INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

Larval growth, silk production and economic traits of *Bombyx mori* under the influence of Nutrilite-enriched mulberry diet

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ABSTRACT

The impact of feeding *Bombyx mori* with Amway nutrilite-enriched mulberry leaves has been examined with reference to larval growth, silk production and economic parameters of sericulture. At a minimum effective dose of 1% in distilled water, nutrilite enhances growth rates in the larval body and silk gland during fifth instar development. The analysis of growth trends in terms of compound periodical growth rates, indicate that nutrilite reinforces the day-to-day larval growth by 3.8 additional percentile points, silk gland growth by 21.74 additional percentile points and the gland-body ratio by 7.89 additional percentile points during fifth instar development. Nutrilite also enhances the rate of silk protein synthesis by 48.46 additional percentile points in the anterior silk gland (ASG), 5.81 additional percentile points in the middle silk gland (MSG) and by 10.45 additional percentile points in the posterior silk gland (MSG). The enhancement in silk gland proteins results in ~41% elevation in core shell protein synthesis and 17% reduction in floss protein synthesis. In doing so, it contributes to sericulture industry by causing improvements in gland-body ratio, cocoon weight, shell weight, raw silk weight, denier and renditta. Nutrilite stimulates growth in both profit-making parameters like shell weight, shell protein, raw silk weight and denier and retards the growth rates in loss making parameters like the floss protein and floss weight. Nevertheless, keeping in view its overall positive impact on all aspects of sericulture, the Amway nutrilite is recommended as a profitable supplementary diet for silkworm.

Keywords: *Bombyx mori*, Gland-body ratio, Larval growth, Nutrilite, Silk proteins, Economic traits of sericulture.

INTRODUCTION

Amway nutrilite is a protein-rich and fat-free commercial nutrient, widely used as a supplemental diet. It is available in amorphous form with added benefits of soya-isoflavones, calcium and iron. The protein constitutes the chief ingredient (80%) of nutrilite, the majority of which includes a soya protein (63%). Every 10g of nutrilite consists of 8g of protein, 0.5g of carbohydrates, 0.3g of fats, 70 mg of calcium and 1mg of iron, and nine essential amino acids, viz., isoluecine (408 mg), leucine (696 mg), lysine (544 mg), methionine and cystiene (224 mg), phenylalanine and tyrosine(722mg), threonine (320mg), tryptophan (104mg), valine (432 mg) and histidine (216 mg)[1].

Nutrition is the single most factor that contributes to the growth and development in ***B. mori***[2, 3]. A variety of nutrients, minerals, antibiotics, vitamins, hormones and other exogenous modulators were successfully applied in sericulture with a view to stimulate growth, metabolism and silk production in ***B. mori***[4-13]. However, the application of commercial nutrilite as a supplemental diet in sericulture has not been explored so far. The present study was therefore undertaken with a view to test the nutritive role of this Amway product on the larval growth of *B. mori* and to adjudicate its potential for the stimulation of silk production and improvements in the economic traits of sericulture.

MATERIAL AND METHODS

The present investigation was carried out on Pure Mysore x CSR_2 hybrid strain of *Bombyx mori* reared under 28°C and 85% RH as per Krishnaswami[14]. After hatching, the worms were fed with M_5 variety of mulberry leaves at 6 AM, 10 AM, 2 PM, 6 PM and 10 PM, under normal 12 hr light and 12 hr dark conditions. After the third moult, the fourth instar larvae were divided into two control and experimental batches of 100 worms each. While the control batch was given normal five feedings per day, the experimental batch was fed with mulberry leaves dipped in 1% minimum effective dose (MED) of nutrilite at their 6 PM diet as recommended by Thulasi and Sivaprasad[15]. Before feeding, the nutrilite-fortified mulberry leaves were dried under cool weather conditions. The experiment was carried out on the fifth instar larvae on alternative days, i.e., on 1st, 3rd, 5th and 7th days of instar life. Since, the body weight is an index of maximal growth, the impact of nutrilite on larval and silk gland (SG) growth rates were determined by recording the mean body weight of 25 randomly selected silkworms and 5 pairs of silk glands in an electronic balance (ELICO: MODEL BL-22OH) and the same was expressed in grams. The impact of nutrilite on the growth of larval body and silk gland was determined by analyzing changes in the gland-body ratios throughout the fifth instar development, while its impact on silk production was determined by analyzing the total protein profiles in the anterior (ASG), middle (MSG) and posterior (PSG) regions of the silk gland.

