<sub>1</sub>, respectively (Fig.4).

**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 niacin at varied concentrations registered notable variation with respect to shell weight. The highest shell weight was observed on mulberry leaves supplemented with niacin at 0.6% in FC<sub>2</sub> and FC<sub>1</sub> (0.36 and 0.33g) followed by 0.4% (0.33 and 0.28g), respectively. While it was least at 0.2% in FC<sub>1</sub> (0.26 and FC<sub>2</sub> (0.31g). The minimum shell weight of 0.24 and 0.30g were noticed in control batches of FC<sub>1</sub> and FC<sub>2</sub>, respectively (Fig.5).

**Shell ratio**

The hybrids FC<sub>2</sub> and FC<sub>1</sub> fed on mulberry leaves fortified with niacin at 0.6% recorded highest shell ratio (22.78 and 20.37) followed by 0.4% (21.29 and 18.18%), respectively. On the other hand, it was lowest for said trait at 0.2% in FC<sub>1</sub> (17.56%) and FC<sub>2</sub> (20.26%) over control batches of FC<sub>1</sub> (16.55%) and FC<sub>2</sub> (19.86%), respectively (Fig.6).

**Filament length**

Filament length is one of the major contributing quantitative traits in silkworms.

The silkworm reared on mulberry leaves supplemented with niacin noticed marked differences in respect of filament length with maximum being in FC<sub>2</sub> and FC<sub>1</sub> at 0.6% (1082 and 1013m) followed by 0.4% (1078 and 1008m), respectively. However, minimum filament length of 1002 and 1063m were recorded at 0.2% in FC<sub>1</sub> and FC<sub>2</sub> when compared to control batches of FC<sub>1</sub> (999m) and FC<sub>2</sub> (1058m), respectively (Fig.7).

**Filament weight**

The hybrids FC<sub>2</sub> and FC<sub>1</sub> expressed higher filament weight on fortified mulberry leaves with niacin at 0.6% (0.29 and 0.25g) followed by 0.4% (0.28 and 0.24g), respectively. As against to this, it was lowest at 0.2% in FC<sub>1</sub> (0.22g) and FC<sub>2</sub> (0.26g) whereas control batches recorded filament weight of 0.23g in FC<sub>1</sub> and 0.27g in FC<sub>2</sub> (Fig.8).

**Denier**

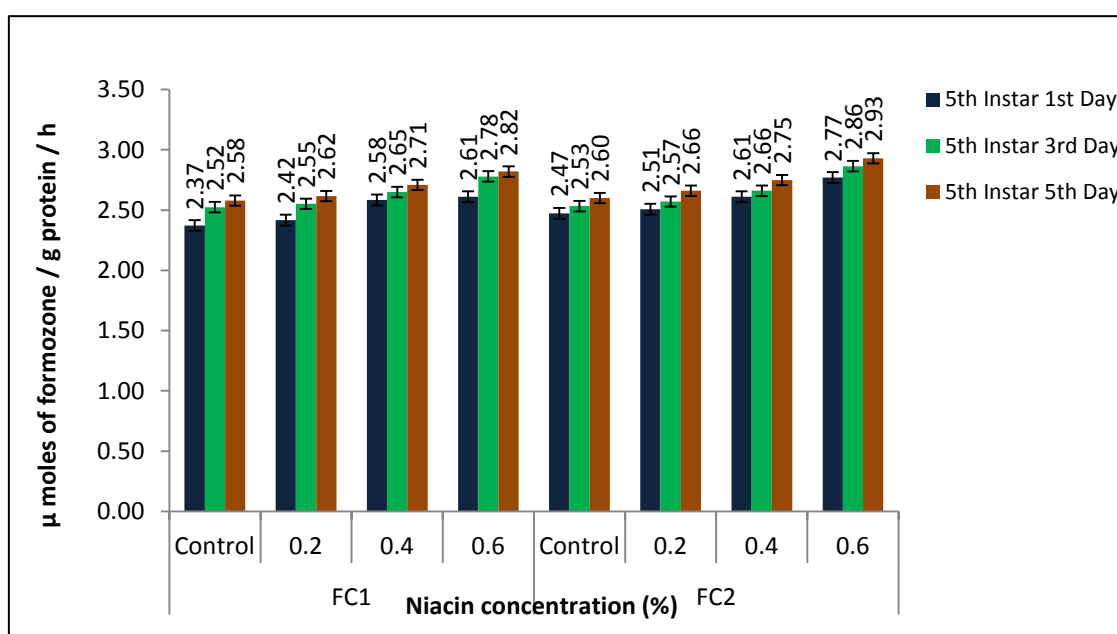
It denotes the size of the filament obtained from cocoons of silkworm breeds. Marginal variation was noticed with respect to denier among hybrids provided with mulberry leaves fortified with niacin at different concentrations. The hybrids FC<sub>2</sub> and FC<sub>1</sub> exhibited lowest denier at 0.2% (2.20 and 2.01) and followed by 0.4% (2.33 and 2.14d). Similarly, highest denier was recorded at 0.6% in FC<sub>2</sub> (2.41d) and FC<sub>1</sub> (2.22d). Whereas lowest denier was recorded in control batches of FC<sub>1</sub> (2.07d) and FC<sub>2</sub> (2.30d), respectively (Fig.9).

**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 niacin at varied concentrations. The hybrids FC<sub>2</sub> and FC<sub>1</sub> expressed lowest renditta at 0.6% (5.44 and 6.24kg) followed by 0.4% (5.53 and 6.41kg), respectively. In contrast, highest renditta was observed at 0.2% in FC<sub>1</sub> (6.72 kg) and FC<sub>2</sub> (5.88 kg). However, control batches recorded renditta of 6.30kg and 5.59kg in FC<sub>1</sub> and FC<sub>2</sub>, respectively (Fig.10).

