EFFECT OF ANTIBIOTIC ADMINISTRATION ON GROWTH AND DEVELOPMENT OF SILK GLAND IN MULBERRY SILKWORM (*BOMBYX MORI* L.)

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ABSTRACT: Nutrition is the important physiological process, which plays a prime role in the growth and in turn it affects the productivity of the silkworm. The supplementation of different materials along with mulberry leaves to silkworm results higher yield because the production of quality silk mainly depends on larval nutrition. The influence of antibiotic on growth and development of silkworm larva and silk gland was assessed. Solution of two concentrations (50 and 100 ppm) of antibiotic (Norfloxacin®) was administered orally along with mulberry leaves to two popular Indian silkworm hybrids (CSR2× CSR4 and BL67×CSR101) during 5th instar larval period. It was found that administration of antibiotic enhanced the growth and development of silkworm and recorded significantly higher silk gland, larval, shell and cocoon weight in treated batches. The growth of the silk gland during different hours of 5th instar was observed and maximum growth was recorded during 144 hrs and it was significantly higher for treated batches. The study summarizes that supplementation of mulberry leaves with antibiotic have a significant improvement in growth and development of silkworm larva, silk gland as well as other economical traits of silkworm.

KEYWORDS**:** Antibiotic administration, Norfloxacin, Growth of silk gland, larval weight, *Bombyx mori* L.

The productivity and quality in sericulture depends on the healthiness, growth of the larvae and the environmental conditions. The quality of silk is affected by cocoon reelability, neatness, non-breakable filament length, cleanness etc. to certain extent. The quantity of available dietary protein is important in feeding of herbaceous insect for its growth, survival and population dynamics. It has been proved that a nutritionally un balanced diet drastically reduce growth rate of herbivorous animals by promising a metabolic load (Naik & Delvi, 1987). Fortification of mulberry leaves is considered as one of the effective method to enrich the silkworm food. In recent years attempts have been made to fortify the leaves with nutrients like proteins, amino acids, vitamins, minerals, carbohydrates crude extracts of jaggery and molasses to harvest better quality of cocoon and silk. (Etebari et al., 2004; Etebari & Matindoost, 2005; Nirmala et al., 2002; Rahmathulla et al., 2012).

Various antibiotics are extensively employed in the nutrition of non-herbaceous animals for raising their productivity. The beneficial effect of the antibiotics has been attributed to their activity in conditioning the composition of intestinal flora, to their potential role as possible growth factors, to their biological efficiency in increased turning over of the feed in to body weight and to their potential disease control activity (Goldberg, 1959; Walton, 1977). The mechanism of action of antibiotics in biomass accumulation is still not completely understood. There is a controversy as to whether the antibiotic acts entirely through its antibacterial property or by favorably affecting the physiology and

metabolism by an increase in the feed efficiency or by the activation of enzymes or through hormones, which control and regulate growth (Verma & Kushwaha, 1971). Many investigators reported biochemical action of antibiotic on aminoacid profile of *B. mori* silk protein (Afrikion, 1960; Walton, 1977). The administration of antibiotic causes the physiological changes in silkworm have a direct bearing on the leaf consumption and its further conversion to cocoon. (Aftab Ahamed et al., 2001). Bohidar & Pradhan (2000) studied the effect of oral administration of four antibiotics on the rearing performance of eri silkworm, *Samia cynthia ricini*.

The natural silk synthesized by the silkworm and spun in the form of a cocoon is originally synthesized in the silk gland. Silk gland of *B. mori* is a typical exocrine gland secreting large amount of silk proteins. It is a paired organ consisting of modified labial/salivary glands located at the two lateral sides under the alimentary canal. Silk inside the silk gland is in liquid state. The change of this liquid silk protein to solid cocoon fibers of certain morphological character is a complex physiological and physicochemical process. The process of spinning of cocoon is a fairly complicated physio-chemical phenomenon and not merely the extrusion of the silk proteins stored in the silk gland.

Silk gland weight is highly correlated to silk production since most of the cell functions are involved in silk production in the mature larva. A high silk gland weight is obtained differently in different strains, either by higher number of cells with cells of small size or by larger cells, which are smaller in number (Prudhomme & Couble, 1979). It is striking that there is only a low correlation between silk production and the relative size of the silk gland to body weight. So, activity of the silk gland is primarily determined by its own characteristics and it remains largely independent of other organs.

In the present investigation, the effect of administration of an antibiotic (Norfloxacin®) on growth and development of silk gland of a productive bivoltine hybrid (CSR2 × CSR4) and a crossbreed hybrid (BL61× CSR101) was studied. The study also evaluated the influence of antibiotic on subsequent increase in other economic traits of silkworm.

