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Effect of feed fortification on nutritional indices traits of silkworm, *Bombyx mori* L.

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Abstract
Dietary efficiency of silkworm is a crucial factor in converting the feed ingested to produce silk of commercial importance. Fortification of mulberry leaves is a constructive technique to increases the nutritional value of the food making it more useful which improves silkworm health, cocoon and silk quality. Silkworms fed with mulberry leaves fortified with *Aloe vera* (100%) recorded the highest nutritional indices parameters *viz.*, ingesta (3.708g), digesta (1.369g), approximate digestibility (36.920%), relative growth rate (0.049), reference ratio (1.585), respiration (0.493), metabolic rate (0.044), efficiency conversion of ingesta to larva (23.350%), cocoon (19.972%), shell (9.673%) and efficiency conversion of digesta to larva (69.109%), cocoon (59.085%), shell (28.518%) with minimum excreta, consumption index, ingesta and digesta required to produce one gram cocoon and shell followed by *Tinospora cordifolia* (2%) compared to untreated control. The plant extracts used in the study beneficially affected the metabolic activities of silkworm which inturn reflected on the qualitative and quantitative improvement of nutritional traits of silkworm.

Keywords: Nutritional indices, ingesta, digesta, *Aloe vera* and *Tinospora cordifolia*

Introduction
Nutrition is a pre-eminent factor, which governs growth and development in commercial sericulture. Information on nutritional ecology of insects is a pre-requisite for a better knowledge of their ethnobiology and physiology. Traditional rearing on mulberry leaf (the regular food of the monophagous silkworm) amplified larval efficiency in renovating food, and high silk production was achieved when the traditional mulberry leaf was added with artificial diet (Alessio *et al*., 2014) [1]. Nutritional intake has direct impact on the overall genetic traits such as larval and cocoon weight, amount of silk production, pupation and reproductive traits (Ramesha *et al.*, 2012) [20]. Efficiency conversion of ingested mulberry leaves into silk or leaf silk conversion rate is a better economic index in cocoon production (Rahmathulla *et al.*, 2005) [19]. Magadum *et al*. (1996) [13] investigated that the total digesta increases with an increased ingesta. Feed conversion efficiency contributes directly or indirectly to the major chunk of the cost benefit ratio of silkworm rearing and is considered to be an important physiological criterion for evaluating the superiority of silkworm breeds/hybrids (Juliang and Xiaofeng, 1992; Trivedy and Nair, 1998) [11, 23]. Food digestion and digestibility and larval growth are inter related and the rate of digestion in silkworm increases with the advancement of instar, which is highest about 65 per cent in the fifth instar (Ueda, 1982) [24].
Miyashita (1986) [17] observed that the productivity of silkworm was controlled by leaf quality (38.20%), climate (37.00%), rearing techniques (9.30%), races (4.20%), eggs (3.10%) and other factors (8.20%). Although mulberry is a complete diet for silkworm, sometimes it is possible that some deficiencies occur due to different reasons. Factors such as weather, agricultural practices, pest and diseases have an immense impact on the nutrient composition of mulberry leaves (Ito and Niminura, 1966) [9]. Fortification of mulberry leaves with live microbial culture of *Bacillus licheniformis* beneficially affected the silkworm by enhancing larval biomass and healthiness which in turn reflects on the qualitative and quantitative improvement of cocoon characters (Mala and Vijila, 2018) [15]. The enrichment of mulberry leaves with an aim of increasing the production of cocoon is a very important aspect (Islam *et al*., 2004) [8] and has been addressed with various attempts of fortifying mulberry leaves with nutrients, antibiotics, juvenile hormones, plant products with JH-mimic principles, anti-juvenile hormones or dusting with plant extracts or extrafoliation of mulberry leaf with sugars,

vertebrate hormones, feed additives, protein rich flours, probiotics etc. Among which use of plant extracts is appropriate for the current scenario as they enhances bioavailability of nutrients, digestion and have direct growth promoting properties. Plant extracts increase the feeding efficiency of silkworms as they have phagostimulant characteristics and contain sterols of different kinds which are required for insect's normal growth and development (Hipparagi *et al*., 2001) [6]. In addition, the pathogens do not develop resistance to botanicals in-spite of their repeated usage (Murakoshi *et al.*, 1975) [18]. Hence, the study focuses to evaluate the effect of locally available eco-friendly aqueous plant extracts on nutritional indices traits of silkworm, *Bombyx mori viz.*, nutrition consumption and conversion efficiency parameters, when administered orally through feed.

