Effect of the Nutritional Fortification of Nanoparticles of Alanine on the Growth and Development of Mulberry Silkworm, *Bombyx mori L.*

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Abstract: The freshly collected mulberry leaves were smeared with solutions of nanoparticles of alanine an aminoacid in different concentrations. The enriched leaves were air dried. The fifth instar larvae of mulberry silkworm *Bombyx mori L.* were fed with enriched leaves thrice a day. Feeding parameters, Food conversion efficiency, growth and economic characters were studied and recorded. Nanoparticles of aminoacids significantly enchanced the growth of the larvae, silkgland weight and cocoon parameters when compared to control. So aminoacid nanoparticles can be used as a fortification agent for improving the silk production.

***Index Terms*:** Alanine, nanoparticles, enrichment, silk glands, silkworm**.**

Introduction

Silkworm, *B.mori* is an oligophagous insect that feeds mainly on the leaves of the mulberry plant. The successful harvest of quality cocoons depends exclusively on the nutrition of the silkworm (Nagesh and Devaiah, 1996). Nutrition of silkworm is the sole factor which almost individually augments the quality and quantity of cocoon production (Laskar and Datta, 2000). *B. mori* feeds on the leaves of the mulberry tree, and serves as an excellent model system because of its short life cycle, inexpensive rearing method, and lack of ethical issues involved (Hiroshi *et al*., 2008). It is an economically important insect. Therefore among all the insects, the mulberry silkworm was considered the most fit organism to carry out the proposed study.

Fortifying mulberry leaves with supplementary agents such as antibiotics, juvenoids, botanicals, feed additive flours, etc., so as to improve the growth of silkworms and in-turn the quality and quantity of silk. Proteins form the main constituents of the silk fibre. The proteins have been reported to improve the growth, development, immunity and thus increase the silk yield (Nasreen *et al*., 1999). Among the various dietary protein supplements tested, casein, glutan and soya flour are the most attractive sources in terms of cost and quality and have been extensively studied and generally recommended for fortification (Gupta *et al*., 1977; Raj *et al*., 2000). Likewise more than 30 different supplementary nutritients have been analyzed and in different regions with different climates (Etebari et al., 2004). The pervious investigations showed that methods of supplementation, had considerable effect on growth and silk yield.

Enrichment of the mulberry leaves is one of the strategies by which cocoon and silk productivity can be increased and the quality can be enhanced and maintained by fortifying amino acids (El-Karaksy and Idriss, 1990; Kabila *et al.*, 1994; Sarkar *et al.*, 1995; Yasmin *et al.*, 1995; Nirwani and Kaliwal, 1996, 1998; Zaman *et al.*, 1996; Basit and Ashfagh, 1999; Goudar and Kaliwal, 1999; Etebari and Matindoost, 2005). These studies show that nutritional supplements have a significant impact on larval growth and cocoon production (Ito and Tanaka, 1962). It has been shown that 75% of mulberry leaf protein is directly converted into silk protein and 25% goes to the body tissues of the silkworm larvae (Tazima, 2001). Although quite a few studies have been conducted on amino acids supplementation, (Sarkar *et al.*, 1995; Yasmin et al., 1995; Zaman *et al.*, 1996; Basit and Ashfagh, 1999; Etebari and Matindoost, 2005). β-alanine is a derivative of B5 vitamin (pantothenic acid) and coenzyme A (Chapman, 1998). Although quite a few studies have been conducted on amino acids supplementation, their results vary (Sarkar *et al*., 1995; Yasmin *et al.*, 1995; Zaman et al., 1996; Basit and Ashfagh, 1999; Etebari and Matindoost, 2005). L-Alanine is the most predominant composition of silk. 34.36% of silk is composed of alanine (shin *et al*,2009)

The addition of aminoacids in low concentrations gave best results regarding weights of larva, percentage of silkgland, crude protein, cocoon and cocoon shells (Moustafa and elkarasky,1900) the supplementation with a mixture of glycine, alanine, sugar, potassium iodide salt, vitamins, soymilk, significantly improved the larval weight, silkgland weight, chemical composition of silkgland and cocoon characters of *Bombyx mori* (Sarkar *et al* 1995). Fortification of mulberry leaves with extra nutrients like glucose, glycine, egg albumin, molasses etc., was found to increasethe larval growth and improve cocoon characters (Verma and Atwal,1963; Sengupta *et al* 1972). The requirements of amino acids in *B. mori* are confirmed by different experiments (Qadar et al., 1994; Eid et al., 1989; Khan & Saha, 1995 and Saha et al.,1994). The absorption, assimilation, and conversion efficiencies will be greater when nanoparticles of nutrients are used as food supplements.The works on the supplementation of nanoparticles in silkworms are scanty.