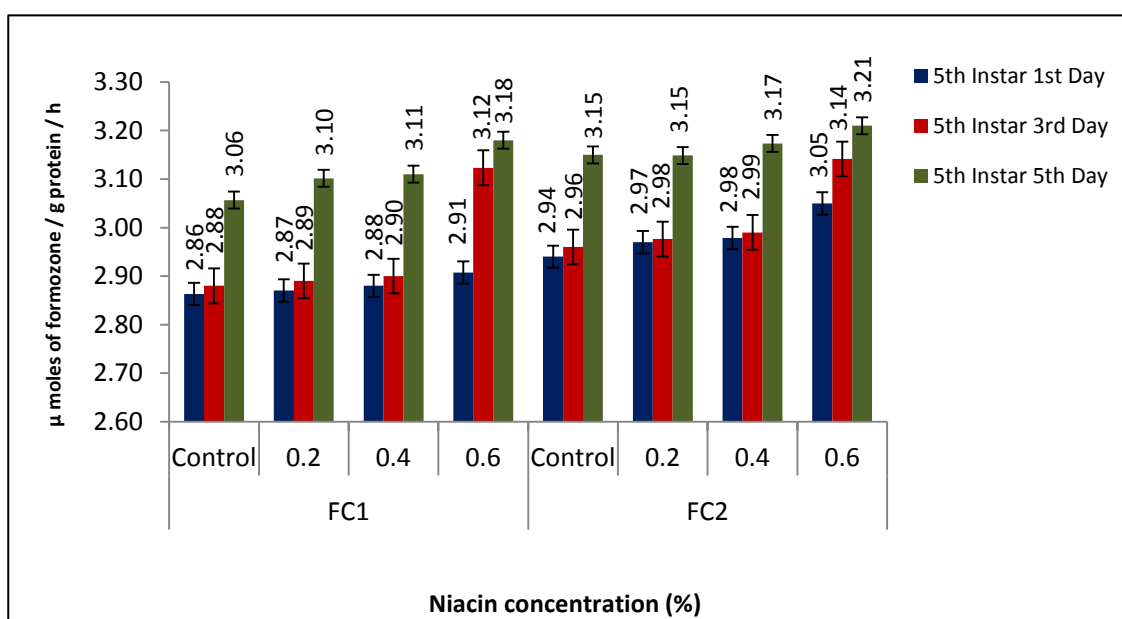
**Table 1: Influence of mulberry leaves supplemented with niacin at varied concentrations on succinate dehydrogenase activity in the fat body of bivoltine hybrids**

Hybrids	Concentration	5 <sup>th</sup> Instar 1 <sup>st</sup> Day	5 <sup>th</sup> Instar 3 <sup>rd</sup> Day	5 <sup>th</sup> Instar 5 <sup>th</sup> Day
FC <sub>1</sub>	0.2	2.42	2.55	2.62
	0.4	2.58	2.65	2.71
	0.6	2.61	2.78	2.82
	Control	2.37	2.52	2.58
FC <sub>2</sub>	0.2	2.51	2.57	2.66
	0.4	2.61	2.66	2.75
	0.6	2.77	2.86	2.93
	Control	2.47	2.53	2.60

**Fig. 1: Effect of mulberry leaf supplemented with niacin at varied concentrations on SDH activity in the fat body of bivoltine hybrids**

**Table 2: Influence of mulberry leaves supplemented with niacin at varied concentrations on lactate dehydrogenase activity in the fat body of bivoltine hybrids.**

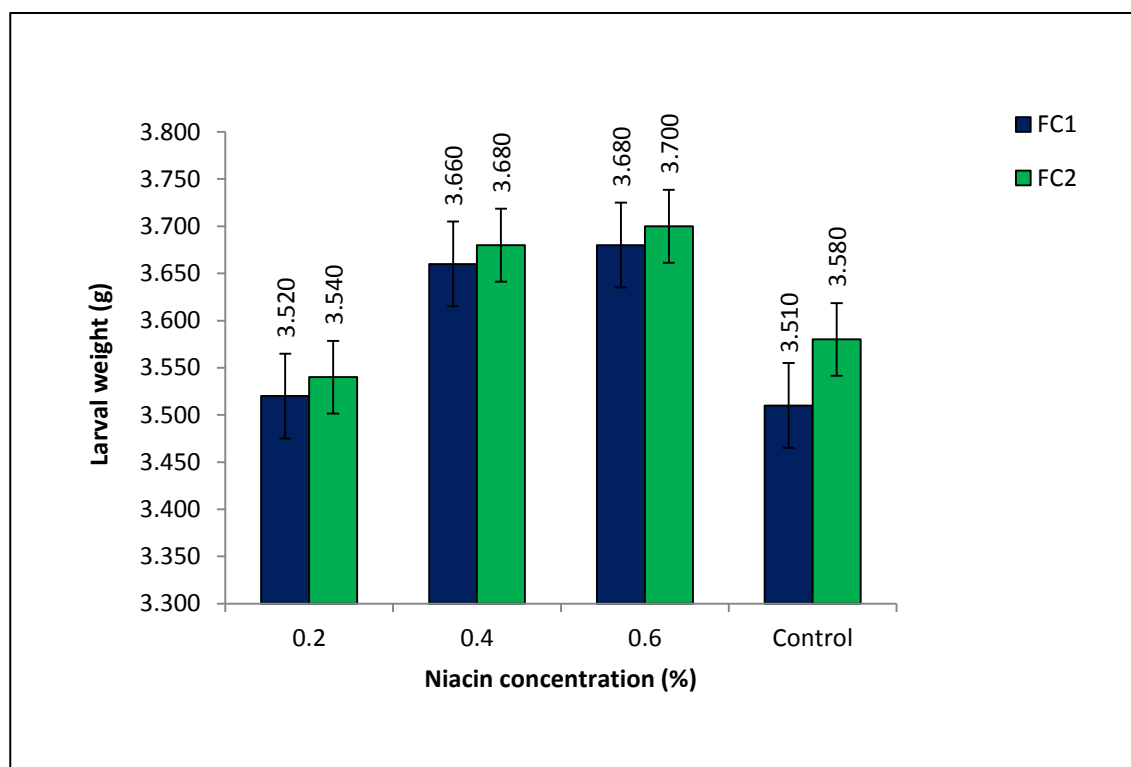
Hybrids	Concentration	5 <sup>th</sup> Instar 1 <sup>st</sup> Day	5 <sup>th</sup> Instar 3 <sup>rd</sup> Day	5 <sup>th</sup> Instar 5 <sup>th</sup> Day
FC <sub>1</sub>	0.2	2.87	2.89	3.10
	0.4	2.88	2.90	3.11
	0.6	2.91	3.12	3.18
	Control	2.86	2.88	3.06
FC <sub>2</sub>	0.2	2.97	2.98	3.15
	0.4	2.98	2.99	3.17
	0.6	3.05	3.14	3.21
	Control	2.94	2.96	3.15