The SG was isolated by mid-dorsal dissection of the larval body in the Silkworm Ringer[16] and separated into anterior (ASG), middle (MSG) and posterior (PSG) regions and their total protein levels were estimated in 1% homogenates by the method of Lowry *et al*[17]., and the same was expressed in mg protein /gram wet weight of tissue. Similarly, the protein content of cocoon was estimated in 1% homogenate in distilled water. Before homogenation, the hard silk cocoons were dipped in diluted sodium hydroxide solution and converted to softened form in order to facilitate its homogenization in distilled water. Some important economic parameters of sericulture such as, gland- body ratio, green cocoon weight, shell weight, shell protein, floss weight, floss protein, pupal weight, raw silk weight, denier, renditta were analyzed as per the standard methods in vogue[18-22]. The experimental data were statistically analyzed online by using Graphpad[23] and Percent Change[24] packages and meaningfully interpreted in terms of compound periodical growth rates (CPGR) as given by Sivaprasad[25].

RESULTS AND DISCUSSION

The impact analysis of nutrilite on the larval growth and silk production stems from the fact that the soya protein, which is the chief ingredient of nutrilite, has potential to modulate silk gene expression and silk protein synthesis in *B. mori* [8]. The findings of the present investigation, depicting the positive influence of nutrilite on the larval growth, gland growth, protein profiles and economic traits of sericulture are presented in tables 1 to 3 and figures 1 to 3.

Nutrilite versus larval growth and gland-body ratio

The gland-body ratio (GBR) has been recognized as an important indicator of growth and silk production in *B. mori*. This growth parameter is computed by dividing the silk gland (SG) weight by the larval body weight[19]. Needless to say, higher GBR reflects higher silk productivity and vice versa. In the silkworm higher GBR could be achieved by enriching mulberry leaves with exogenous modulators and nutrients[21-22].The studies on larval body growth vis-a-vis silk gland growth, as indicated by changes in their respective weights during fifth instar development demonstrated the potential influence of nutrilite on silkworm growth and development (Table 1and Fig.1). Under natural conditions, the larval body weight grew continuously throughout the fifth instar and registered an overall growth rate (OGR) of ~324% and a CPGR of 61.92% at the end. At the same time the SG recorded an OGR of ~1729% and a CPGR of 192.40%. Consequent on the growth trends in larval body and SG, the GBR showed an OGR of ~335% and a CPGR of 63.19% in the control batch. Under the influence of nutrilite, at its minimum effective dose (i.e., 1% in distilled water), the larval body weight recorded an OGR of ~351% and a CPGR of 65.72% during the same period and the SG recorded an OGR of ~2157% and a CPGR of 214.14%. Similar growth trends were recorded in the GBR, which showed an OGR of ~401% and a CPGR of 71.08% during fifth instar larval development (Table 1). Further, analysis of experimental data in terms of elevations in growth trends indicate that the CPGR of the larval body grew by 3.8 percentile points (65.72-61.92%), SG by 21.74 additional percentile points (214.14-192.40%) and the GBR by 7.89 percentile points (71.08-63.19%) under the influence of nutrilite-enriched mulberry diet. The current study demonstrates that the nutrilite acts as a growth modulator in *B. mori* and contributes to higher GBR by boosting growth rates in body and gland (SG) during fifth instar development (Table 1 and Fig.1).

Nutrilite versus silk gland proteins

The silk gland is the major site of silk protein synthesis. It synthesizes and stores 93 different proteins that are implicated in larval growth and development including the two silk proteins; fibroin and serecin[26-31]. The enormous growth in the GBR during fifth instar larval development has resulted in similar growth trends in the protein profiles of anterior (ASG), middle (MSG) and posterior (PSG) regions of the silk gland (Table 2). During fifth instar larval growth, the protein profiles of control batch recorded an OGR of ~29% and a CPGR of 8.99% in ASG, an OGR of ~183% and a CPGR of 41.37% in MSG and an OGR of ~114% and a CPGR of 28.95% in PSG. In the experimental batches, the OGR and CPGR grew respectively by ~290% and 57.45% in ASG, ~220% and 47.28% in MSG and by ~171% and 39.40% in PSG during the same period (Table 2 and Fig. 2).

