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Effect of dietary lactic acid, folic acid, sucrose supplement on growth and cocoon quality of *Antheraea proylei* Lepidoptera Saturniidae

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Abstract

The silkworm, *Antheraea proylei*, was used to examine the effects of sucrose, lactic acid, and folic acid on its growth rate pattern. Third, fourth, and fifth instar larvae fed on sucrose (5%, 10%, 15%), lactic acid (10%, 15%, 20%), and folic acid (0.2%, 0.4%, 0.5%), had their growth, cocoon quality evaluated. The purpose of the study was to determine the effect of different feeding supplementation on the growth rate pattern of *Antheraea proylei*. The study's findings showed the length and weight of larvae. Result showed that lactic acid supplementation positively affects growth and cocoon quality, with increased larval weight and length which achieve the objective of Sericulture is to generate of large number of high quality of cocoons which in turn generate high quality of raw silk.

Keywords: Growth performance, sericulture, cocoon yield, dietary effect, silk quality, nutrient impact

1. Introduction

Sericulture is method of rising silkworms on plants either mulberry or non-mulberry to produce silk fibers. The semi-economy can benefit from a significant quantity of sericulture by products from silkworm forming to post - cocoon technologies albeit it is primarily employed for the textile industry ^[1].

Oak leaves have been used as a known food source for the silkworm *Antheraea proylei* for a very long time. Artificial diets have been suggested as an alternative for silkworms because there may be certain restrictions on the growth of oak leaves ^[2]. In the 1960s, research on artificial diets for silkworms started and Fukuda first raised them in 1953. The silkworm successfully transitioned from egg to moth on an artificial feed. However, because there was little knowledge of the nutritional needs at the time, diets were very simple in composition ^[3]. The impact of various sugars and associated substances on the silkworm's feeding was examined using an agar- diet that included starch casein, leaf powder, cellulose powder, and sugar, sucrose stimulated feeding the most, followed by fructose and raffinose out of more than twenty compounds tested, including monosaccharides, disaccharides, trisaccharides, glycosides, and sugar. In relation to the silkworm's dietary preferences the significance of the nutrients as feeding triggers was emphasised ^[4].

The silkworm larvae continuously observed showed that feeding took place at regular intervals throughout larval growth the actions of larvae deficient in food were also examined in orders to investigate potential factor affecting meal timing ^[5]. Longer initial mealtimes after diet replacement were the effect of food deprivation although feeding patterns were unaffected ^[6]. There are about 1000 variety of silkworm (*Bombyx mori*.) among them bivoltine, multivoltine races are particularly used for rearing in India ^[7].

A monophagous insect known as the silkworm plays a specific role in the sericulture sector. There are number of food ingredient included in the artificial diet of silkworm. Sucrose is naturally occurring disaccharides that is by far the most readily available and sucrose is low molecular weight carbohydrates ^[8].

For the silkworm, sucrose was a very ineffective stimulator. The concentration of sugar has an increasing impact.

It was further demonstrated that sucrose may induce feeding in the larva even when leaf was not included in the diet [4]. The anaerobic metabolism of glucose producer lactic acid, a naturally occurring (3 hydroxy carboxylic acid), in almost all living things. Commercially, it is a colourless to slightly yellow, hygroscopic liquid that comes in solutions of 50 to 90 weight percent [9].

Lactic Acid is a common occurrence in nature and is created by variety of organisms, including bacteria and human cells. It was discovered in 1780 as a component of sour milk and has since been employed in a variety of products for the food, beverage, pharmaceutical, cosmetic, chemical and electronic sectors, such as free acids, salts and esters [10].

In various oxidative states, Folic Acid acts as a source of single carbon units and is engaged in single carbon transfer processes. These cofactor's uptake, transportation and intracellular metabolism are complicated process [11]. Animals who consume a lot of folic acid may experience an alternation in their DNA and histone methylation, which will manifest as phenotypic changes in future generations [12]. Spraying a folic acid solution on mulberry leaves and feeding the resulting larvae to silkworm had a noticeable positive impact on weight, size, and growth rate [13]. Linnaeus in 1758 studied the folic acid effects on nutritional efficiency metrics and nutritional indexes of the silkworm *Bombyx mori* [14]. Folic acid increased the cocoon weight and shell weight. Artificial nutrition encourages tiny landless farmers to switch to sericulture and lower labour costs.

2. Materials and Methods

Eggs of silkworms (*Antheraea proylei*), Weighing machine, Disinfectant sprayer, Trays, BOD incubator, Beaker Glass bottle, Chemicals (Lactic Acid, Sucrose, Folic Acid), Distilled water, Brush, Spray bottle. *Antheraea proylei* eggs were gathered in clean cloth net bags. 0.2% of formalin solution was used to disinfect eggs and they were then washed in water to eliminate the chemical. Eggs were incubated at $(24 \pm 2^\circ\text{C})$ and 70 - 80% relative humidity.