Nanotechnology has started getting momentum in the advancement of functional feed which deliver the nutrients effectively. Nanotechnology affords novel ways to enhance the growth and production in the field of nutrition. Though the technology is attaining constant development with varied application studies in nutrition of silkworm are limited. Considering the key role played by amino acid absorption in silkworm nutrition and development, attempts have been made here to assess the effects of alanine nanoparticle supplementation on the growth and the economic traits of the silkworm, *B. mori L.*

Materials and methods

2.1 Silkworm Rearing

The commercial breed of the mulberry silkworm i.e., the double hybrid CSR6 × CSR26 × CSR2 × CSR27 collected from the silkworm rearing Department, Rayanur, Karur district was used for this study. The silkworm rearing was followed by the standard protocol suggested by Regional Sericulture Research Station, Salem.

Alanine was purchased from Loba-Chemie Indo Austranal Co, Bombay. In this work, the size of the alanine particles was reduced to the size of 267nm by ball milling method using Pulverisette 7. Such nanoparticles of alanine were supplemented to the silkworm larvae along with mulberry leaves in different concentrations. Fortification of such alanine nanoparticles started from the first day of V instar larvae and were reared both in control and experimental groups. The experimental group larvae were divided into 12 groups with each groupcontaining 30 larvae to evaluate the effects on the growth of the silkworm.

2.2 FEED EFFICACY AND COCOON PARAMETERS

The quantity of mulberry leaves offered to all groups was similar and the larvae were fed three times a day (9.00 AM, 1.00 PM and 5.00 PM). Unfed leaves larvae and faecal pellets were weighed daily and recorded. Based on the data recorded feed efficacy parameters like approximate digestibility, Efficiency of conversion of digested food, Efficiency of conversion of ingested food, rate of consumption, rate of assimilation, rate of production and rate of metabolism, food consumption Index, coefficient of food utilization and tissue somatic Index were calculated by using the formulae of Macfadyen and Petrusewicz (1970).

Cocoon parameters like cocoon weight, cocoon shell weight, cocoon shell ratio, and pupal weight were recorded by using standard methodology described by Etebari *et al*, (2002)

2.3 STATISTICAL ANALYSIS

Collected data were subjected to statistical analysis such as ‘t’ test to find out the significant difference between control and experimental groups.

RESULTS AND DISCUSSION

3.1 Feeding Parameters

Feeding parameters of the larvae were recorded in the Table 2. Alanine is present at a level of 1.23% in dry mulberry leaves which is what a silkworm requires for its normal growth (Ito, 1978). In general, silkworms do not encounter alanine deficiency (Hamamura, 2001). Feeding parameters like Consumption rate, Assimilation rate, Production rate, Metabolism rate were significantly lesser in the alanine supplemented larvae and less concentrated (8%, 17%, 6%, 54%) in sucrose supplemented larvae than that of the control. Though significant difference in these factors was observed among the larvae supplemented with different concentration of nanoparticles of alanine, there was no regular increase or decrease in these parameters with the increasing concentration of the alanine. This indicates the increase in concentration of nanoparticles of alanine had no effect on feedinng parameters.

Approximate digestibility was found to be about 1% lesser in sucrose supplemented larvae than that of the control, whereas it was greater in alanine supplemented larvae. No significant difference was noticed in Approximate digestibility among the larvae of the experimental sets. This indicates that increase in concentration of nanoparticles of alanine had no effect on the digestibility.

Efficiency of conversion of digested food and Efficiency of conversion of ingested food were significantly greater in the sucrose supplemented larvae and less concentrated in alanine supplemented larvae than that of the control and experimental sets. Efficiency of conversion of digested food and ingested food were found to be decreasing with increasing concentration, so lower concentrations of (5µg/ml) alanine increase the Efficiency of conversion of digested food and Efficiency of conversion of ingested food.

Food consumption index was significantly greater in the sucrose supplemented larvae and less concentrated in alanine supplemented larvae than that of the control and experimental sets. Food consumption index was found to be decreasing with increasing concentration, so lower concentrations of 5µg/ml alanine increase the food consumption index.

Coefficient of food utilization was found to be 1% lesser in the sucrose supplemented larvae and greater in alanine supplemented larvae than that of the control. Lower concentrations of (5µg/ml) alanine increased the coefficient of food utilization significantly.

The increase in food consumption and utilization might be due to the supplementation of nanoparticles of alanine, they increase the proportion of food nutrients that pass across the gut tissue and body tissue rather than passing the food directly through the digestive system unused. Nanoparticles may pass into the cells more readily than their larger counter parts. This would have accelerated their assimilation process.