**Fig. 2: Effect of mulberry leaf supplemented with niacin at varied concentrations on LDH activity in the fat body of bivoltine hybrids**



**Table 3: Influence of mulberry leaves supplemented with niacin at varied concentrations on larval and cocoon characters**

Treatment (%)	Hybrids	Matured larval Weight (g)	Cocoon weight (g)	Shell weight (g)	Shell Percentage (%)	Filament Length (m)	Filament Weight (g)	Denier	Renditta
0.2	FC <sub>1</sub>	3.52±0.01	1.48±0.01	0.26±0.01	17.56±0.65	1002±3.67	0.22±0.01	2.01±0.05	6.72±0.22
	FC <sub>2</sub>	3.54±0.01	1.53±0.01	0.31±0.00	20.26±0.22	1063±3.90	0.26±0.01	2.20±0.05	5.88±0.11
0.4	FC <sub>1</sub>	3.66±0.03	1.54±0.03	0.28±0.01	18.18±0.36	1008±3.34	0.24±0.01	2.14±0.04	6.41±0.12
	FC <sub>2</sub>	3.68±0.03	1.55±0.01	0.33±0.00	21.29±0.18	1078±4.79	0.28±0.02	2.33±0.01	5.53±0.08
0.6	FC <sub>1</sub>	3.68±0.01	1.56±0.02	0.33±0.01	20.37±0.65	1013±1.52	0.25±0.01	2.22±0.05	6.24±0.14
	FC <sub>2</sub>	3.70±0.02	1.58±0.01	0.36±0.01	22.78±0.37	1082±4.00	0.29±0.01	2.41±0.03	5.44±0.08
Control	FC <sub>1</sub>	3.51±0.01	1.45±0.01	0.24±0.01	16.55±0.72	999±1.38	0.23±0.01	2.07±0.05	6.30±0.19
	FC <sub>2</sub>	3.58±0.02	1.51±0.01	0.30±0.02	19.86±1.15	1052±2.53	0.27±0.01	2.30±0.06	5.59±0.09