2.1 Rearing Process

The rearing of larva has been done on the leaves of oak plant. During the larval period the larva progressed through five instar stages. The observing result on the various rearing methods including the development of the larval stage and economic aspects such as a SEM examination of cocoon's length and weight, shell weight, larval weight has been carried out. The outcome results have been recorded as a result. In rearing process three artificial diets were given i.e. Sucrose (5%, 10%, 15%), Lactic Acid (10%, 15%, 20%), Folic Acid (0.2%, 0.4%, 0.5%) solutions of different concentration and sprayed on fresh leaves of oak plant for the feeding of silkworm larvae, that improved larval weight, length of larva, growth rate, cocoon weight and shell weight. The grown-up larva was collected for cocoon harvesting from different diet. For this purpose, we use different artificial diet (Sucrose, Lactic Acid, Folic Acid). Firstly the 3 samples of different solution i.e. Sucrose (5%, 10%, 15%), Lactic Acid (10%, 15%, 20%), Folic Acid (0.2%, 0.4%, 0.5%) with different concentration. Sucrose, Lactic Acid and Folic Acid respectively were used. We diluted all three solutions with distilled water to prepare different concentration. Then different concentration of each solution was placed in different clean glass containers. Fresh leaves

were collected, and each solution was sprayed with different concentration on Oak leaves (*Quercus serrata*). Then the larva were picked up with the help of brush and placed on the nine batches of oak leaves which sprayed with different concentration of three samples for 24 hours. After that larvae attached themselves to the edge of leaves where they start feeding. They show continuous movement over the leaves then observed their feeding process and repeated this feeding process along with changing fresh leaves after every 24 hours. Larvae were shows growth and start moulting process. When larvae show third instar stage then measured the weight length and growth pattern of larvae After 3rd instar repeated the process of feeding to the 4th - 5th instar of larvae and again measure the length and growth of larva. After the 5th instar, larvae stop feeding to itself and start the spinning process of cocoon. Collect the cocoon, and measure their length, weight, and shell weight. Oak silkworm (*Antheraea proylei*) were raised from hatching to the adult stage on a diet that include Sucrose in different concentration (5%, 10%, 15%), Lactic Acid (10%, 15%, 20%) and Folic Acid (0.2%, 0.4%, 0.5%).

3. Results and Discussion: As a consequence of the current research, it was discovered that *Antheraea proylei*'s capacity to lay eggs increased as temperature increased (Average temperature 25°C). *Antheraea proylei* morphological characteristics exhibit a very consistent structure throughout each larval stage, but they differ depending on their size and colour. Different larval instar was measured for length width and weight in 53-60 days. The entire life cycle was completed. The oak Tasar silkworm *Antheraea proylei* rearing abilities produced better result in term of larval growth and economic features. The study was undertaken to evaluate their growth rate pattern of silkworm. Growth rate pattern of 3rd, 4th, 5th instar larvae was studied under the influence of Sucrose, Lactic Acid and Folic Acid.

Mean -Sum of given data / total number of data.

$$\bar{x} = \sum x / n$$

Standard deviation (s)

$$S = \frac{\sqrt{\sum (x - \bar{x})^2}}{n-1}$$

The larval stage of silkworm is a crucial time for growth and development, and the weight of the larvae might indicate how the larva is progressing. In this study from 3rd to 5th instar larvae we were measure the weight and length of larvae. After supplementation with different concentration (5%, 10%, 15%) of sucrose solution and calculated the average weight and length. In 5% concentration (1.55g $\pm$ 0.01g & 2.0cm $\pm$ 0.18cm), (7.23g $\pm$ 0.22g & 8.23cm $\pm$ 0.60cm), (8.48g $\pm$ 0.46g & 8.63cm, $\pm$ 0.61cm). In 10% concentration (1.24g $\pm$ 0.03g & 1.8cm $\pm$ 0.08cm), (6.0g $\pm$ 0.44g & 6.6cm $\pm$ 0.70cm), (8.18g $\pm$ 0.58g & 9.1cm $\pm$ 0.29cm). In 15% (1.53g $\pm$ 0.02g & 2.0cm $\pm$ 0.08cm), (6.35g $\pm$ 0.66g & 8.18cm $\pm$ 0.25cm), (8.83g $\pm$ 0.53g & 10.95cm $\pm$ 0.12cm) respectively. In 3rd & 4th instar larva 5% concentration increased weight and length and in 5th instar larva highest (15%) concentration shows increased weight and length. As shown in (Table 3.1).

Feeding responses in Lactic acid supplementation (10%, 15%, 20%) in 3rd, 4th & 5th instar, larvae shows average weight and length with different concentration is (1.34g ± 0.01g & 2.05cm ± 0.12cm) (9.73g ± 0.51g & 9.2cm ± 0.77cm) (12.1g ± 0.26g & ± 10.28cm ± 0.35cm), (1.25g ± 0.01g & 1.98cm ± 0.17cm), (10.43g ± 0.79g & 9.05cm ± 0.31cm) (11.28g ± 0.38g & 9.7cm ± 0.45cm), (0.94g ± 0.01g & 1.88cm ± 0.09cm) (9.03g ± 0.77g & 8.2cm ± 0.29cm) (9.03g ± 0.34g & 10.65 cm ± 0.20cm) respectively. In 10% concentration 3rd and 5th instar larvae have increased weight and length but in 4th instar larvae medium concentration (15%) shows highest value. So, we found that (Table 3.2) shows lowest concentration in 3rd, 4th and 5th instar shows better growth and development during larval stage.

In folic acid supplementation (0.2%, 0.4%, 0.5%) in 3rd, 4th, 5th instar, larvae show average weight and length with different concentration is (1.32g ± 0.01g & 1.89cm ± 0.12cm) (7.75g ± 2.1g & 6.05 cm ± 0.31cm) (8.95g ± 1.38g & 8.78cm ± 0.38cm), (1.5g ± 0.01g & 1.93cm ± 0.22cm) (8.85g ± 0.75g & 8.18cm ± 0.53cm) (11.9g ± 1.14g & 11.18cm ± 0.40cm), (1.52g ± 0.01g & 2.05cm ± 0.12cm) (8.33g ± 0.33g & 7.18cm ± 0.27cm) (11.18g ± 0.53g & 10.5 cm ± 0.25cm) respectively. In 0.4% concentration 4th and 5th instar larvae have increased weight and length but in 3rd instar larvae concentration (0.5%) shows highest value (Table 3.3).