Tissue somatic index (TSI) signify the percentage of tissue in entire body and it was found to be significantly greater in the alanine supplemented larvae and less in sucrose supplemented larvae than that of the control. Tissue somatic index was found to be increasing with increasing concentration, so higher concentrations of (15µg/ml) alanine increase the tissue somatic index.

3.2 Larval weight and Silk gland weight of the V instar larva

The larvae of the control sets and experimental sets were found to be increasing in weight daily. While comparing with the control larvae, significant increase in weight of the larvae was observed in all experimental sets. Single larva weighed about 0.4 to 0.9 g during first day of V instar, weight of the larva was found to be increasing daily and at the last day of V instar the weight was about 2.33g in control and 2.42g in15µg/ml alanine supplemented larvae. Fortification of alanine resulted in increased larval weight in all 3 groups, Amino acid I (66%), Alanine II (17.391%), Alanine III (28.985%). Statistically the results were significant. Silk gland weights were found to be higher in all aminoacid treated sets than the control. Highest silk gland weight with 86.075% increase over control was recorded in Alanine III (15µg/ml). Tissue somatic index was also evaluated with the data recorded, result data was found to be higher in Alanine III (15µg/ml) with 79.170% increase than control.

The average weight of the larva fed with normal mulberry leaves (control) was found to be 1.38g. though the larval weight was found to be greater in sucrose supplemented larvae the increase was not significant statistically. The weight of the aminoacid supplemented larva was significantly greater than that of the control and sucrose supplemented larvae. Highest larval weight was recorded in the larvae supplemented with 15µg/ml alanine. Larval weight was found to be increasing with increasing concentration, significant difference was noticed in larval weight among the larvae in the experimental sets over control. This indicates the increase in concentration of nanoparticles of alanine had a significant effect on the larval weight.

Silk gland weight was greater (86.075%) in nanoparticles of alanine (15µg/ml) supplemented larvae (1.47g) and minimum in control (0.79 g). Silk gland weight was significantly greater in both sucrose supplemented larvae and alanine supplemented larvae than that of the control. Silk gland weight was found to be increasing with increasing concentration, so higher concentrations of (15µg/ml) alanine increased the silk gland weight.

While comparing the results with Rouhollah Radjabi (2010), it is clear that when the size of alanine was reduced the intestinal absorbance of alanine became greater, The present study showed a better results on larval weight and silk gland weight when alanine was fortified without reducing the size (Table 3). The increase in growth of the larvae might be due to the conversion of alanine into aspartate or glutamate through transamination. The increase in growth can be correlated with the role of Alanine in glucose, tryptophan and organic acid metabolism.

3.3 Cocoon parameters

After complete spinning of larvae cocoons were formed, those cocoons were analysed, the results revealed that the weight of the control cocoon was 0.71g and cocoon weight was found to be maximum in the larvae fed with mulberry leaves supplemented with 15µg/ml alanine (1.21g) This indicate the increase in concentration of nanoparticles of alanine had no effect on the cocoon weight.

Shell weight was found to be about 6% greater in sucrose supplemented larvae than that of the control, whereas it was greater in alanine supplemented larvae. This indicate the increase in concentration of nanoparticles of alanine had significant effect on the shell weight.

Pupa weight was significantly greater in both sucrose supplemented larvae and alanine supplemented larvae than that of the control. Pupa weight was found to be decreasing with increasing concentration, so lower concentrations of (5µg/ml) alanine increase the pupal weight.

The weight of the pupa formed from the larvae with 5µg/ml alanine was found to be greater than control. The maximum pupal weight was found in 5µg/ml and 15µg/ml alanine supplementation (1.11 g) and (0.95 g) as compared to control (0.56 g). These findings are of great significance from the view point of rearing silkworms for production of seed cocoons as fecundity of silk moth is dependent on pupal weight. This necessitates the application of feed additives to silkworm at an optimum level. The present findings are in agreement with the finding of Sridhar and Radha (1986) and Babu (1994), who also reported higher pupal weight in silkworms reared on glycine fortified leaves. So such pupae with more nutritious substances would be more valuable as a fish feed and poultry feeds

Shell ratio depends upon the quantity of the silk produced from each cocoon and was found to be lesser in sucrose supplemented and alanine supplemented larvae and was found to increasing with increasing concentrations, so higher concentration of (15µg/ml) alanine increase the shell ratio.

In silkworms, silk fibroin is derived mainly from four amino acids: alanine, serine, glycine and tyrosine (Kirimura, 1962) which come from their dietary source of protein and amino acids (Ito, 1983). Silkworms obtain 72-86% of their amino acids from mulberry leaves. More than 60% of the absorbed amino acids is used for silk production (Lu and Jiang, 1988).Kabila et al. (1994) indicated that the addition of aspartic acid in concentration of 1 or 2% to mulberry leaves increased the economic characteristics of the silkworms.