**Fig. 3: Effect of mulberry leaf supplemented with niacin at varied concentrations on larval weight**

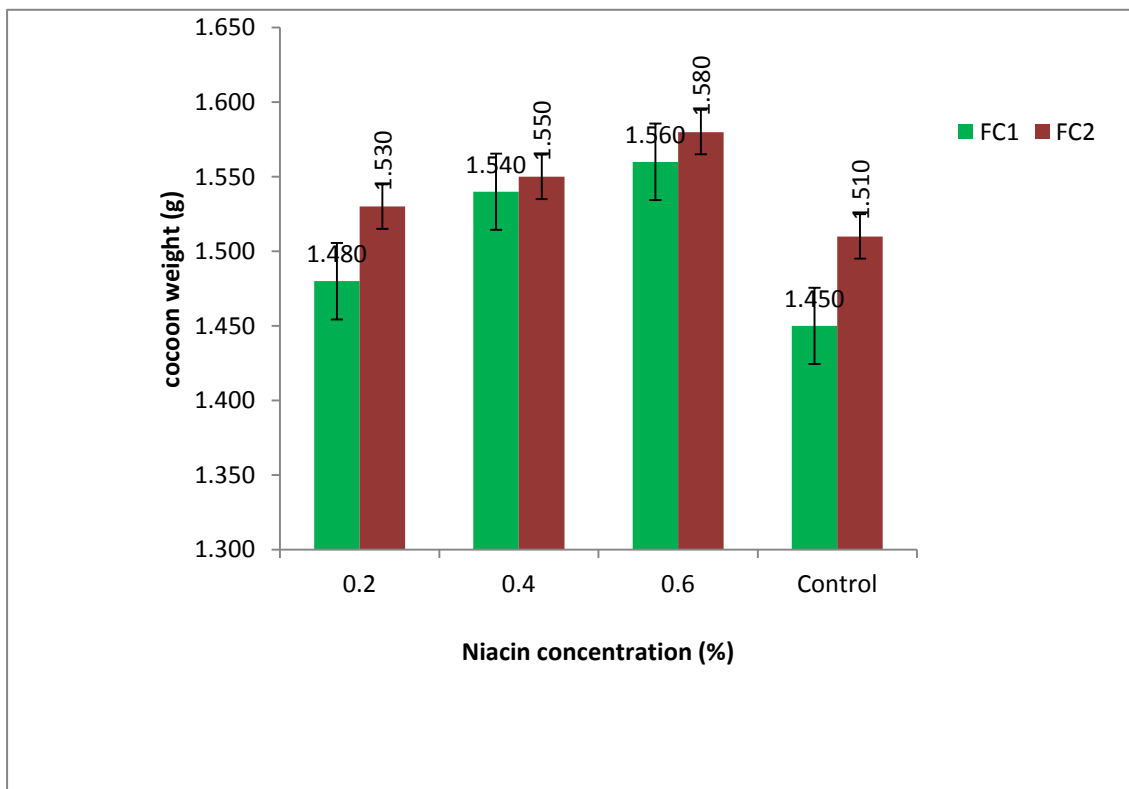


Fig. 4: Effect of mulberry leaf supplemented