The result of cocoon parameters in sucrose solution with different concentration having average weight and length (4.15g ± 0.23g & 4.85cm ± 0.12cm), (3.78g ± 0.49g & 4.98cm ± 0.41cm), (5.55g ± 0.23g & 5.13cm ± 0.22cm) respectively. Then the cocoon parameters demonstrated that the weight and length of the cocoon is increase in highest concentration (15%) Table (3.4).

The result of cocoon parameters in lactic acid solution with different concentration having average weight and length (4.9g ± 0.49g & 4.65cm ± 0.26cm), (5.33g ± 0.31g & 4.9cm ± 0.21cm), (4.7g ± 0.49g & 5.13cm ± 0.29cm) respectively (Table 3.5). Then the cocoon parameters demonstrated that the weight of the cocoon was increased in middle concentration and length of the cocoon was increase in highest concentration. The result of cocoon parameters in Folic acid solution with different concentration having average weight and length (5.1g ± 0.29g & 3.75cm ± 0.62cm), (3.9g ± 0.31g & 4.0cm ± 0.35cm), (6.33g ± 0.31g & 3.9cm ± 0.43cm) respectively (Table 3.6). Then the cocoon parameters demonstrated that the weight of the cocoon was increased in highest, and length of the cocoon is increase in middle concentration. The result of the shell weight under the influence of different solution i.e. sucrose, lactic acid and folic acid with different concentration. In sucrose

solution (5%, 10%, 15%) having weight is (0.4g ± 0.08g, 0.35g ± 0.05g, 0.5g ± 0.08g) In lactic acid (10%, 15%, 20%) having weight is (0.5g ± 0.05, 0.5 ± 0.08g, 0.6g ± 0.08g). In folic acid (0.2%, 0.4%, 0.5%) having weight is (0.38g ± 0.09, 0.38g ± 0.09g, 0.4g ± 0.08g) respectively. Then the shell weight of the lactic acid solution is more in the comparison of folic acid and sucrose solution as shown in (Table 3.7).

Previous study clearly shows that significantly different parameters found in the larvae fed with different concentration diet such as number of faces per day, body weight, sucrose activity [18].

In present study, concluded that sucrose solution shows better result in different parameters in lowest concentration in 3rd and 4th instar larval stage and in 5th instar larval stage highest concentration shows the better result in different parameters. According to Zhen, a low lactic acid concentration promotes larval growth, whereas a high lactic acid concentration prevents larval eating and reduces larval growth. Because of the potential for reduced leaf quality brought on by a high lactic acid content (10%), mulberry leaves may be preferred by the larvae [19].

The result of the present study also correlated with [19], where lactic acid shows better result in growth and development in lower concentration whereas sucrose and folic acid shows decrease growth and development. High concentration of lactic acid reduces the quality of leaf it effects the growing pattern of larva.

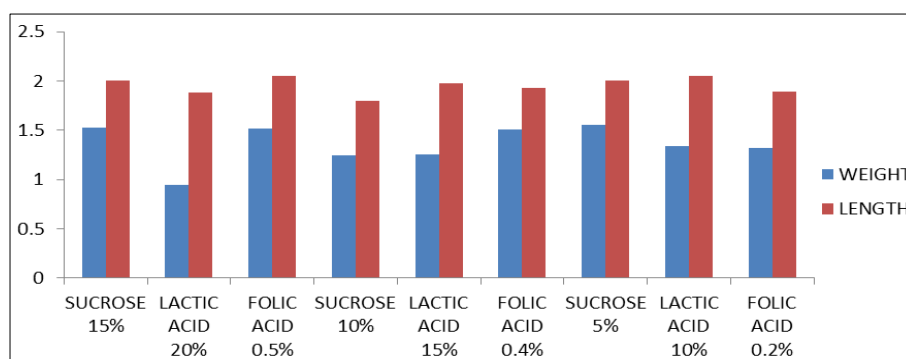
Rahmathulla V.K studied that folic acid act as a growth promoter and significantly increases the yield per weight of cocoon. The result also shows that folic acid application on leaf does not affect survivability of the silkworm larvae [13].

In our study, we concluded that the growth parameters show variation in all instars and shows significant result in 4th and 5th instar larval stage but non-significant result in 3rd instar larval stage.

When we compared all the 3 concentrations higher, lower, and middle we concluded that it shows the significant result in 4th and 5th in star and also in cocoon and shell weight. Non-significant result shows in 3rd instar stage.