Materials and Methods

Disease free layings (DFLs)

The disease free layings (DFLs) of double hybrid race (CSR6 x CSR26) x (CSR2 x CSR27) were procured from Silkworm Seed Production Centre, Coimbatore.

Disinfection of rearing room and appliances

Before commencement of silkworm rearing, the rearing room along with the rearing equipments (*viz*., trays, stand and mountage) was thoroughly washed with water and one per cent bleaching powder solution and then disinfected with 2.5 per cent chlorine dioxide at the rate of 1.5 litre per square meter (Dandin *et al*., 2003) [5].

Rearing of silkworm with mulberry leaves fortified with plant extracts

The plant extracts used in the study were *Aloe vera* and *Tinospora cordifolia*. The larvae were reared from first to fifth instar under hygienic conditions in range of 25-28 ºC and relative humidity of 75-85% in rearing room (Krishnaswami, 1978) [12]. The mulberry leaves of V1 variety were fed 3-4 times a day and were divided into three groups; one group reared with mulberry leaves fortified with *Tinospora cordifolia* at 2 per cent concentration (600 ml Kg^{-1} of mulberry leaves), the second group on mulberry leaves fortified with *Aloe vera* 100% pure gel extract (725 ml Kg^{-1} of mulberry leaves) and the third group reared with only mulberry leaves and served as control. The treatment was given for the first feed on the first and third day of 4th and 5th instars respectively. The remaining feed was given only with mulberry leaves. During rearing, optimum spacing was provided according to the age of silkworm. Fifty worms of 4th instar were separated and maintained in trays for different treatments at seven replications per plant extract.

Analysis of nutritional indices traits of silkworm (*Bombyx mori* L.) with plant extracts fortified mulberry leaves

On the 1st day of fifth instar, 50 healthy silkworm larvae per treatment in seven replications were selected for nutritional traits analysis. Accurately weighed fresh mulberry leaves were fed 3 times a day to the experimental batches and the control. Simultaneously, an additional batch of larvae for each treatment was maintained to determine the dry weight on subsequent daily increase in larval weight recorded separately as suggested by Maynard and Loosli (1962) [16]. The healthy larvae were counted daily in each replicate and missing larvae were replaced from the reserve batch. Left over leaves and excreta were collected on each subsequent day, separated manually and dried in a hot air oven daily at about 100 °C until they reached constant weight using an air-tight electronic balance. When the larvae finished feeding they were shifted to the mountage for spinning at normal ambient temperature of 25±2 °C and RH 65±5%. Cocoons were harvested 5 days later after completion of cocoon spinning. Harvested cocoons were accessed for quantitative traits using the equations detailed below. The dry weight of left over leaves, excreta, larvae, cocoon, and shell in each of the treatment was recorded.

The equations with brief description of the nutritional indices evaluated during the study are given below.

Nutrition consumption traits

a. Ingesta (g)

Total intake of the dry weight (g) of mulberry leaves by silkworm larvae during the 5th stage up to spinning or ripening stage.

I (g) = Dry weight of leaf fed-Dry weight of left over leaf.

b. Digesta (g)

Total assimilated dry food from the intake or ingesta of dry weight of mulberry leaves by silkworm larva during the 5th stage until spinning or ripening.

D (g) = Dry weight of leaf ingested-dry weight of litter.

c. Excreta (g)

The non-utilized mulberry leaves in the form of litter from the ingested mulberry leaves of a silkworm.

E (g) = Ingesta-Digesta.

d. Approximate digestibility (AD) (%)

It directly indicates the assimilation efficiency of mulberry leaves and depends on the passage rate of food through gut in silkworm.

$$AD\ (\%) = \frac{\text{Dry weight of digesta}}{\text{Dry weight of food ingested}} \times 100$$

e. Relative growth rate (RGR)

Larval gain biomass and the efficiency of conversion of nutrition into larval biomass.

$$RGR = \frac{\text{Weight gain of the larva during feeding period}}{\text{5th stage mean fresh larval weight (g) x 5th stage larval duration in days}}$$

f. Reference ratio (RR)