with niacin at varied concentrations on cocoon weight

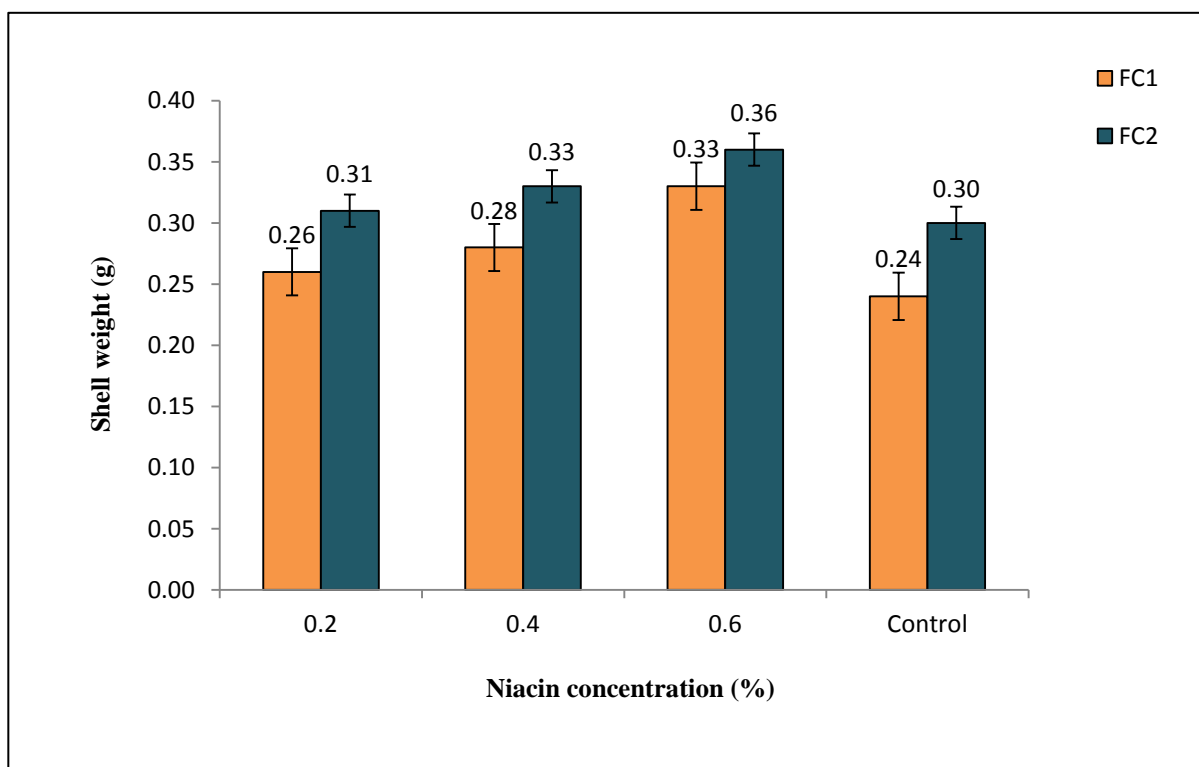
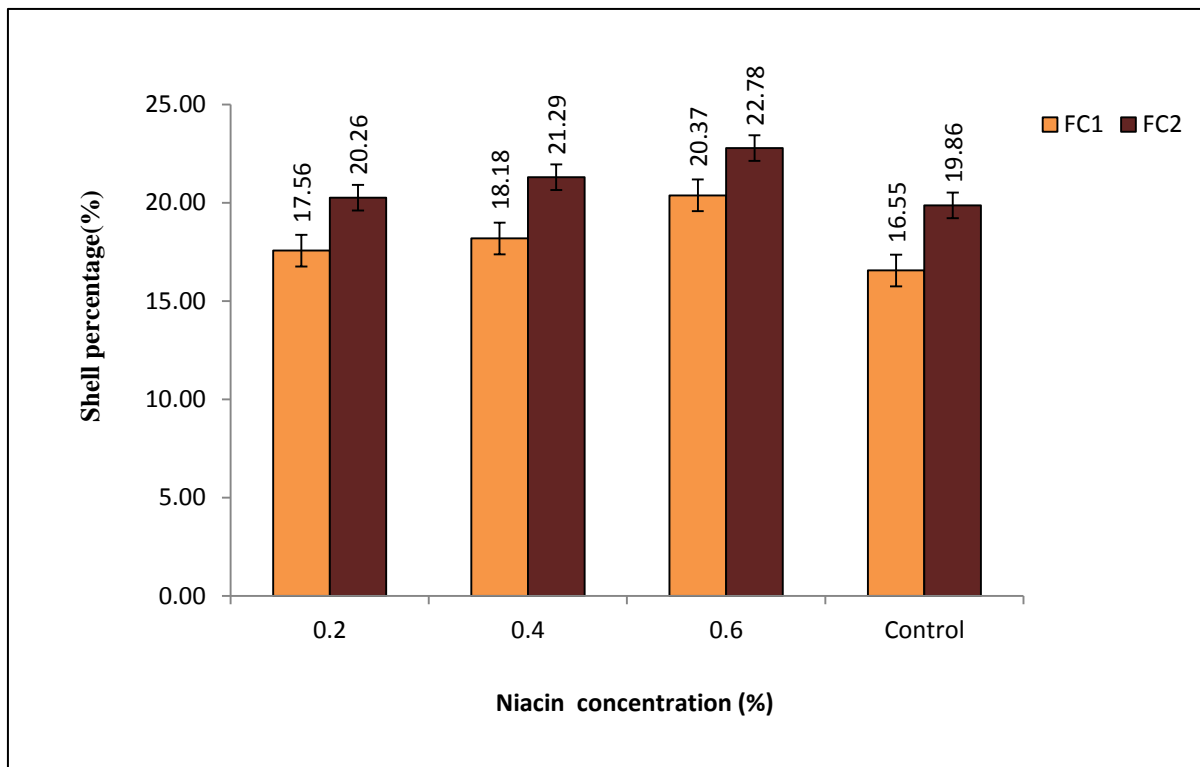
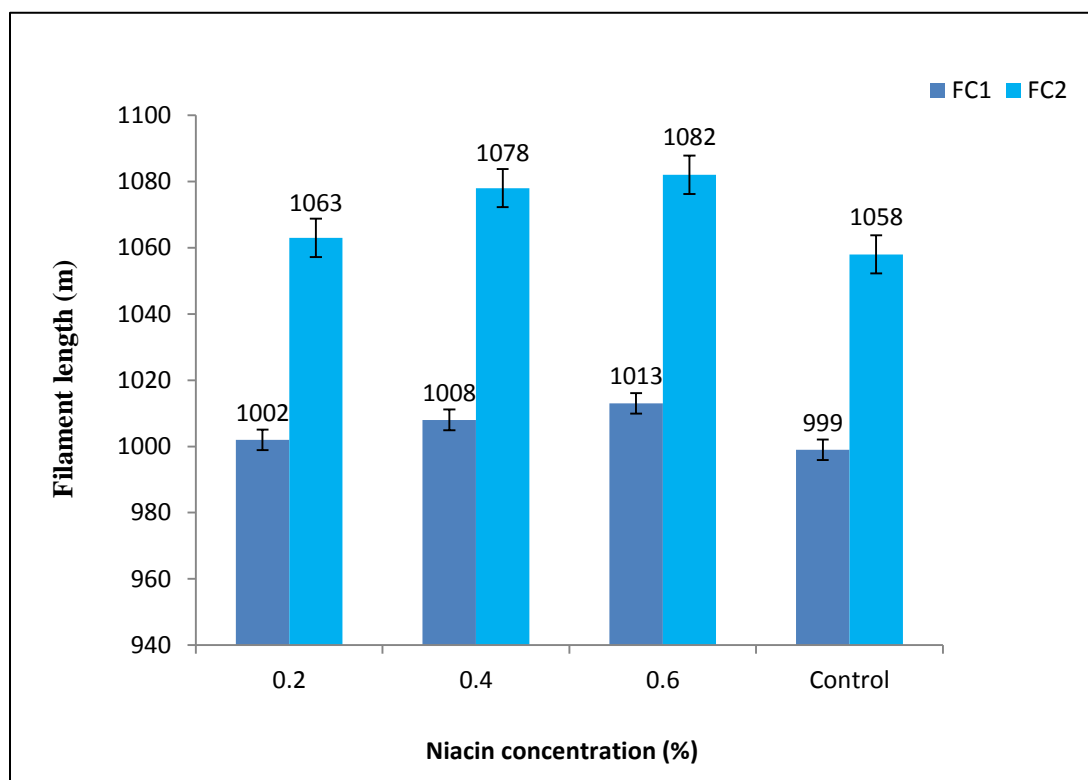