MATERIALS AND METHODS

Study material

A productive bivoltine hybrid silkworm (CSR2×CSR4) developed by breeders of Central Sericultural Research and Training Institute, Mysore, India under the collaboration of Japanese experts was used as one of the study material. This hybrid is suitable to rear during favorable season (August–February) under Indian environmental condition and is popular for its high survivability, yield and silk ratio and also producing quality bivoltine silk matching with the international standards. The crossbreed hybrid is used in the present study is a new multivoltine × bivoltine breed (BL67 × CSR101) and can be reared throughout the year under Indian conditions.

Young age silkworm rearings was conducted by following the new standard package and practices (Rajan et al., 2001) by providing fresh tender leaves of V1 mulberry variety with high moisture content of 75-80%. The temperature (27±1°C) and humidity (85– 90%) was maintained during young age rearing. During late age rearing it was maintained 25 ±1°C and 70± 5 % respectively.

Antibiotic administration

The experiment was conducted at Central Sericultural Research and Training Institute, Mysore (2002-03), Norfloxacin® is a broad-spectrum antibiotic called the quinolones (Fig. 1) and it is active against both gram positive and negative

bacteria. It is a synthetic chemotherapeutic agent occasionally used to treat common as well as complicate urinary tract infections. It works by entering the bacterial cell and inhibiting a chemical called DNA-gyrase, which is involved in the production of genetic material (DNA). Therefore it prevents the bacteria from reproducing and their further growth is stopped.

The freshly moult out fifth instar larvae were grouped in to three batches for each hybrid. Each batch was separated with three replications of 100 larvae and reared at temperature of 25±1°C and a relative humidity of 75±5%. The batch 1 & 2 were experimental batches (T-1 and T-2), and the larvae were fed with mulberry leaves fortified with two different concentrations of antibiotic Norfloxacin® (50 and 100 ppm). However, the batch-3 (Control) larvae were considered as a carrier control fed with normal mulberry leaf sprayed with distilled water. The solution was prepared by adding powdered antibiotic in distilled water and made the required concentration. The known quantity of leaf as per the standard recommendation (Rajan et al., 2001) was sprayed with freshly prepared solution (50 ml for each batch/feeding) and dried the leaf samples of treatments and control for 15 minutes after keeping in shade. The treatment was initiated on the first day of fifth instar and was continued up to spinning. A parallel batch for each treatment and control were maintained separately and these silkworm batches were mainly used to dissect out silk gland. Growth rate pattern of silkworm was studied daily by taking observation of weight of 5 male and female larvae. For determination weight of silk gland, every day 3 healthy larvae from additional batches of each treatment were dissected and observed the weight of silk gland after dry out moisture from the gland with the help of a tissue paper. Fully matured larvae were mounted separately replication and treatment wise in plastic collapsible montages for cocoon spinning. The cocoon harvesting and assessment was done on 6th day after mounting. The cocoon weight, shell weight and shell ratio were calculated and sample of each treatment was subjected for reeling operation and calculated the filament length. The experiment was designed under the Randomized Block Design (RBD) and was repeated two times in different season (rainy and summer) and analysis of variance (Anova) was worked out to arrive at the treatment significance levels. The treatment means values were compared by using values of critical difference (C.D.). The standard error (SE±) and critical difference were worked out by using following formulae.

C.D at 5% level of significance = S.E difference × t_5% level of significance

SE± = $\sqrt{V_E (1/r_1+1/r_2)}$ where r_1 and r_2 are numbers of the replications of the treatments to be compared.

The detailed plan of the experiment (Table 1) and formulae for calculation of different parameters are described below.

$$\text{Weight of single larva} = \frac{\text{Weight of 10 larvae (g)}}{\text{Total no. of larvae weighed (10)}}$$

$$\text{Growth index} = \frac{\text{Final weight of larvae (g)} - \text{Initial weight of larvae (g)} \times 100}{\text{Initial weight of larvae (g)}}$$

$$\text{Weight of single cocoon} = \frac{\text{Weight of 10 male cocoons + Weight of 10 female cocoons (g)}}{\text{No. of cocoons weighed (20)}}$$

$$\text{Single shell weight} = \frac{\text{Total shell weight of 10 male cocoons + Total shell weight of 10 female cocoon shell (g)}}{\text{Total no of cocoon shell weighed (20)}}$$

$$\text{Tissue somatic index} = \frac{\text{Silk gland weight (g)} \times 100}{\text{Larval weight (g)}}$$

$$\text{Silk conversion index} = \frac{\text{Shell weight (g)} \times 100}{\text{Silk gland weight (g)}}$$

$$\text{Average filament length} = \frac{\text{Total filament length (m)}}{\text{Total no. of cocoons reeled}}$$

RESULTS AND DISCUSSION

After the administration of antibiotic, day-to-day increase in weight of silkworm larva as well as silk gland was observed during 5th instar for bivoltine and crossbreed hybrid silkworm. Maximum increase in larval weight was observed at 48 hrs with respect to the weight of previous day in treatments and control (Fig. 2). In bivoltine hybrid it was recorded higher in treated batches when compared with control. Similar, results were also observed in cross breed hybrid (Fig. 3). Similar, to larval weight maximum growth of silk gland with respect to previous day was observed at 72 hrs and also it was significantly higher for treated batches (Fig. 4). The same trend was repeated in cross breed hybrid (Fig. 5).