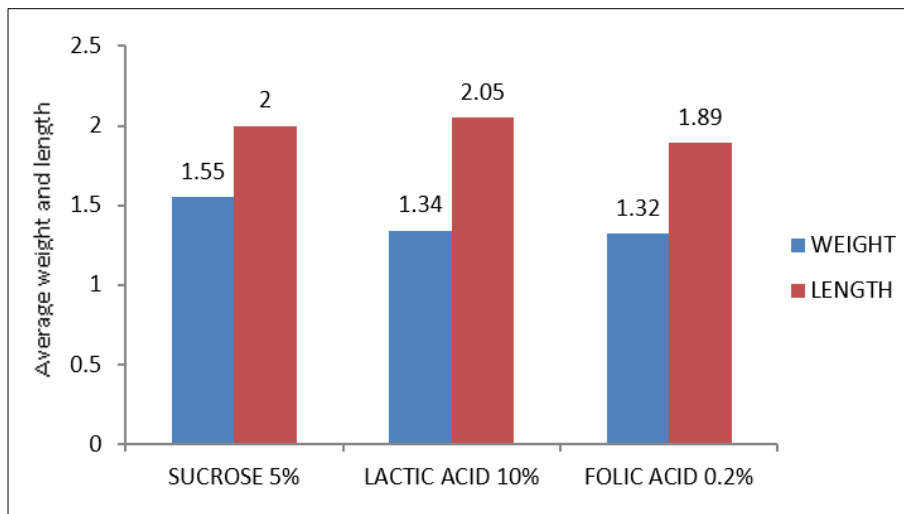
In the current study we investigated how different solution affected *Antheraea proylei* development and growth. We discovered that lactic acid feeding shows maximum growth and development in all larval stages and cocoon.

The present study shows that the calcium deposition is maximum in folic acid and lactic acid as compared to sucrose solution. The pores are more evident in folic acid and sucrose solution as compared to the lactic acid solution.

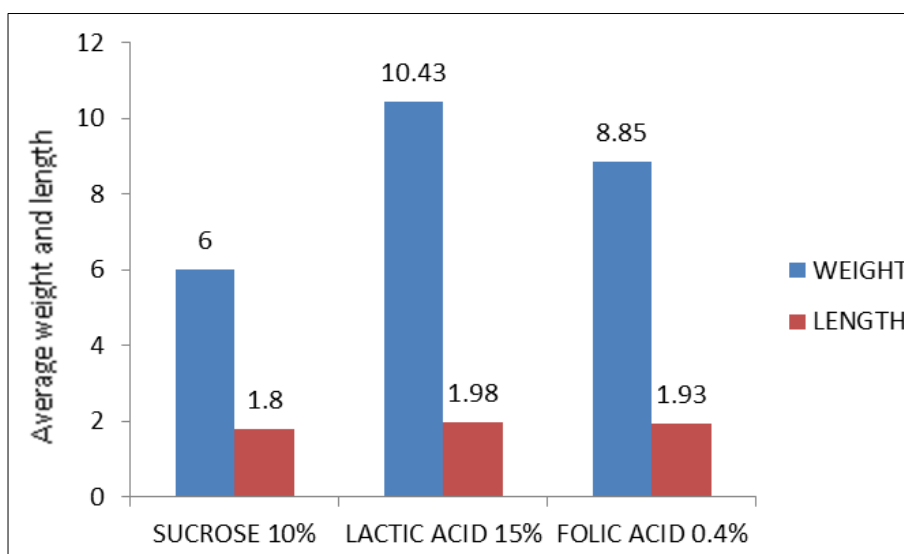


Different concentration of solution in third instar larva

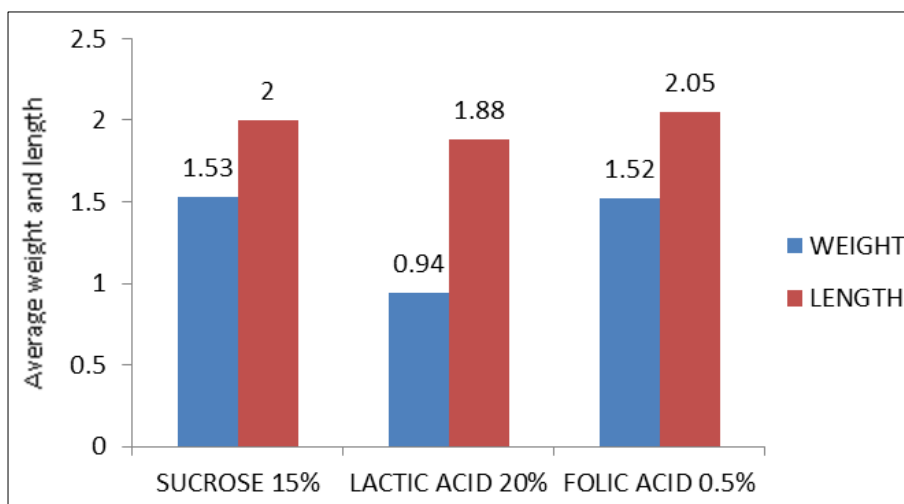
(A)



(B)

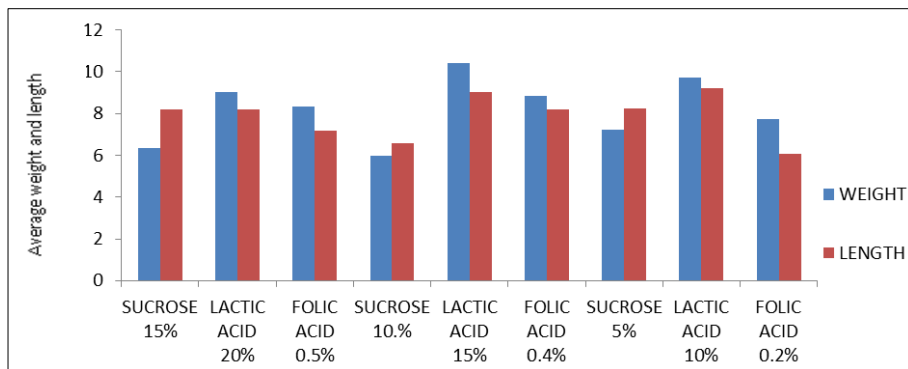


(C)