While comparing the results with Rouhollah Radjabi (2010), it is clear that when the size of alanine is reduced the absorbance of alanine is seen to be more, The present study showed a better results on cocoon parameters than fortifiying the alanine without reducing the size (Table 4). The nanoparticles of alanine can reach the haemolymph easily and hence they are effectively used for the synthesis of silk protein. They can inhibit the bacterial attachment to intestinal cells. Nanoparticles offer a very larger surface for absorption because of their ultra fine structure and hence function very effectively than larger particles.

3.4 Filament parameters

Filament length and weight were significantly greater in both sucrose supplemented larvae and alanine supplemented larvae than that of the control. Filament length was found to be increasing with increasing concentration, so higher concentrations of (15µg/ml) alanine increase the filament length. Maximum silk filament weight was found in silk produced by the larvae supplemented with (15µg/ml) alanine concentration. The highest silk filament weight was found in larvae supplemented with (15µg/ml) (0.49g) which is 172.22% higher than control, The minimum filament weight was in control (0.18 g). Higher silk filament weight was increased due to amino acid supplement through the mulberry leaves at an optimum level which resulted in higher silk filament length in turn resulted in higher silk filament weight.

Denier is the unit of measure the linear mass density of fibers, is the mass in grams per 9000 meters of the fiber and is used to estimate the number of cocoons required to reel the silk. Denier was significantly greater in both sucrose supplemented larvae and alanine supplemented larvae than that of the control. Denier was found to be increasing with increasing concentration, so higher concentrations of (15µg/ml) alanine. The maximum denier was recorded (5.582) and minimum was in (3.903). Among the treatments, mulberry leaves treated with (15µg/ml) alanine concentration was having higher denier (5.582) with 43.018% increase than control and least was recorded in absolute control (3.903). The significant increase in the denier may be due to fineness of the silk filament. As silk filament length and silk filament weight increased the denier also increased over the control. The similar findings are found in case of Chakrabarty and Kaliwal (2012) who experimented application of Arginine, Histidine and their mixtures to the silkworms and found improved denier at a rate of 150µg/ml over the control.

Rouhollah Radjabi (2010) reported that the weight of larvae fed with alanine was 1.291g during v instar. But when alanine was given in the form of nanoparticles the larval weight results were found to be increasing with increasing concentration. Rouhollah Radjabi (2009) reported that when larvae were fed with alanine silk gland weight was about 0.866g but in our study when aminoacid was fed in the form of nanoparticles the silk gland attained its maximium weight up to 1.47g. Cocoon characters were also found as a negative results in Rouhollah Radjabi (2010) cocoon weight (1.174g) pupa weight (0.89g) shell weight (0.275g) and shell ratio (23.42) where as our experiment gave a better results cocoon weight (1.21g) pupa weight (0.95g) shell weight (0.25) and shell ratio (20.661) the reason behind this may be due to the size of the alanine treated, when the size of the alanine is less absorbance is found to be more.

Conclusion

Amino acids are closely related to the biosynthesis of silk proteins as well as to the growth of silk glands of *B. mori* (Ito, 1972). It has been experimentally determined by Bose et al. (1989) that silkworms require 18 amino acids for their adequate nutrition. Amino acids and their derivatives participate in intracellular functions as diverse as nerve transmission, regulation of cell growth and the biosynthesis of various compounds in silkworm (Rodwell, 1993).

The increase in quantity of silk yield, decrease in some feeding parameters in larvae supplemented with nanoparticles of alanine over control larvae can be correlated with the greater efficiencies of conversion of digested food and ingested food. This confirms the fact that alanine increases the efficiency of conversion and metabolic rate.

Since the absorbance of alanine is more when the size of alanine is less, it is concluded that alanine nanoparticle supplementation increased the growth of the larvae and increased the quantity and quality of the silk. Therefore alanine nanoparticle shall be recommended as a fortifying agent to the sericulture farmers after analyzing the whole molecular mechanism of the role of alanine in improving the silk yield. Organic nanoparticles increase the nutritional value, they can be encapsulated and commercially manufactured on a large scale. Such capsules enclosing nanoparticles increase the bioavailability, shelf life and deliver the nutrients without affecting the taste.

Table – 1: Control and Experimental groups used in this study to determine the effects of nanoparticles of Alanine in silkworm larvae.