An indirect expression of absorption and assimilation of food. Expresses the ingesta required per unit excreta produced.

$$RR = \frac{\text{Dry weight of food ingested}}{\text{Dry weight of excreta}}$$

g. Consumption index (CI)

The rate of food intake to the mean weight of the larvae during the feeding period.

$$CI = \frac{\text{Ingesta}}{\text{5th stage mean fresh larval weight (g) x 5th stage larval duration in days}}$$

h. Respiration
A catabolic reaction in which total oxidation of the digested or assimilated food for releasing energy required for all the biological activities by break down of macromolecules into simpler molecules.
Respiration = Dry weight of food digested-Maximum dry weight of larvae.

i. Metabolic rate (MR)
Measure of total biochemical reactions involving both catabolic and anabolic reactions of an organism, associated with the degradation of macromolecules into smaller unit and vice versa.

$$MR = \frac{\text{Respiration}}{\text{5th stage mean fresh larval weight (g) x 5}^{th}\text{ stage larval duration in days}}$$

Nutritional efficiency conversion traits
a. Efficiency conversion of ingesta to larva (%)
Associated with the efficiency conversion of ingested nutrition into biomass or body matter at different stages and expressed in percentage.

$$\text{ECI larvae (\%)} = \frac{\text{Maximum dry weight of larva}}{\text{Dry weight of ingesta}} \times 100$$

b. Efficiency conversion of digesta to larva (%)
The expression of efficiency conversion of digesta into larval biomass:

$$\text{ECD larvae (\%)} = \frac{\text{Maximum dry weight of larva}}{\text{Dry weight of digesta}} \times 100$$

c. Efficiency conversion of ingesta to cocoon (%)
This is the most economically important trait used by the sericulture industry.
It is the expression of efficiency conversion of ingesta into cocoon, also referred to as the leaf-cocoon conversion rate. This nutritional trait was kept as the ultimate index for assessing the superiority of treatment for nutritional efficiency in this investigation.

$$\text{ECI cocoon (\%)} = \frac{\text{Dry weight of cocoon}}{\text{Dry weight of ingesta}} \times 100$$

d. Efficiency conversion of digesta to cocoon (%)
It is the expression for efficiency conversion of digesta into cocoon.

$$\text{ECD cocoon (\%)} = \frac{\text{Dry weight of cocoon}}{\text{Dry weight of digesta}} \times 100$$

e. Efficiency conversion of ingesta to shell (%)
It is the expression of efficiency conversion of ingesta into shell. It is also referred to as the leaf-shell conversion rate.

$$\text{ECI shell (\%)} = \frac{\text{Dry weight of shell}}{\text{Dry weight of ingesta}} \times 100$$

f. Efficiency conversion of digesta to shell (%)
It is the expression of efficiency conversion of digesta into shell.

$$\text{ECD shell (\%)} = \frac{\text{Dry weight of shell}}{\text{Dry weight of digesta}} \times 100$$

g. Ingesta per gram cocoon (g)
It is another important trait of economical significance to assess silkworm performance in nutritional analysis. It was the expression of total ingesta required for the production of one gram of cocoon.

$$\text{I/g cocoon} = \frac{\text{Dry weight of ingesta}}{\text{Dry weight of cocoon}}$$

h. Digesta per gram cocoon (g)
It is the total digesta requisite for the production of one gram of cocoon.

$$\text{D/g cocoon} = \frac{\text{Dry weight of digesta}}{\text{Dry weight of cocoon}}$$

i. Ingesta per gram shell (g)
It is the total ingesta requisite for the production of one gram of shell:

$$\text{I/g shell} = \frac{\text{Dry weight of ingesta}}{\text{Dry weight of shell}}$$

j. Digesta per gram shell (g)
It is the total digesta requisite for the production of one gram of shell:

$$\text{D/g shell} = \frac{\text{Dry weight of digesta}}{\text{Dry weight of shell}}$$

Results
Nutrition consumption traits of silkworm (*Bombyx mori* L.) fed with plant extracts fortified mulberry leaves
Mulberry leaves fortified with *Aloe vera* (100%) and *Tinospora cordifolia* (2%) extracts when fed to silkworms showed profound influence on the nutritional consumption traits. In this study, the highest nutritional consumption parameters *viz.*, ingesta (3.708g), digesta (1.369g), approximate digestibility (36.920%), relative growth rate (0.049), reference ratio (1.585), respiration (0.493) and metabolic rate (0.044) were recorded in *Aloe vera* (100%) supplemented batches of silkworm followed by *Tinospora cordifolia* (2%) compared to control. The both plant extracts treated batches recorded significantly higher nutritional consumption parameters *viz.*, over the control with minimum consumption index. The effect of fortified mulberry leaves fed to silkworms on nutritional consumption characteristics are presented in Tables 1 and 2.