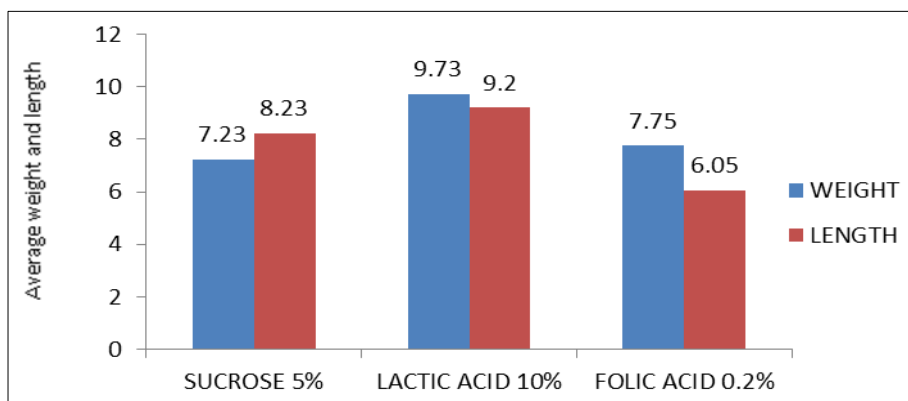
(D)

Fig 1: Graphical representation of third instar larva.

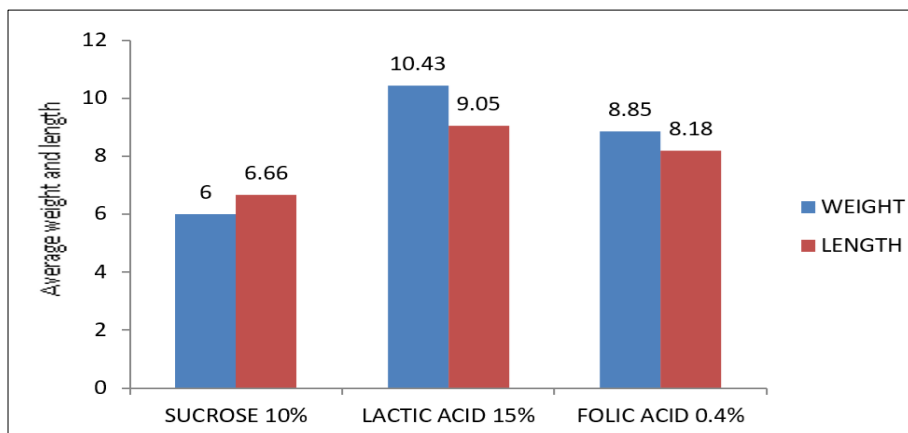


(A)

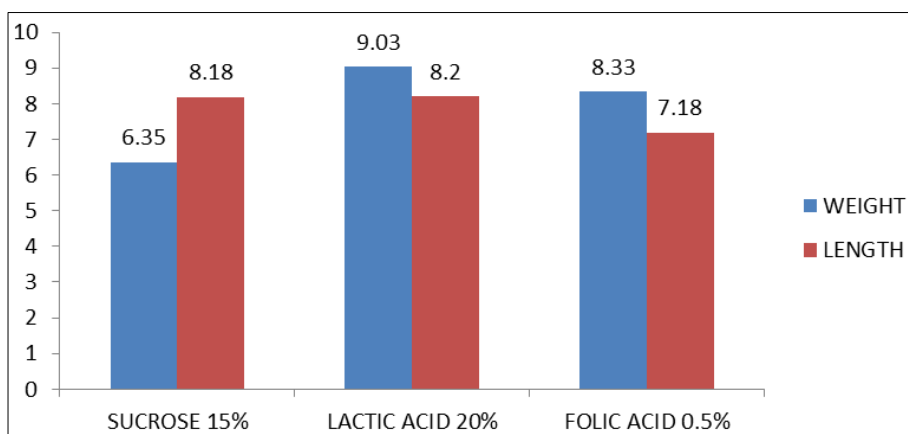
Different concentration of solution



(B)

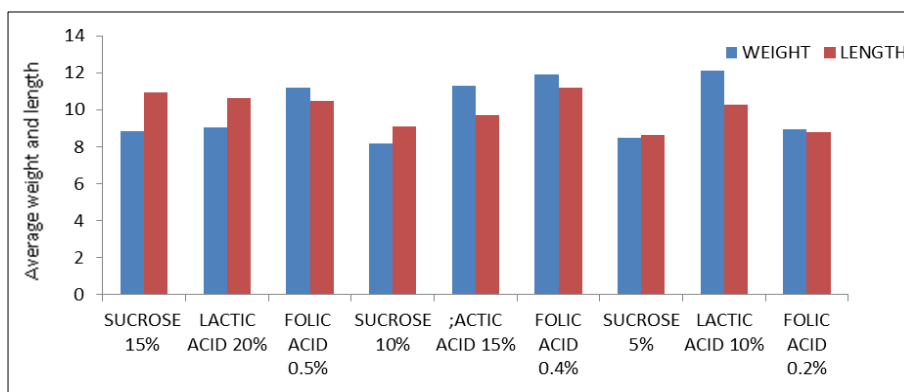


(C)