Groups of Larvae	**Description**
Control	Mulberry leaves without any supplementation.
Sucrose (2g/100ml)	Mulberry leaves smeared with 2% Sucrose solution.
Alanine I (5µg/ml)	Mulberry leaves smeared with prepared (5µg/ml) Alanine solution.
Alanine II (10µg/ml)	Mulberry leaves smeared with prepared (10µg/ml) Alanine solution.
Alanine III (15µg/ml)	Mulberry leaves smeared with prepared (15µg/ml) Alanine solution.

Table-2: Effect of nanoparticles of Alanine on the feed efficacy of the Mulberry silkworm *Bombyx mori L.*

GROUPS→ PARAMETERS↓	CONTROL	SUCROSE	Nanoparticles of Alanine I (5µg/ml)	Nanoparticles of Alanine II (10µg/ml)	Nanoparticles of Alanine III (15µg/ml)	'F' value
Consumption Rate (CR) mg/day	181.708 ±3.300	152.264 ±2.10 [-8.917%] t= 18.440*	176.112 ±0.92 [-3.080 %] t= 4.002*	171.579 ±0.78 [-5.574 %] t= 7.315*	174.344 ±0.357 [-4.052%] t= 5.433*	58.703*
Assimilation Rate(AR) mg/day	139.026 ±0.489	114.998 ±0.719 [-17.283%] t= 67.639*	137.662 ±0.968 [-0.981%] t= 2.808*	134.048 ±0.582 [-3.580%] t= 14.542*	136.159 ±0.766 [-2.062%] t= 7.049*	31.788*
Production Rate (PR) mg/day	80.381 ±0.853	75.151 ±0.610 [-6.506%] t= 12.215*	83.208 ±0.725 [3.517%] t= -6.159*	79.713 ±0.512 [-0.831%] t= 1.684**	74.935 ±0.700 [-6.775%] t= 12.115*	242.87*
Metabolism Rate (MR) mg/day	102.296 ±0.774	46.804 ±0.929 [-54.246%] t=112.221*	60.018 ±0.583 [-41.329%] t= 106.624*	54.331 ±0.636 [-46.888%] t= 117.131*	61.224 ±1.134 [-40.150%] t= 73.160*	120.61*
Approximate Digestibility (AD) %	76.510 ±9.952	75.525 ±15.65 [-1.287%] t= 0.415**	78.167 ±5.716 [2.165%] t= -0.664**	78.126 ±3.090 [2.112%] t= -1.086**	78.098 ±4.373 [2.075%] t= -1.054**	0.231**
Efficiency of conversion of Digested food (ECD) %	57.817 ±47.741	65.349 ±35.296 [13.027%] t= 0.608**	60.443 ±10.551 [4.541%] t= 0.0328**	59.466 ±22.77 [2.852 %] t= 0.4112**	55.034 ±26.408 [-4.813%] t= 0.547**	0.473**
Efficiency of conversion of Ingested food (ECI) %	44.236 ±11.042	49.355 ±8.399 [11.572%] t= 0.424**	47.247 ±5.036 [6.80%] t= -0.208**	46.458 ±17.340 [5.023%] t= 0.161**	42.981 ±18.985 [-2.837%] t= 0.364**	0.438**
Food Consumption Index (FCI)	13.125 ±0.482	19.705 ±0.297 [50.133%] t= 28.353*	18.272 ±0.569 [39.215%] t=-16.828*	16.898 ±0.839 [28.746%] t= -9.488*	14.284 ±0.806 [9.615%] t= -3.399*	41.581*
Coefficient of food utilization (CFU)	76.512 ±0.924	75.524 ±0.424 [-1.291%] t= 2.372*	78.664 ±0.420 [2.812%] t= -5.191*	78.124 ±0.442 [2.106%] t= -3.873*	78.100 ±0.684 [2.075%] t= -3.399*	2.116**
Tissue Somatic Index	60.769 ±0.488	84.72 ±0.545 [39.413%] t= 79.944*	53.327 ±0.188 [-12.205%] t=34.254*	81.504 ±0.114 [34.121%] t=-101.598*	108.88 ±0.386 [79.170%] t=-169.091*	46.841*

Note: Values with ± sign is Standard deviation
Values inside the square brackets indicate the % of change over the control
*Significant at the level of $p < 0.05$, **Not significant at the level of $p < 0.05$.