Nutritional conversion efficiency characteristics of silkworm fed with plant extracts fortified mulberry leaves
The nutritional conversion efficiency of silkworm hybrid differed significantly due to application of *Aloe vera* (100%)

and *Tinospora cordifolia* (2%) extracts of different concentrations, which inturn when fed to silkworm through mulberry leaves. The efficiency conversion of ingesta and digesta into larva, cocoon and shell are represented in Table 3.

Efficiency conversion of ingesta and efficiency conversion of digesta to larval biomass

The efficiency of mulberry leaves ingested converted into silkworm larval biomass varied significantly among the treatments. The highest efficiency conversion of ingesta (ECI) to larva was recorded in *Aloe vera* (23.350%) followed by *Tinospora cordifolia* (21.709%) administered batches of silkworm and the least in control (18.944%). With regard to efficiency conversion of digesta (ECD) to larva, significant increase was observed among the treatments and more efficient conversion of digested food into larval biomass in *Aloe vera* (69.109%) followed by *Tinospora cordifolia* (64.793%) administered batches of silkworm and less efficient in control (61.430%) were noticed.

Efficiency conversion of ingesta and efficiency conversion of digesta to cocoon

Efficiency conversion of ingesta (ECI) to cocoon revealed higher in *Aloe vera* (19.972%) treated batches of silkworm followed by *Tinospora cordifolia* (18.124%) and lower in the control (16.769%). Efficiency conversion of digesta (ECD) to cocoon varied significantly among the treatments which ranged between 59.085% for *Aloe vera*, 54.032% for *Tinospora cordifolia* supplemented larvae and 49.261% for control.

Efficiency conversion of ingesta and efficiency conversion of digesta to shell

The efficiency of mulberry leaves ingested converted into silkworm shell biomass varied significantly among the treatments. Efficiency conversion of ingesta (ECI) to shell was observed to be maximum in *Aloe vera* (9.673%) followed by *Tinospora cordifolia* (9.268%) and minimum in control (8.864%). With regard to efficiency conversion of digesta (ECD) for shell, high conversion is observed in *Aloe vera* treated lots (28.518%) followed by *Tinospora cordifolia* (27.054%) and low in control with 25.960 per cent was observed.

Ingesta/g and Digesta/g to cocoon and shell

Significant differences were revealed with respect to ingesta and digesta required to produce one gram of cocoon and shell and data represented in Table 4. The highest ingesta and digesta required to produce one gram cocoon was observed in control (6.248 and 2.283 g) and minimum required by treated lots of *Aloe vera* (5.930 and 1.810 g) followed by *Tinospora cordifolia* (6.043 and 2.014 g). Similarly, the highest ingesta and digesta per gram shell was recorded in control (11.514 and 3.997 g) and minimum required by treated lots of *Aloe vera* (10.541 and 3.631 g) followed by *Tinospora cordifolia* (11.048 and 3.825 g).

Discussion

Food ingesta is a very important physiological and economic trait as far as sericulture is concerned. The food intake is regulated by the physical nature and presence of phagostimulants in food (Scriber and Slansky, 1981) [21]. The diet has significant impact on the gut bacterial community and enzyme activity. It was observed that, fortification with *Aloe vera and Tinospora cordifolia* increased the total bacterial count as well as percent dominance of cellulase, amylase and lipase with respect to the growth of *B. mori* (Mala and Vijila, 2017) [14]. *Aloe vera* (L) contains over 75 nutrients and 200 active compounds, including vitamins, enzymes, minerals, sugars, lignin, anthraquinones, saponins, salicylic acid and amino acids. These bio compounds might activate the velocities of bio chemical reactions catalyzed by the mid gut enzymes and thereby increase the digestibility in the fifth instar larvae of silk worm, *Bombyx mori* (L) (Vitthalrao and Anil, 2012) [25]. Higher food intake in the treated category reflects the high silk production ability, as feeding influences the synthesis of total DNA, RNA and protein synthesis (Chavancy and Fournier, 1979) [4].