Fig. 5: Effect of mulberry leaf supplemented with niacin at varied concentrations on shell weight



**Fig. 6:** Effect of mulberry leaf supplemented with niacin at varied concentrations on shell percentage



**Fig. 7:** Effect of mulberry leaf supplemented with niacin at varied concentrations on filament length

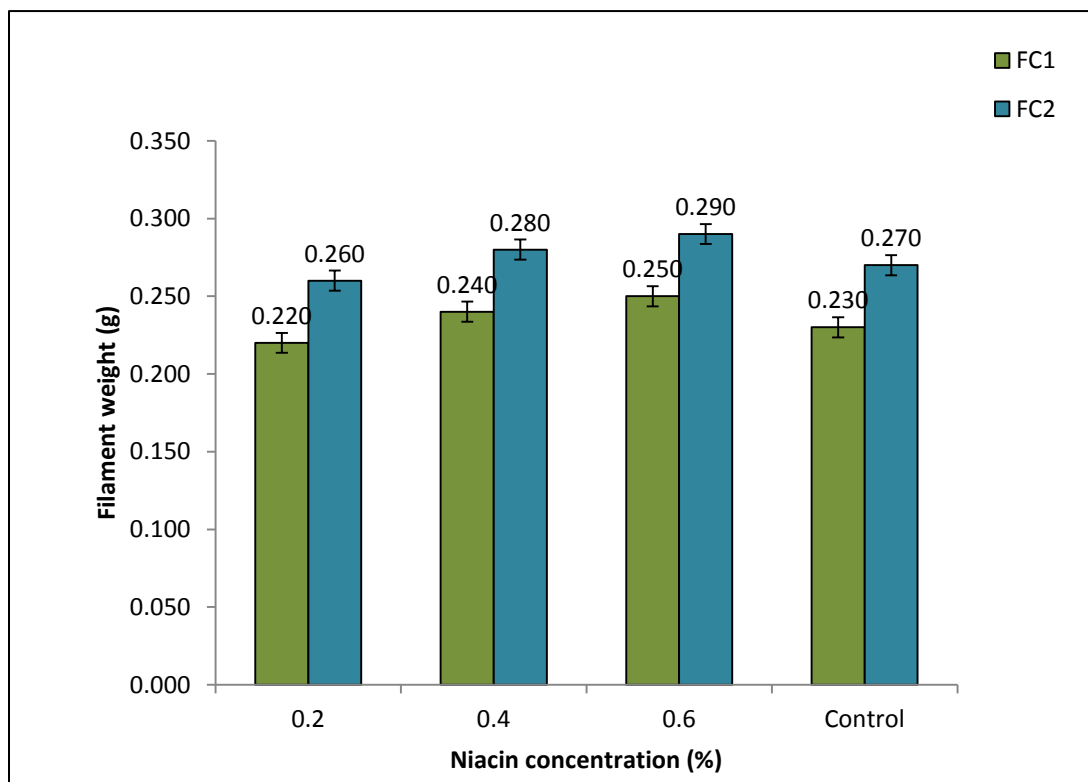


Fig. 8: Effect of supplemented mulberry leaf with niacin at varied concentrations on filament weight

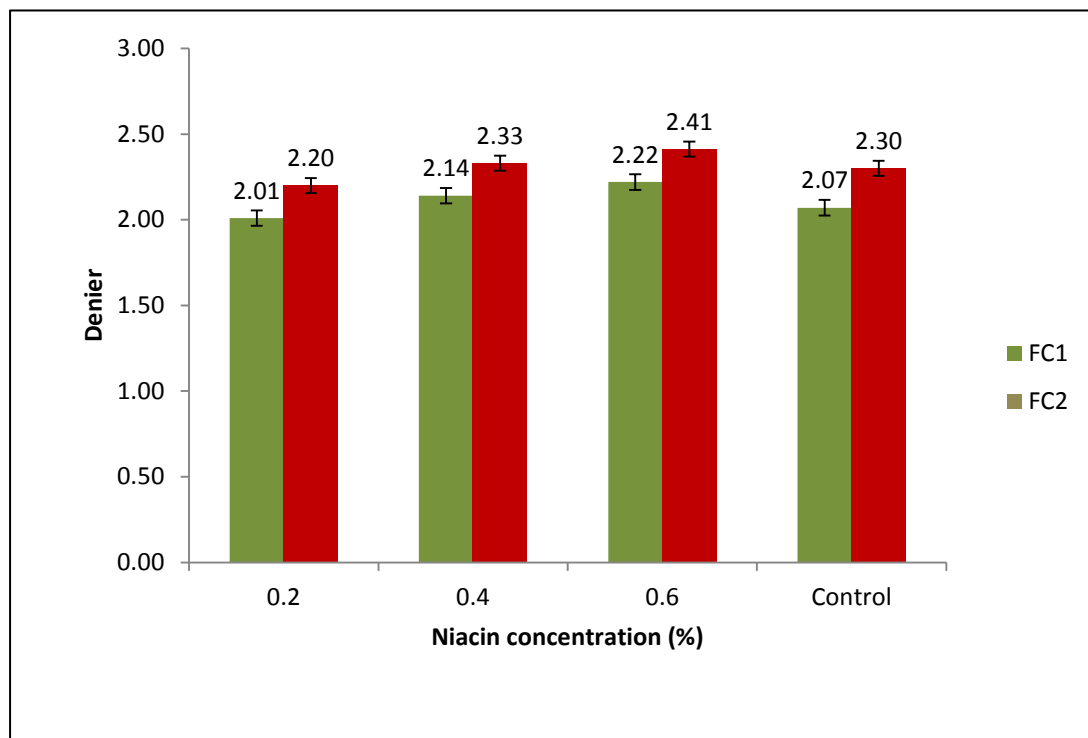
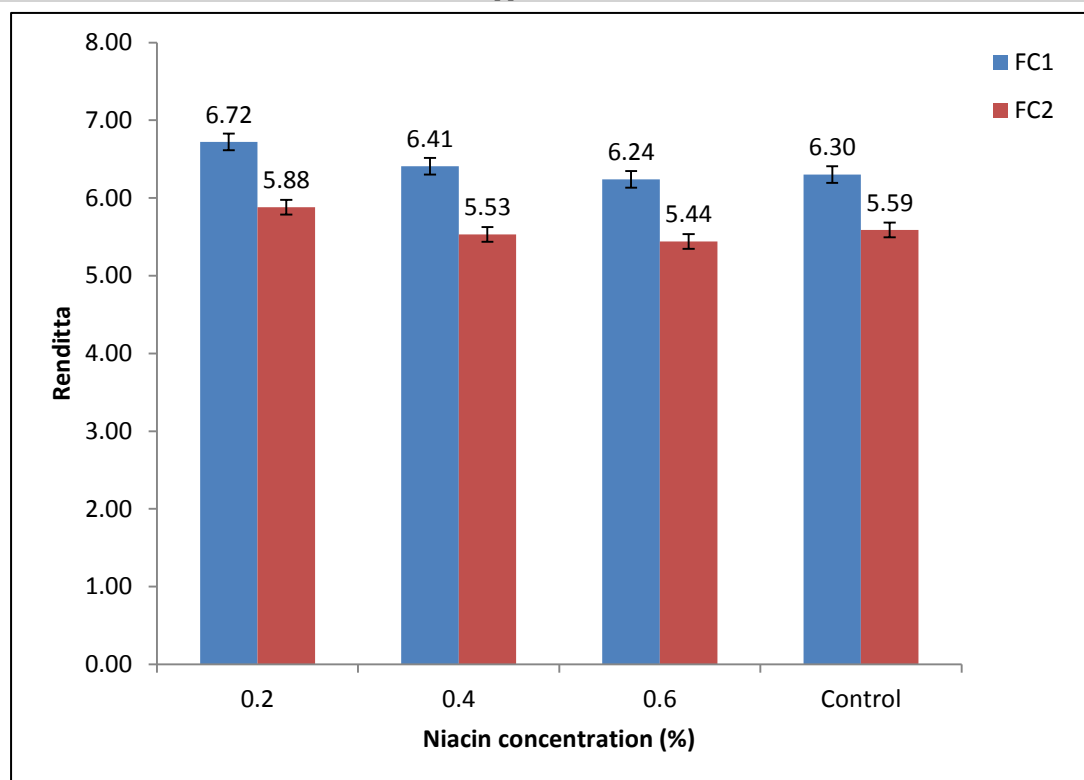