Maximum larval and silk gland weight was observed at 144 hrs of larval development and it was recorded higher for treated batches of bivoltine hybrid as well as cross breed hybrid (Figs. 2-5). Slight decrease in silk gland weight was observed after 144 hrs of development during full maturation period of silkworm. The fully matured larval weight in bivoltine hybrids was recorded significantly higher for T2 (6.18g) followed by T1 (5.85g) and least weight was recorded for control (5.43g) (Table 2). Similar trend was observed in cross breed hybrid also and it was recorded higher for T2 (4.82g) followed by T1 (4.65g) and control (4.08g) (Table 3). Similarly, weight of silk gland also recorded at the end of the 5th instar larval period and it was recorded significantly higher in antibiotic administered batches of bivoltine hybrid (1.97g for T2 and 1.885g for T1) when compared with the control (1.745g) (Table 2). Similar, observations were recorded in the case of cross breed hybrid and it was significantly higher in antibiotic treated batches (1.541g for T2 and 1.484g for T1) (Table 3). This explains the allometric growth of silk gland and extra production silk on treatment with antibiotics.

Daily increment of larval and silk gland weight was calculated based on day to day observation and from these data growth index with respect to initial weight of larva and silk gland were calculated (Tables 4 & 5). Maximum growth index of larva was recorded at 144 hrs of development and it was also significantly higher in treated batches (526.77 for T2, 493.30 for T1 and 439.55 for control). Same trend was observed in cross breed hybrid (479.32 for T2, 458.89 for T1 and 380.76 for control). Similar, observations were made for growth index of silk gland of bivoltine hybrid and it was recorded maximum at 144 hrs (Table 4) and recorded significant difference between treated and control batches (839.15 for T2, 789.15 for T1 and 697.16 for control). Similar, observations were also made in

the case of cross breed hybrid and it was recorded significantly higher for T2 (775.56) followed by T1 (740.90) and control (660.79)(Table 4). Sailaja et al. (1991) reported that antibiotic terramycin having an effect on the development and organic composition of silkworm *B.mori.* Oral treatment with antibiotic terramycin increased larval and cocoon weight in *B.mori.* It changes in biochemical composition indicated increased protein synthesis and an increase in carbohydrate uptake from the blood.

Comparatively shorter larval duration of 5th instar silkworm was observed in treated batches of bivoltine hybrid and it was recorded as 140 hrs and 142 hrs for T2 and T1 respectively. In cross breed hybrid also same trend was repeated and it was recorded shortest larval duration in T2 (138 hrs) (Table 2). Earlier studies of Verma & Kushwaha (1971) and Radhakrishna Rai & Devaiaha (1998) reported there was no influence of antibiotic administration on larval duration. Banuprakash et al. (1999) recorded that the larval duration was shorter in antibiotic (chloramphenicol) treated silkworms, but the difference was not so vivid. Aftab Ahamed et al. (2001) reported that food assimilated, assimilation rate, assimilation efficiency, food converted, conversion rate, and conversion efficiencies were significantly higher in the antibiotic treated silkworm batches, though the dry food consumed is on par with the carrier control. A notable feature of the results is that the enhancement in the major commercial traits was not accompanied by a prolonged larval duration. This might be due to a direct stimulating effect of antibiotic on protein synthesis in silk gland.

Tissue somatic index represent the ratio between silk gland and larval weight and there was no significant difference between control and treatments (32.22 for T1, 32.15 for T2 and 32.13 for control) in bivoltine hybrid (Table 2). Similar observations were made in the case of cross breed hybrid also.

Silk conversion index is the percentage of silk in the fiber to the laminal silk in the silk gland. It was recorded higher in treated batches of bivoltine hybrids (27.26 for T1 and 28.22 for T2) when compared with control (25.94) (Table 2). Similarly, in cross breed hybrid also it was recorded higher in treated batches (26.21 for T1 and 26.54 for T2) (Table 3). Aftab Ahamed et al., (2001) reported that administration of chloramphenicol resulted in increased conversion of food in to shell content, indicating its beneficial results of higher silk synthesis. The total consumption during larval period of *B. mori* over 80% is consumed during the final instar and the silk, which is spun out finally as cocoon, is synthesized during fifth instar. Prudhomme et al. (1985) reported that silk produce in early instar is degraded during subsequent moults, hence supplementation in the earlier instars does not improve the cocoon production in addition to increasing the cost of rearing.