Table-3: Effect of nanoparticles of Alanine on the Larval parameters of silkworm, *Bombyx mori L.*

GROUPS→ PARAMETERS↓	CONTROL	SUCROSE	Nanoparticles of alanine I (5µg/ml)	Nanoparticles of alanine II (10µg/ml)	Nanoparticles of alanine III (15µg/ml)	Alanine (1000µg/ml) ***
Larval Weight (g)	1.38 ±0.092	1.457 ±0.097 [5.579%] t=-1.229**	1.66 ±0.169 [66%] t= -3.212*	1.62 ±0.226 [17.391%] t= -2.108*	1.78 ±0.108 [28.985%] t=-6.199*	1.291
Silk Gland Weight (g)	0.79 ±0.092	1.19 ±0.116 [50.632%] t= -6.197*	1.25 ±0.089 [58.227%] t=-8.099*	1.27 ±0.074 [60.759%] t= -9.135*	1.47 ±0.159 [86.075%] t=-8.360*	0.866

Note: Values with ± sign is Standard deviation

Values inside the square brackets indicate the % of change over the control
*Significant at the level of $p < 0.05$, **Not significant at the level of $p < 0.05$.
*** (Rouhollah Radjabi 2010.)

Table 4: Effect of nanoparticles of Alanine on the Cocoon parameters of silkworm, *Bombyx mori L*

GROUPS→ PARAMETERS↓	CONTROL	SUCROSE	Nanoparticles of alanine I (5µg/ml)	Nanoparticles of alanine II (10µg/ml)	Nanoparticles of alanine III (15µg/ml)	Alanine (1000µg/ml) ***
Cocoon Weight(g)	0.71	0.82 [15.492%] t=-1.946*	1.17 [64.788%] t=-7.529*	0.88 [23.94%] t=-4.018*	1.21 [70.42%] t=-10.833*	1.174
Shell Weight(g)	0.15	0.16 [6.66%] t=-0.372**	0.06 [-60%] t=2.542*	0.05 [-66.66%] t=3.021*	0.25 [66.66%] t=-2.847*	0.275
Pupa Weight (g)	0.56	0.66 [17.857%] t=-1.614**	1.11 [98.214%] t=-7.654*	0.83 [48.214%] t=-4.122*	0.95 [69.642%] t=-5.587*	0.89
Shell Ratio%	21.126	19.512 [-7.639%] t=8.536*	5.128 [-75.726%] t=5.527*	5.681 [-73.108%] t=5.674*	20.661 [-2.201%] t=3.702*	23.42

Note: Values inside the square brackets indicate the % of change over the control
*Significant at the level of $p < 0.05$, **Not significant at the level of $p < 0.05$.
*** (Rouhollah Radjabi 2010.)

Table 5: Effect of Nanoparticles of Alanine on the Filament parameters of silkworm, *Bombyx mori L*

GROUPS→ PARAMETERS↓	CONTROL	SUCROSE	Nanoparticles of alanine I (5µg/ml)	Nanoparticles of alanine II (10µg/ml)	Nanoparticles of alanine III (15µg/ml)
Silk Filament Weight (mg)	0.18	0.25 [38.8%]	0.44 [144.44%]	0.48 [166.66%]	0.49 [172.22%]
Filament Length (m)	415	538 [29.638%]	763 [83.855%]	787 [89.638%]	790 [90.3661%]
Denier	3.903	4.182 [7.14%]	5.190 [32.974%]	5.489 [40.635%]	5.582 [43.018%]

Note: Values inside the square brackets indicate the % of change over the control.