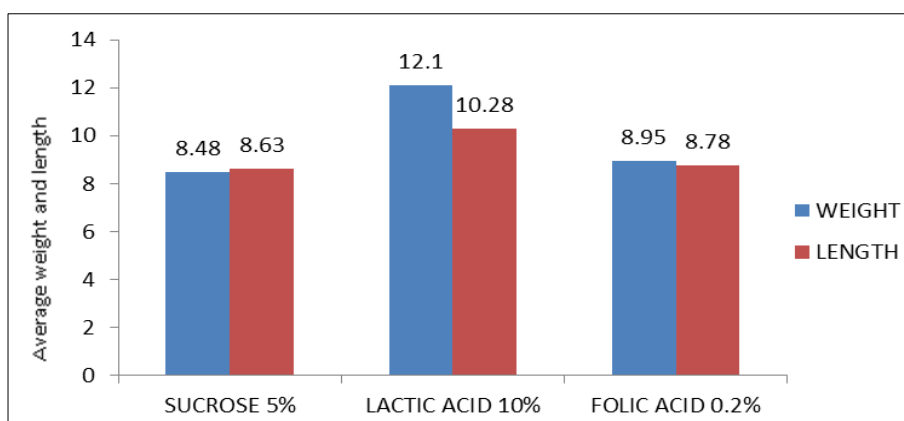


(D)

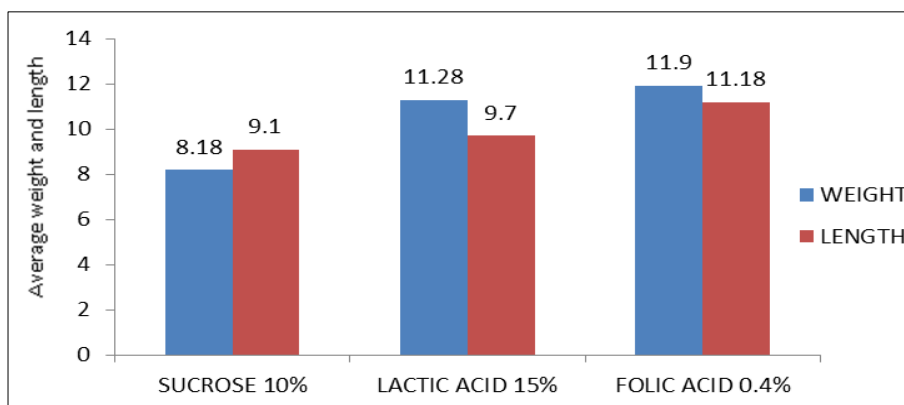
Fig 2: Graphical representation of fourth instar larva.

Different concentration of solution

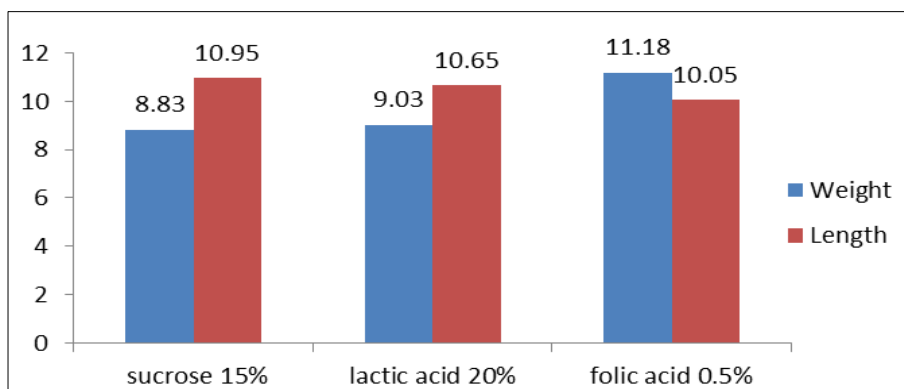
(A)



(B)

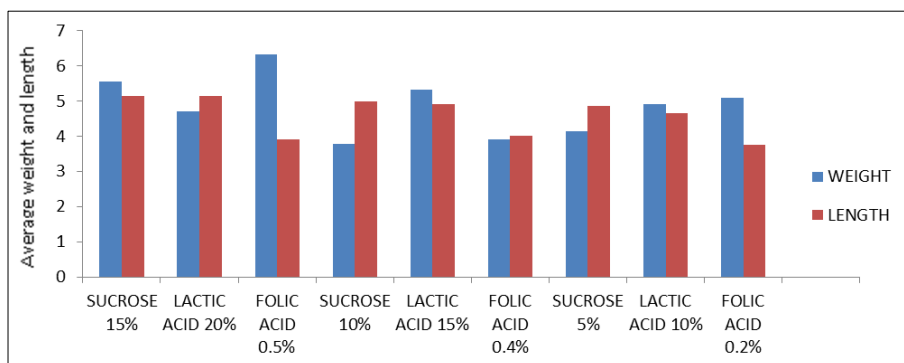


(C)