Fig. 9: Effect of mulberry leaf supplemented with niacin at varied concentrations on denier



**Fig. 10: Effect of mulberry leaf supplemented with niacin at varied concentrations on renditta**

Vitamins are organic compounds required in trace amounts in the diet for proper growth and development. 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. The results on the “Niacin as a nutrient supplement with mulberry leaf and its impact on the succinate dehydrogenase activity and lactate dehydrogenase economic traits of *Bombyx mori* L.” are discussed in the light of earlier workers and presented in the following paragraphs:

#### **Influence of supplementation of mulberry leaves with niacin on succinate and lactate dehydrogenase activity.**

The fat body has a storage function of reserve materials such as glycogen, proteins, etc. to carry 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. Hence, in the present investigation lactate and succinate dehydrogenase activity was estimated in fat body and obtained results are discussed below.

The silkworm hybrid FC<sub>1</sub> fed on mulberry leaves supplemented with niacin recorded highest activity level of SDH and LDH in the fat body at 0.6% concentration (2.82  $\mu$  moles of formozone /g protein/hour) and (3.18  $\mu$  moles of formozone /g protein/hour) in 5<sup>th</sup> instar 5<sup>th</sup> day larvae followed by 5<sup>th</sup> instar 3<sup>rd</sup> day and 5<sup>th</sup> instar 1<sup>st</sup> day, respectively. Similar trend was also noticed in FC<sub>2</sub> at 0.6 % concentration (2.93  $\mu$  moles of formozone /g protein/hour) and (3.21  $\mu$  moles of formozone /g protein/hour).

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. 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.

In the present study SDH activity was relatively higher in both the hybrids due to additional supplementation of niacin which enhance the activity of coenzymes such as NAD and NADP. These coenzymes play an important role in metabolism by acting as hydrogen and electron transfer agents by means of reversible oxidation and reduction. Further, FC<sub>2</sub> expresses higher enzyme activity levels of SDH compared to FC<sub>1</sub>. It clearly indicates that the of niacin differed genetically among the hybrids. The present findings are in conformity with the findings of Anil Kumar et al. (2017) who have reported that supplementation of pyridoxine at 1000ppm concentration enhances SDH activity (3.353 units /h) in FC<sub>2</sub> bivoltine hybrids over control batch (3.033 units/h).

In both the hybrids, the activity levels of SDH was highest in V-instar 5<sup>th</sup> day, followed by V-instar 3<sup>rd</sup> day and V-instar 1<sup>st</sup> day over control batches. It clearly indicates that effective utilization of additional supplementation of niacin with advancement of age. These results are in line with the earlier observations of Anil Kumar et al. (2017) who have observed that silkworm hybrids FC<sub>2</sub> and FC<sub>1</sub> supplemented with niacin increases the SDH activity with advancement of age. Similarly increase in protease activity with advancement of age was also reported in silkworm breeds by Anil Kumar (2009).

Lactate dehydrogenase enzyme belongs to oxido reductases group which converts lactate into pyruvate which is

important compound for kreb's cycle and gluconeogenesis. Increased in activity of LDH might be due to addition supplementation of niacin which is also enhance activities of coenzymes such as NAD and NADP. Further the activity levels of LDH are relatively higher than the SDH in FC<sub>2</sub>. It clearly indicates that niacin enhance LDH activity when compared to SDH. Similar to SDH, the LDH activity increases with the advancement of age indicating more utilization of niacin.

In both hybrids, the LDH and SDH activities were highest in 5<sup>th</sup> instar 5<sup>th</sup> day, followed by 5<sup>th</sup> instar 3<sup>rd</sup> day and 5<sup>th</sup> instar 1<sup>st</sup> day over control batches. It clearly indicates that effective utilization of additional supplementation of niacin varies with advancement of age. These results are in line with the earlier observations of Anil Kumar (2009) who has opined that increase in protease activity with advancement of age. Further, the activity level of LDH and SDH were maximum in the silkworm hybrid FC<sub>2</sub> and FC<sub>1</sub> with niacin supplementation at 0.6%. These results are showed that utilization of niacin differs genetically among the hybrids.