The higher value of approximate digestibility indicates the greater suitability of food plants. High value of reference ratio indicates high rate of digestion and absorption of food. Growth rate directly influences the speed of development of larvae which in turn depends on the quality of leaf and physiological stages of the larvae. The difference in the relative growth rate of *Aloe vera* supplemented larvae from the control observed in the present study indicated that the *Aloe vera* (L) supplementation resulted in higher protein utilization. On similar series Jadhav *et al.* (2016) [26] reported that the *Asteracantha longifolia* leaf extract at 1:50 concentration daily feeding recorded significantly higher food consumption (0.591gm dry wt. larva^{-1}day^{-1}), food absorption (0.256 gm dry wt. larva^{-1}day^{-1}), relative growth rate (0.033 gm dry wt. larva^{-1}day^{-1}) and approximate digestibility (39.11%) over control. Silkworms fed on mulberry leaves supplemented with pectin (0.5%) proved to be considerably effective showing higher food consumption (4.595 g), digestibility (1.565 g), reference ratio (1.573) and growth index (4.546) compared to control (Indira *et al.*, 2013) [7].

Higher nutritional efficiency values are necessary as the larva has to maintain metabolic demand during metamorphosis (transformation stages from larvae to pupa, pupa to moth). Efficiency conversion of ingested mulberry leaves into silk or leaf silk conversion rate is a better economic index in cocoon production which contributes directly or indirectly on the cost benefit ratio of silkworm rearing (Rahmathulla *et al.*, 2005) [19]. The results of present study showed that the efficiency conversion of ingesta (ECI) and efficiency conversion of digesta (ECD) of the plant extracts treatments were superior over control. The higher ECD values might be due to higher absorption ability, higher digestibility and higher larval weight influenced by the treatment.

Maximum ECI to larva (23.350%), cocoon (19.972%), shell (9.673%) and ECD to larva (69.109%), cocoon (59.085%), shell (28.518%) were recorded in *Aloe vera* treated batches of silkworm with minimum consumption index (0.253), ingesta and digesta required to produce one gram of cocoon (5.930 and 1.810 g) and shell (10.541 and 3.631 g) compared to control. Silkworm batch which had less consumption index with higher efficiency conversion were considered to be nutritionally efficient one. The results are in accordance with Jadhav *et al.* (2016) [26] who reported that the *Asteracantha longifolia* leaf extract at 1:50 concentration daily feeding recorded significantly higher efficiency of conversion of ingested food (24.15%) and efficiency of conversion of digested food (55.25%). Ananda Kumar and Ann (2011) [3] noticed that the higher ECI (24.42%) and ECD (45.18%) were recorded in serifeed dusted mulberry leaves than control (23.06 and 40.64%). Sheebha *et al.* (2008) [22] revealed that the food consumption, tissue growth and efficiency conversion of ingested food increased by 19.36, 19.27 and

23.8 percent respectively in the larvae treated with antibiotics. Maximum food consumption (97.30%), assimilation efficiency (96.72%), AD (94.75%), ECD (36.30%) and ECI (34.40%) were observed over control when 10 per cent amway protein was administrated to the larvae (Amala Rani *et al*., 2011a) [2].

Table 1: Effect on nutrition consumption characteristics of silkworm fed with plant extracts fortified mulberry leaves

Treatments	Ingesta/larva (g)	Digesta/larva (g)	Excreta/larva (g)	Approximate Digestibility (%)	Relative Growth Rate
Mulberry + *Tinospora cordifolia* (2%)	3.591^{b} (±0.005)	1.245 ^{b} (±0.004)	2.346 ^{a} (±0.008)	34.670 ^{b} (±0.067)	0.045 ^{a} (±0.004)
Mulberry + *Aloe vera* (100%)	3.708^{a} (±0.004)	1.369 ^{a} (±0.007)	2.339 ^{b} (±0.005)	36.920 ^{a} (±0.054)	0.049 ^{a} (±0.004)
Mulberry alone (control)	3.487 ^{c} (±0.009)	1.206 ^{c} (±0.006)	2.281 ^{c} (±0.004)	34.585 ^{c} (±0.063)	0.038 ^{b} (±0.003)
SEd	0.0041	0.0035	0.0036	0.0332	0.0024
CD (0.05%)	0.0086	0.0074	0.0076	0.0698	0.0051