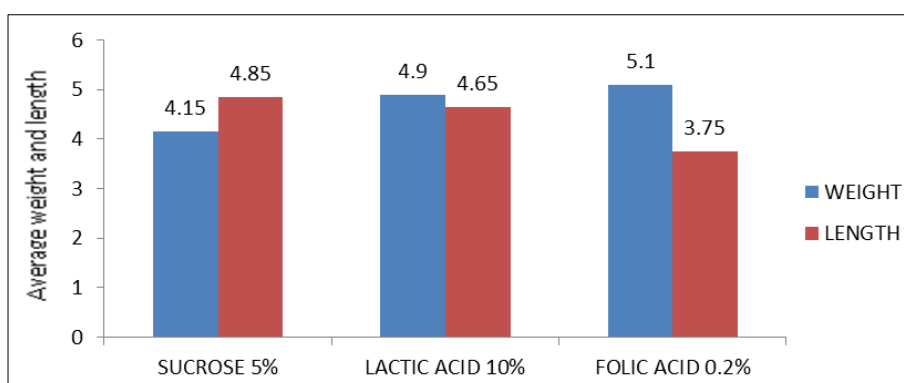


(D)

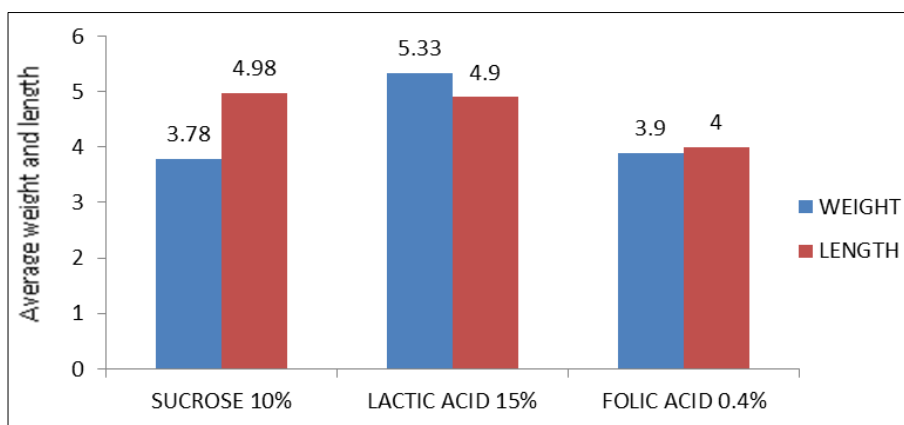
Fig 3: Graphical representation of fifth instar larva.

Different concentration of solution

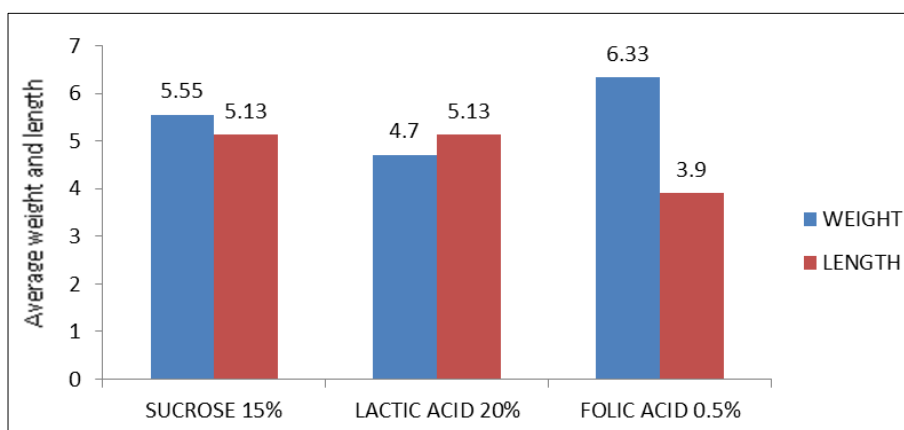
(A)



(B)



(C)



(D)

Fig 4: Graphical representation of Cocoon weight and length.

Different concentration of solution

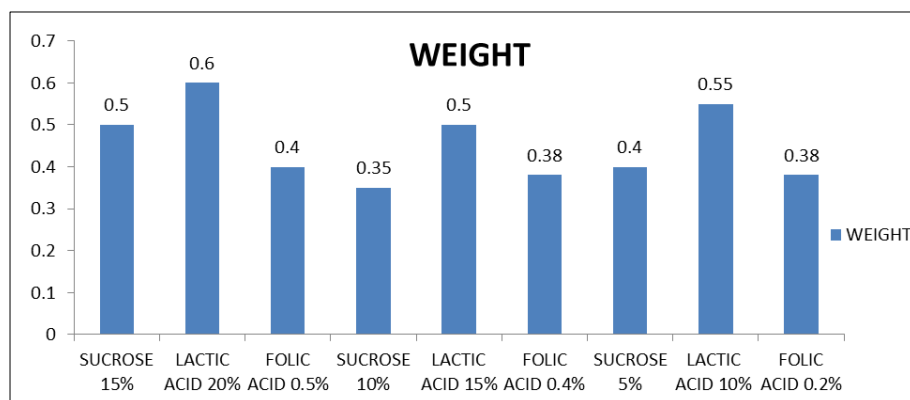


Fig 5: Graphical representation of Shell weight.

Table 3.1: Effect of sucrose with different concentration on different larval stages of the silkworm.

Larval stage	Weight (g)			Length (cm)		
	5%	10%	15%	5%	10%	15%
3 rd instar	1.54 ± 0.01	1.24 ± 0.03	1.53 ± 0.02	2.0 ± 0.18	1.8 ± 0.08	2.0 ± 0.08
4 th instar	7.23 ± 0.22	6.0 ± 0.44	6.35 ± 0.66	8.23 ± 0.60	6.6 ± 0.70	8.18 ± 0.25
5 th instar	8.48 ± 0.46	8.18 ± 0.58	8.83 ± 0.53	8.63 ± 0.61	9.1 ± 0.29	10.9 ± 0.12

Table 3.2: Effect of lactic acid with different concentration on different larval stages of the silkworm.