#### **Larval weight**

Silkworm hybrids reared on fortified mulberry leaves with niacin at different concentrations exhibited notable influence on larval weight with maximum being in FC<sub>2</sub> (3.70 g) and FC<sub>1</sub> (3.68 g) at 0.6% concentration over control batch. The increase in larval weight might be due to additional supplementation of niacin along with mulberry leaves. The present findings were in conformity with the findings of Balasundaram et al. (2013b) 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 (CSR<sub>2</sub> x CSR<sub>4</sub>). Similar results were also observed with supplementation of folic acid, para-amino benzoic acid and combination of both in the silkworm larvae NB<sub>4</sub>D<sub>2</sub> by Singaravelu et al. (2001).

**Cocoon weight**

Supplementation of niacin at varied concentrations on silkworm hybrids registered encouraging results on cocoon weight. The larvae reared on niacin at 0.6% expressed higher cocoon weight of 1.58 g and 1.1.56 g in FC<sub>2</sub> and FC<sub>1</sub>, respectively. The increase in cocoon weight in both breeds might be due to increase in absorption of niacin 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) 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., 2013).

**Shell weight**

Silkworms nourished with mulberry fortified with niacin at different concentrations registered notable influence on shell weight. The worms supplemented with niacin at 0.6% exerted higher shell weight (0.36g) in FC<sub>2</sub>. On the other hand, FC<sub>1</sub> recorded highest shell weight of 0.33g over control batch. The increase in shell weight might be due to additional supplementation of niacin which enhances the biosynthesis of silkworm protein. These results corroborate the earlier findings of Balasundaram et al. (2013a) who have opined that supplementation of mulberry leaves with ascorbic acid at the rate 0.2% enhance shell weight of 0.80g over control batch (0.63g) in the silkworm hybrid CSR<sub>2</sub> x CSR<sub>4</sub>. Similar trend also noticed in some other vitamins by (Prasad et al., 1994; Nirwani and Kaliwal, 1998; Singaravelu et al., 2001; Rai et al., 2002; Rahmathulla et al., 2007; Tantra and Kanika Trivedy 2011) (Fig 4).

**Shell ratio**

Silkworms reared on mulberry leaves extra foliated with niacin exerted marked influence

on shell ratio. A concentration of niacin at 0.6% resulted in higher shell ratio of 21.78 and 20.37% in FC<sub>2</sub> and FC<sub>1</sub>, respectively compared to control batch. Increase in the shell ratio might be due to enhanced silk productivity by additional supplementation of niacin. These results are supported by the observations of Rahmathulla et al. (2007) who have observed that the bivoltine hybrid CSR<sub>2</sub> x CSR<sub>4</sub> 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 (CSR<sub>2</sub> x CSR<sub>4</sub>) (Balasundaram et al., 2013).

**Filament length**

Filament length has positive correlation with shell weight. The silkworm feed on mulberry leaf fortified with niacin at varied concentrations registered marked influence on filament length. The bivoltine hybrids FC<sub>2</sub> and FC<sub>1</sub> supplemented with niacin at 0.6% exerted longer filament length 1082 and 1013m over other concentrations and control batches. The increase in filament length might be due to higher rate of silk protein synthesis by additional supplementation of niacin. These results are in conformity in the finding of Rai et al. (2012) 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 (CSR<sub>2</sub> x CSR<sub>4</sub>) supplemented with folic acid with 0.2% enhanced filament length 965m over control batch 919m (Balasundaram et al., 2013a).

**Filament weight**

Silkworm breeds reared on fortified mulberry leaves with niacin at varied concentration exhibited notable impact on filament weight. In the current study, FC<sub>2</sub> and FC<sub>1</sub> expressed gain in the filament weight of 0.290 and 0.250g, respectively with niacin supplementation at 0.6%. 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 niacin. These results are agreement in the findings of Singaravelu et al. (2001) who have 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 extra foliated with niacin at lower concentration registered encouraging results, than the higher. The larvae reared on niacin supplementation at 0.2% expressed lower denier of 2.20 in FC<sub>2</sub> and 2.01 in FC<sub>1</sub>. These results are in conformity in the findings of Balasundaram et al. (2013a)

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).

#### Renditta

The silkworms reared on fortified mulberry leaves with niacin at different concentrations expressed encouraging results in respect of renditta. The lowest renditta of 5.88 and 6.24kg were recorded in FC<sub>2</sub> and FC<sub>1</sub>, respectively. The improvement for this trait in both bivoltine hybrids at 0.6% of niacin 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) who have noticed that silkworm reared on mulberry leaf supplemented with glycine at 10 ppm concentration significantly reduces the renditta.

#### Conclusion

The bivoltine hybrids FC<sub>1</sub> and FC<sub>2</sub> performed better in respect of economic traits such as larval weight, cocoon weight, shell weight, filament length, filament weight, renditta except denier at 0.6% of niacin supplementation over other concentrations as well as control batches. Further, FC<sub>2</sub> scored better for these traits compare to FC<sub>1</sub>. The silkworm larvae reared on mulberry leaves

fortified with niacin at 0.6% concentration recorded highest activity levels of SDH and LDH activities over other concentration as well as control batch. Both the enzymes activities were relatively higher in V instar 5<sup>th</sup> day, V instar 3<sup>rd</sup> day and V instar 1<sup>st</sup> day in both the hybrids. Further, the activity levels were maximum in FC<sub>2</sub> when compared to FC<sub>1</sub>. The results of the present study revealed that, fortification of mulberry leaf with niacin at 0.6 % enhances economic parameters of mulberry silkworms.

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