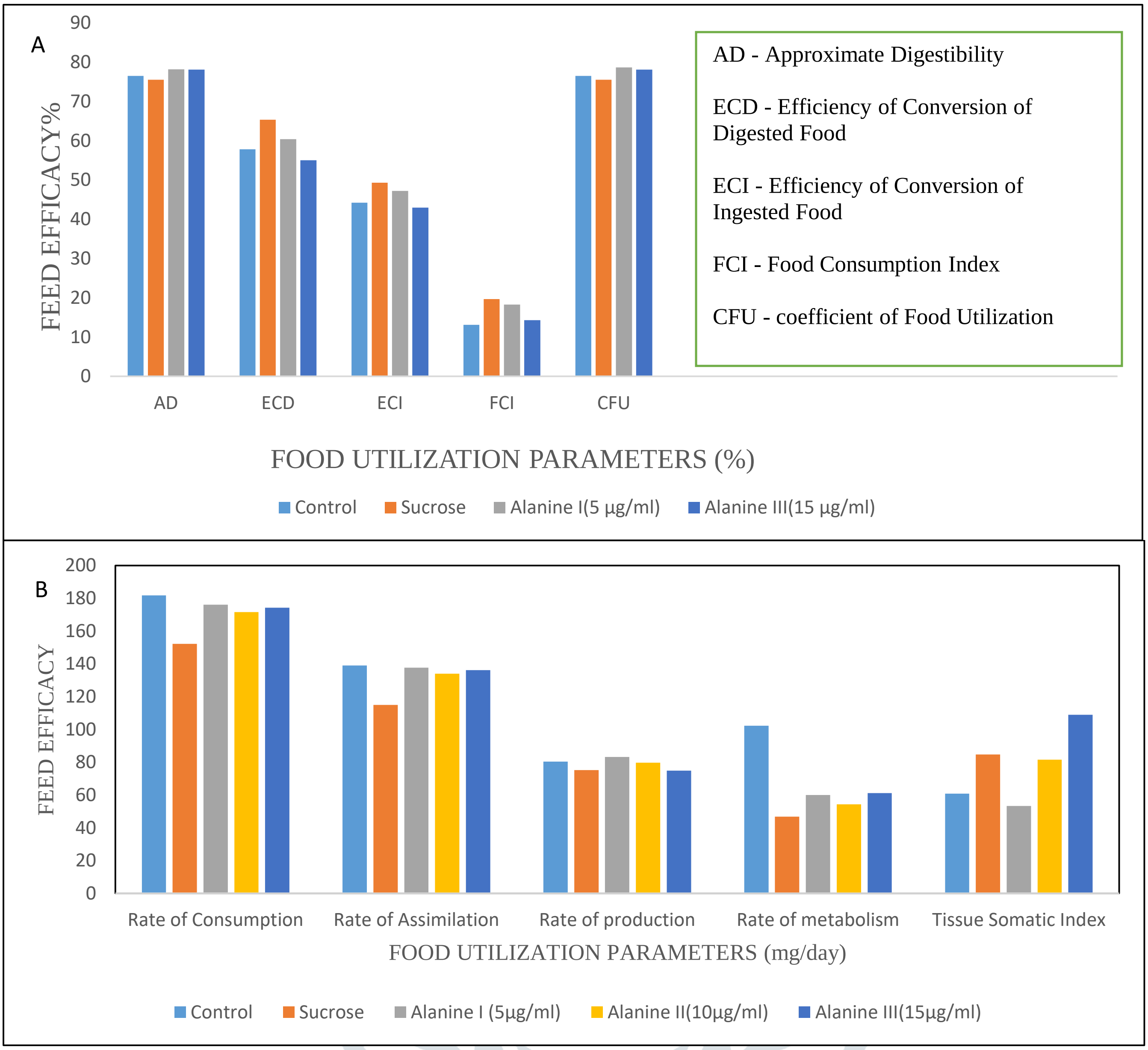

Fig 1: A & B Effect of the Nanoparticles of Alanine on food utilization parameters of the mulberry silkworm *Bombyx mori L*

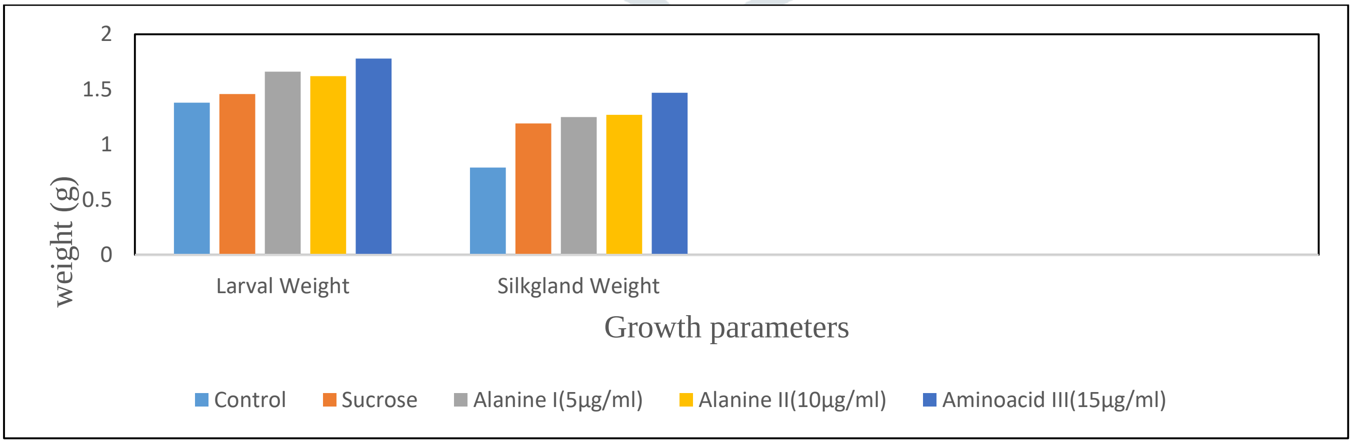

Figure .2- Effect of the nanoparticles of alanine on the Larval growth and silkgland weight of the Mulberry silkworm *Bombyx mori L.*