It has been demonstrated that the ASG, which is relatively inert, shows low protein levels, while the middle (MSG) and posterior (PSG) regions acts as protein reservoirs in *B. mori* (Shimura, 1993). The current study revealed that nutrilite activates protein synthesis more prominently in the ASG compared to the other two regions (MSG and PSG). The analysis of data in terms of CPGRs indicate that the nutrilite enhanced to day-to-day protein synthetic rate maximally by 48.46 percentile points (57.45-8.99%) in the ASG compared to 5.81 percentile points (47.28-41.37%) in MSG and 10.45 percentile points (39.40- 28.95%) in PSG (Fig.2). Likewise, the nutrient enhanced the OGRs in the SG proteins by 260.84 additional percentile points (290.3-29.46%) in ASG, 36.97 percentile points (219.50-182.53%) in MSG and by 56.49 additional percentile points (170.89-114.40%) in PSG. Thus, the growth stimulating effect of nutrilite is more specific and targeted towards the ASG, which is considered metabolically inert and synthesizes proteins at a slower rate under natural conditions. Nevertheless, the nutrilite has potential to activate silk protein synthesis in all the three regions (ASG, MSG and PSG) of the silk gland. By and large, similar improvements were obtained in the body growth, silk gland growth, silk protein synthesis and economic traits sericulture, when the silkworm larvae were fed with exogenous nutrients like honey and alfalfa at their minimum effective concentrations[32-35]. Though, the reason for the growth stimulating nature of nutrilite has not been explored so far, the positive impact is attributed to its constituent proteins and minerals, as reported earlier for ascorbic acid, lemon juice, alfalfa and honey[22-23, 31-35].

Nutrilite versus economic traits of sericulture: The higher growth rate of 26.93% (351.41-324.48%) in the larval body weight, observed during the fifth instar development has been extended to the quiescent pupal stage, with ~1.4% improvement in its body weight (Table 3). The same impact has been felt in the economic parameters of sericulture with significant gains in silk output and quality. Clearly, feeding the silkworm larvae with nutrilite- enriched mulberry leaves confers a two-fold advantage to the sericultural industry. Firstly, it yields higher outputs in profit-making economic parameters like the cocoon weight, shell weight, pupal weight, shell protein, raw silk weight, denier and renditta and secondly, it minimizes the sericultural loss by reducing floss output. As observed in the present study, the green cocoon weight increased by ~4%, shell weight ~8%, shell protein by ~41%, pupal weight by ~1%, raw silk weight by ~5% and the denier by ~22% under the influence of nutrilite. The positive impact of nutrilite, so reflected, is further reinforced by reduction in floss weight by ~6% and floss protein content by ~17% and renditta by ~5% (Table 3).

Profitable gains in the economic traits of sericulture, under the impact of nutrilite, are achieved by its growth stimulating effect on *B. mori*, more particularly with reference to the silk protein synthesis. Further, nutrilite positively reinforces silk synthesis in ASG, MSG and PSG, more particularly in the former with concomitant improvements in the levels of shell protein that constitutes fibroin. At the same time, the nutrilite seems to retard the synthesis of floss (-16%) that culminated in low sericin and floss output as observed in the present investigation (Table 3, Fig. 3). Probably, because of this reason, the raw silk weight recorded an elevation of 5% and floss weight (sericultural wastage) declined by the same proportion under the influence of nutrilite. Obviously, this dual effect of nutrilite is responsible for the marginal reduction (~5%) in the renditta and considerable increase in the denier value (~22%), both reflecting positive gains in the silk quality and productivity.

Conclusion

The commercial Amway nutrilite has potential to stimulate growth, protein synthesis and metabolism in ***B. mori,*** at a minimum effective dose (MED) of 1% in distilled water. It selectively accelerates the growth rate in the silkworm larvae, at a time when it is naturally slower. Similarly, it stimulates silk protein synthesis in the ASG, MSG and PSG, more significantly in the former, which is otherwise inert under normal conditions. By virtue of its accelerating role on growth and silk protein synthesis, nutrilite improves the productivity and quality of silk produced by ***B. mori*** (Fig.3). The possibility of its use as an enriched mulberry diet for silkworm could be explored after judging its economic implications for the sericulture industry in the actual field conditions.

ACKNOWLEDGEMENT
N. Thulasi thanks University Grants Commission, New Delhi for providing financial assistance in the form of Rajiv Gandhi National Fellowship for this research work.

Table 1
Effect of 1% nutrilite on body weight, silk gland (SG) weight and gland- body ratio (GBR) in *B. mori*, during fifth instar larval development.