Values represent data mean ± standard deviation
Values not sharing a common superscript letter within each column differ significantly at $P< 0.05$ (DMRT)

Table 2: Effect on reference ratio, consumption index, respiration and metabolic rate of silkworm fed with plant extracts fortified mulberry leaves

Treatments	Reference Ratio	Consumption Index	Respiration (g)	Metabolic Rate
Mulberry + *Tinospora cordifolia* (2%)	1.530^{b} (±0.020)	0.258^{b} (±0.002)	0.454^{b} (±0.004)	0.039^{b} (±0.003)
Mulberry + *Aloe vera* (100%)	1.585^{a} (±0.012)	0.253^{c} (±0.003)	0.493^{a} (±0.005)	0.044^{a} (±0.004)
Mulberry alone (control)	1.528^{c} (±0.010)	0.270^{a} (±0.009)	0.363^{c} (±0.004)	0.035^{b} (±0.004)
SEd	0.0081	0.0032	0.0026	0.0022
CD (0.05%)	0.0170	0.0067	0.0055	0.0047

Values represent data mean ± standard deviation.
Values not sharing a common superscript letter within each column differ significantly at $P< 0.05$ (DMRT).

Table 3: Effect on nutritional conversion efficiency characteristics of silkworm fed with mulberry leaves fortified with plant extracts

Treatments	Larva (%)		Cocoon (%)		Shell (%)	
	ECI	ECD	ECI	ECD	ECI	ECD
Mulberry + *Tinospora cordifolia* (2%)	21.709 ^{b} (±0.049)	64.793 ^{b} (±0.043)	18.124 ^{b} (±0.077)	54.032 ^{b} (±0.065)	9.268 ^{b} (±0.051)	27.054 ^{b} (±0.043)
Mulberry + *Aloe vera* (100%)	23.350 ^{a} (±0.046)	69.109 ^{a} (±0.052)	19.972 ^{a} (±0.128)	59.085 ^{a} (±0.041)	9.673 ^{a} (±0.051)	28.518 ^{a} (±0.051)
Mulberry alone (control)	18.944 ^{c} (±0.039)	61.430 ^{c} (±0.094)	16.769 ^{c} (±0.083)	49.261^{c} (±0.043)	8.864 ^{c} (±0.003)	25.960 ^{c} (±0.034)
SEd	0.0265	0.0366	0.0530	0.0274	0.0277	0.0234
CD (0.05%)	0.0558	0.0769	0.1115	0.0576	0.0581	0.0492

Values represent data mean ± standard deviation.
Values not sharing a common superscript letter within each column differ significantly at $P< 0.05$ (DMRT).

Table 4: Analysis of total ingesta and digesta required for production of one gram of cocoon and shell of silkworm through plant extracts supplementation

Treatments	Cocoon (g)		Shell (g)	
	Ingesta/g	Digesta/g	Ingesta/g	Digesta/g
Mulberry + *Tinospora cordifolia* (2%)	6.043^{b} (±0.035)	2.014^{b} (±0.074)	11.048^{b} (±0.048)	3.825^{b} (±0.058)
Mulberry +*Aloe vera* (100%)	5.930^{c} (±0.046)	1.810^{c} (±0.054)	10.541^{c} (±0.320)	3.631^{c} (±0.049)
Mulberry alone (control)	6.248^{a} (±0.037)	2.283^{a} (±0.072)	11.514^{a} (±0.062)	3.997^{a} (±0.076)
SEd	0.0195	0.0363	0.1018	0.0334
CD (0.05%)	0.0409	0.0763	0.2139	0.0702

Values represent data mean ± standard deviation.
Values not sharing a common superscript letter within each column differ significantly at $P< 0.05$ (DMRT).

Conclusion

Perusal of study revealed that supplementation of aforesaid promising aqueous extracts of *Aloe vera and Tinospora cordifolia* through mulberry leaves to 4th and 5th instar larvae of silkworm have proportionately increased apparent digestibility which in turn resulted into enhanced digestion, absorption, assimilation and utilization of food energy in to larval bio-mass and thereby the cocoon characteristics.

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