Larval stage	Weight (g)			Length (cm)		
	10%	15%	20%	10%	15%	20%
3 rd instar	1.34 ± 0.01	1.25 ± 0.01	0.95 ± 0.25	2.05 ± 0.12	1.98 ± 0.17	1.88 ± 0.09
4 th instar	9.73 ± 0.51	10.4 ± 0.79	9.03 ± 0.77	9.2 ± 0.77	9.05 ± 0.31	8.2 ± 0.29
5 th instar	12.1 ± 0.26	11.2 ± 0.38	9.03 ± 0.34	10.2 ± 0.35	9.7 ± 0.45	10.6 ± 0.20

Table 3.3: Effect of folic acid with different concentration on different larval stages of the silkworm.

Larval stage	Weight (g)			Length (cm)		
	0.2%	0.4%	0.5%	0.2%	0.4%	0.5%
3 rd instar	1.33 ± 0.01	1.5 ± 0.01	1.51 ± 0.01	1.85 ± 0.12	1.93 ± 0.22	2.0 ± 0.12
4 th instar	7.75 ± 2.14	8.85 ± 1.75	8.33 ± 0.33	6.05 ± 0.31	8.18 ± 0.53	7.18 ± 0.27
5 th instar	8.95 ± 1.38	11.9 ± 1.14	11.18 ± 0.53	8.78 ± 0.38	11.18 ± 0.40	10.5 ± 0.25

Table 3.4: Cocoon quality parameter of sucrose solution with different concentration (cocoon weight and length).

Cocoon	Weight(g)			Length (cm)		
	5%	10%	15%	5%	10%	15%
	4.15 ± 0.23	3.78 ± 0.49	5.55 ± 0.23	4.85 ± 0.12	4.98 ± 0.41	5.13 ± 0.22

Table 3.5: Cocoon quality parameter of lactic acid solution with different concentration (cocoon weight and length).

Cocoon	Weight(g)			Length (cm)		
	10%	15%	20%	10%	15%	20%
	4.9 ± 0.49	5.33 ± 0.31	4.7 ± 0.49	4.65 ± 0.26	4.9 ± 0.21	5.13 ± 0.29

Table 3.6: Cocoon quality parameter of folic acid solution with different concentration (cocoon weight and length).

Cocoon	Weight(g)			Length (cm)		
	0.2%	0.4%	0.5%	0.2%	0.4%	0.5%
	5.1 ± 0.29	3.9 ± 0.31	6.33 ± 0.31	3.75 ± 0.62	4.0 ± 0.35	3.9 ± 0.43

Table 3.7: Shell weight of different concentration of different solution.

Shell Weight	Sucrose			Lactic Acid			Folic Acid		
	5%	10%	15%	10%	15%	20%	0.2%	0.4%	0.5%
	0.4 ± 0.08	0.35 ± 0.05	0.5 ± 0.08	0.5 ± 0.05	0.5 ± 0.08	0.6 ± 0.08	0.38 ± 0.09	0.38 ± 0.09	0.4 ± 0.08

4. Conclusion

Sericulture's purpose is to generate a huge number of huge quality cocoon, which in turn provides high grade raw silk. Sericulture is a two-step process that involves the cultivation

of Oak trees and the raising of silkworm on Oak leaves to generate cocoons. It is determined that the *Antheraea proylei* eggs that were raised in the Chauntra Research Extension Centre (Distt.Mandi) exhibit variation at various

larval instar stages. The early stages of the egg's development saw them as oval and light brown in colour. In between 53 and 60 days, the entire life cycle was completed. It was shown that *Antheraea proylei*'s ability to lay eggs improved as temperature increased (average temperature 25°C). The size and colour of *Antheraea proylei*, cause their morphological characteristics, which are essentially identical in structure throughout all of the larval instar stages, vary.

In larval feeding of different solution with different concentration data revealed that feeding with lactic acid solution showed comparatively better development and growth with regards to folic acid and sucrose solution.

The experiment showed that lactic acid larvae have more weight, length and also have bright green colour and their feeding process is also better than the other larvae. In cocoon and shell quality parameter demonstrated that lactic acid shows better result as compared to the weight and length of sucrose and folic acid.

It is studied that larva feeding also affect the quality of cocoon. In this feeding process of larva, the quality of cocoon is good and most cocoon were oval shape and typically had a light brown tint. The dimensions and shapes of the cocoon were very similar. The cocoons either have a multilayer or single layer structure. The double layered cocoons structure of *Antheraea proylei* consists of a huge a typical outer bag that encloses and independent inner cocoon shell. The number of larvae is correlated with the weight of the cocoon (without pupa). It will see in the future how the productivity of rearing influences both the amount and quality of Tasar silk output.

According to the results of the SEM analysis study, the cocoon of a silkworm has a multilayer structure. It has non-woven, very fibrous structure. The cocoon filament is made up of two layers: a fibron inner layer and an outer sericin layer. The exterior surface of the cocoon clearly shows calcium oxalate crystals about 1 millimetre in size. The *Antheraea proylei* exhibits calcium deposition in all samples, with folic acid and lactic acid exhibiting the greatest deposition compared to sucrose solution. The pores are more evident in folic acid (0.2%) and sucrose (5%).

This is concluded from the above study that the rearing of silkworm on *Quercus serrata*, with different feeding pattern increase the growth and the development of *Antheraea proylei*.

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