Day	Statistical tool	Control			Experimental (1% Nutrilite)		
		Larval body weight	Silk gland weight	Gland-body ratio	Larval body weight	Silk gland weight	Gland-body ratio
1	Mean ± S.D	0.494 ± 0.001	0.014 ± 0.001	2.83 ± 0.008	0.494 ± 0.001	0.014 ± 0.001	2.83 ± 0.008
3	Mean ± S.D	1.13 ± 0.01*	0.070 ± 0.001*	6.19 ± 0.01*	1.14 ± 0.02*	0.085 ± 0.002*	7.45 ± 0.01*
5	Mean ± S.D	1.64 ± 0.01*	0.129 ± 0.0009*	7.86 ± 0.02*	1.66 ± 0.01*	0.164 ± 0.001*	9.87 ± 0.01*
7	Mean ± S.D	2.08 ± 0.01*	0.256 ± 0.001*	12.30 ± 0.01*	2.23 ± 0.02*	0.316 ± 0.002*	14.17 ± 0.01*
OGR (%)		324.48	1728.57	334.62	351.41	2157.14	400.70
CPGR (%)		61.92	192.40	63.19	65.72	214.14	71.08

* Statistically significant; **Statistically not significant
Each value is a mean, ± standard deviation of four individual observations. (P value < 0.001). The percent changes were calculated taking the control as the base value and the compound periodical growth rates (CPGR) were computed on the basis of first and seventh day values as per Sivaprasad, 2012.

Table 2
Effect of 1% nutrilite on the total protein levels of the anterior (ASG), middle (MSG) and posterior PSG) regions of the silk gland in the fifth instar larva of *B. mori.*

Day	Statistical tool	ASG		MSG		PSG	
		Control	1%Nutrilite	Control	1%Nutrilite	Control	1%Nutrilite
1	Mean ± S.D	9.40 ± 0.06	9.40 ± 0.06	17.23± 0.28	17.23± 0.28	16.94 ± 0.10	16.94 ± 0.10
3	Mean ± S.D	10.88 ± .83*	30.92 ± 1.44*	33.24±0.31*	35.41 ± 0.86*	30.04 ± 0.52*	32.28 ± 0.62*
5	Mean ± S.D	11.72 ± 0.59**	32.90 ± 0.40*	35.55±1.14*	41.84 ± 3.30*	33.20 ± 0.76*	35.55 ± 1.60*
7	Mean ± S.D	12.17 ± 0.43**	36.69± 1.28*	48.68±1.56*	55.05 ± 2.08*	36.32 ± 1.35*	45.89 ± 1.18*
OGR (%)		29.46	290.3	182.53	219.50	114.40	170.89
CPGR (%)		8.99	57.45	41.37	47.28	28.95	39.40

* Statistically significant: **Statistically not significant
Each value is a mean, ± standard deviation of four individual observations. (P value < 0.001). The percent changes were calculated taking the control as the base value and the compound periodical growth rates (CPGR) were computed on the basis of first and seventh day values as per Sivaprasad, 2012.

Table3
Effect of 1% nutrilite on the economic traits of *Bombyx mori.*

S.No	Economic parameters	Statistical parameters	Control	1% Nutrilite
1.	Green cocoon Weight (g)	Mean ± S.D	0.86 ± 0.01	0.89 (3.5) ± 0.02*
2.	Shell weight (g)	Mean ± S.D	0.13 ± 0.001	0.14 (7.7) ± 0.001*
3.	Shell protein (mg/g)	Mean ± S.D	30.30 ± 0.51	31.98 (40.8) ± 0.06*
4.	Floss weight (g)	Mean ± S.D	0.018 ± 0.0005	0.017 (-5.6) ± 0.0009*
5.	Floss protein (mg/g)	Mean ± S.D	6.36 ± 0.12	5.29 (-16.8) ± 0.01*
6.	Pupal weight (g)	Mean ± S.D	0.73 ± 0.003	0.74 (1.4) ± 0.003*
7.	Raw silk weight (g)	Mean ± S.D	11.78 ± 0.009	12.41(5.3) ± 0.008*
8.	Denier	Mean ± S.D	11.0 ± 0.16	13.39 (21.7) ± 0.52*
9.	Renditta	Mean ± S.D	7.46 ± 0.005	7.11(-4.7) ± 0.01*

*Statistically significant: ** Statistically not significant

Each value is a mean ± standard deviation of four individual observations. (P value < 0.001). The weights of the cocoon, shell and floss represent the mean of 25 individual cocoons. The values in parentheses represent the percent changes from the control.