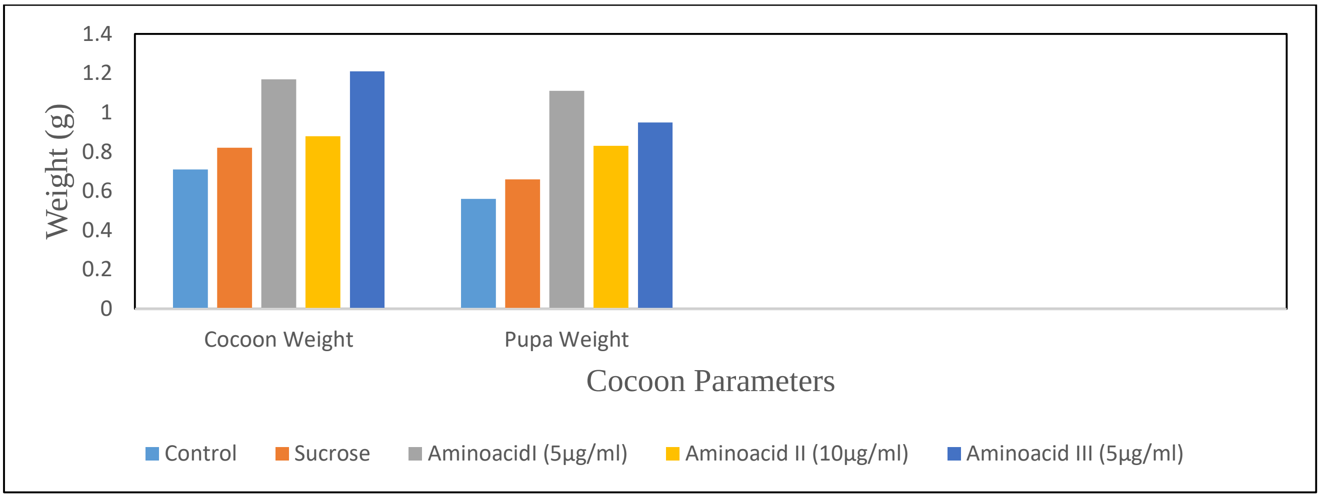

Fig : 3 - Effect of nanoparticles of alanine on the Cocoon weight and pupa weight of the mulberry silkworm *Bombyx mori L.*

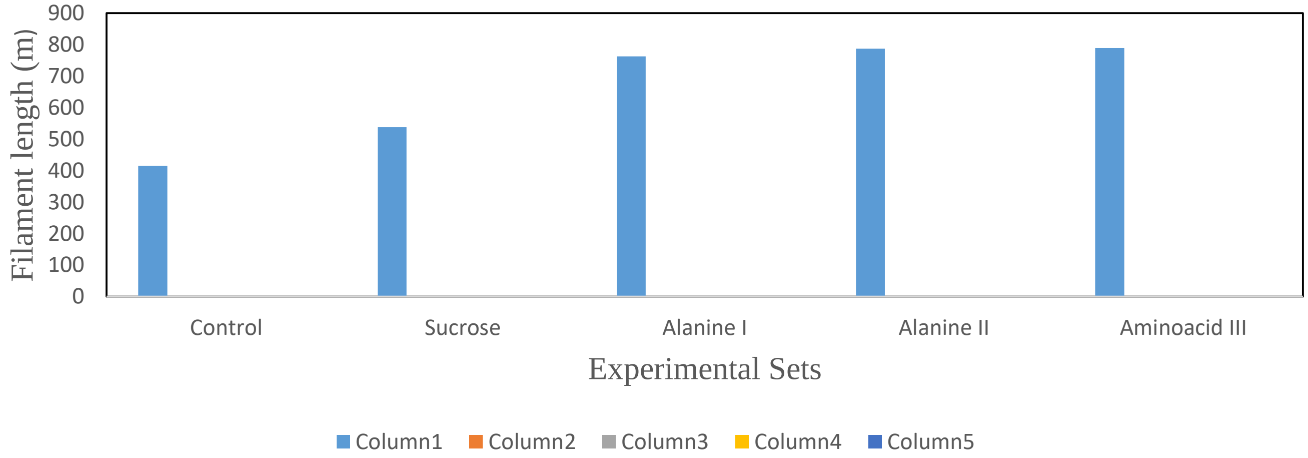

Fig :4- Effects of nanoparticles of alanine on the Filament length of the Mulberry silkworm *Bombyx mori L.*

ACKNOWLEDGEMENT

The authors express their sincere thanks to the Principal, Head and staffs of PG and Research Department of Zoology of Government Arts College (Autonomous), Karur, Tamilnadu for providing the laboratory facilities to cary out this research work.

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