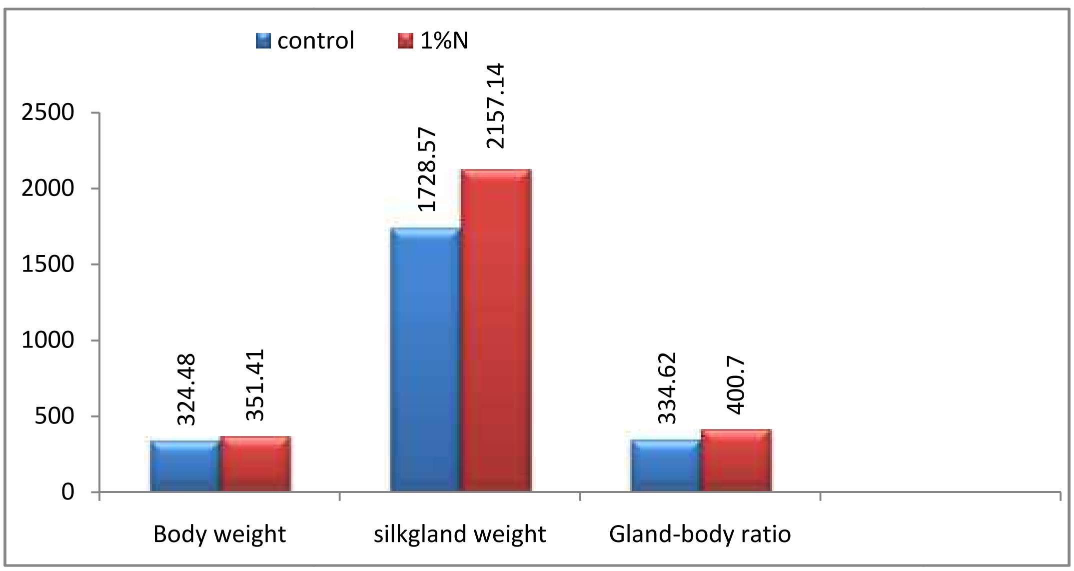

Fig. 1
Effect of nutrilite on the silk gland-body ratio in *B. mori* during fifth instar larval development

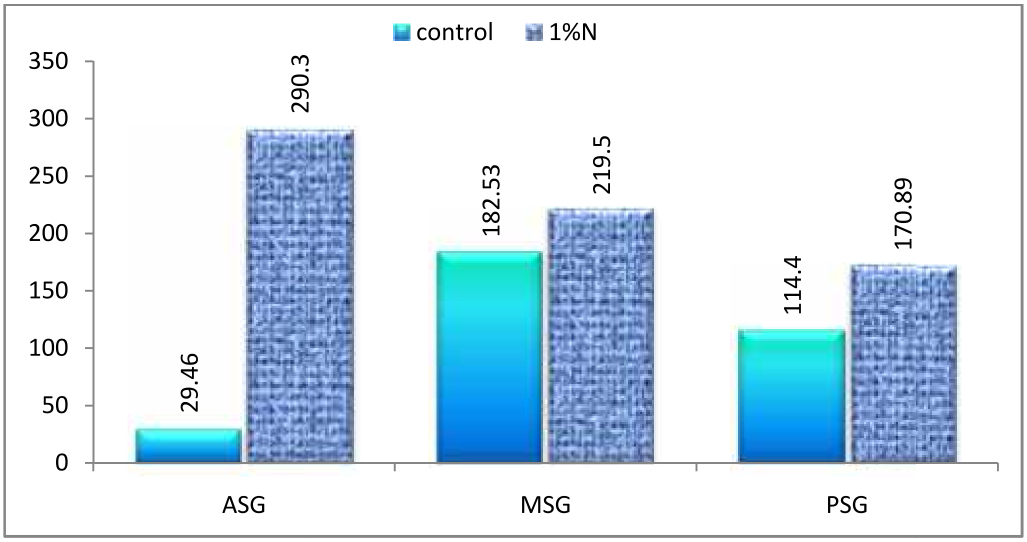

Fig. 2
Effect of different concentrations (5%, 3%, 2%, 1%) nutrilite on the the anterior (ASG), middle (MSG) and posterior (PSG) regions of the silk gland in the fifth instar larva of *B. mori*

Fig.3
Raw silk (A) reeled from 100 green cocoons produced by *B. mori* in the control and nutrilite treated conditions. Note significant increase in the output of raw silk under treatment conditions.

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