The enhanced growth and development of larval weight and silk gland reflected in commercial characters of silkworm and subsequent productivity in sericulture. The shell weight represents actual silk content of the cocoon and it was significantly higher in treated batches (0.556g for T2 and 0.514g for T1) when compared with control (Table 2). Same trend was observed in the case of cross breed hybrid (0.409 for T2 and 0.387 for T1). The cocoon weight was also recorded significantly higher in treated batches (2.03 for T2, 2.07g for T1 and 1.97g for control) of bivoltine hybrid. Similar observations were made in the case of cross breed hybrid. Bohidar & Pradhan (2000) studied the effect of four different antibiotics on eri silkworm and were found Norfloxacin to be the best antibiotic among. Aftab Ahamed et al. (2001) recorded that 25 and 50 ppm of chloramphenicol administration enhance significantly the cocoon weight. Govindan et al. (1990) reported that the antibiotic administrated silkworm larvae

had higher digestive amylase activity in the intestine and later better utilization of food in to larvae and pupae.

The average filament length (AFL) of both hybrids was recorded after the process of reeling and it was found that significantly higher in treated batches of bivoltine hybrid (1230 m for T2 and 1064 m for T1) when compared with control (974m) (Table 2). Similar observation was made in cross breed hybrid (973m for T2, 939 m for T1 and 890 m for control). The increased cocoon shell weight is aptly reflected in the filament length of treated batches. So the results clearly indicated that the antibiotic administration causes an increase in filament length. The study results were in support with the earlier studies (Verma & Kushwaha, 1971; Tayade et al., 1988; Banuprakash et. al., 1999).

The study results concluded that the administration of antibiotic Norfloxacin enhanced growth and development of larval and silk gland of silkworm. This growth of larva and silk gland subsequently enhanced commercial characters of silkworm such as cocoon weight, shell weight, silk ratio and filament length in treated batches. This application can be used for getting higher productivity in sericulture.

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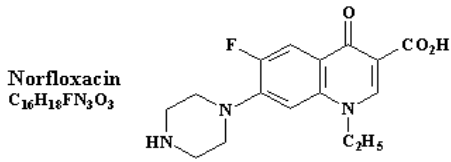


Figure 1. Chemical structure of Norfloxacin.

Table 1. Detailed plan of the experiment.

Race	Treatments with two concentrations of antibiotic		Control
Bivoltine hybrid (CSR2 × CSR4)	50 ppm (T1)	100 ppm (T2)	Distilled water sprayed
Cross breed hybrid (Bl67 × CSR101)	50 ppm (T1)	100 ppm (T2)	Distilled water sprayed

Table 2. Influence of antibiotic on growth and development of bivoltine hybrid silkworm.

Treatments	Maturation (hrs.)	Matured Larval Weight (g)	Matured Silk gland weight (g)	Growth index	Tissue somatic index	Shell weight (g)	Cocoon weight (g)	Silk conversion index	Filament length (m)
T1 (50ppm)	142	5.85	1.885	493.30	32.22	0.514	2.029	27.26	1064
T2 (100ppm)	140	6.18	1.971	526.77	32.15	0.556	2.069	28.22	1230
Control	144	5.43	1.745	439.55	32.13	0.455	1.973	25.94	974
SE±	0.333	0.083	0.018	7.93	0.440	0.006	0.019	0.123	9.658
CD 5%	1.1	0.261	0.042	25.00	1.22	0.019	0.047	0.456	30.4
F-test	**	*	**	*	NS	**	*	*	**

* Significant at 5% level, ** Significant at 1% level, NS- Non significant, SE±=Standard error

Table 3. Influence of antibiotic on growth and development of crossbreed hybrid silkworm.

Treatments	Maturation (hrs.)	Matured Larval Weight (g)	Matured Silk gland weight (g)	Growth index	Tissue somatic index	Shell weight (g)	Cocoon weight (g)	Silk conversion index	Filament length (m)
T1 (50ppm)	144	4.65	1.484	458.89	31.91	0.387	1.98	26.21	939
T2 (100ppm)	136	4.82	1.541	479.32	31.97	0.409	2.01	26.54	973
Control	144	4.08	1.291	380.76	31.61	0.335	1.72	25.96	890
SE±	0.494	0.076	0.015	0.933	0.284	0.005	0.009	0.122	4.991
CD 5%	1.60	0.239	0.035	24.994	1.23	0.016	0.039	0.421	29.88
F-test	**	*	**	*	NS	**	**	NS	**

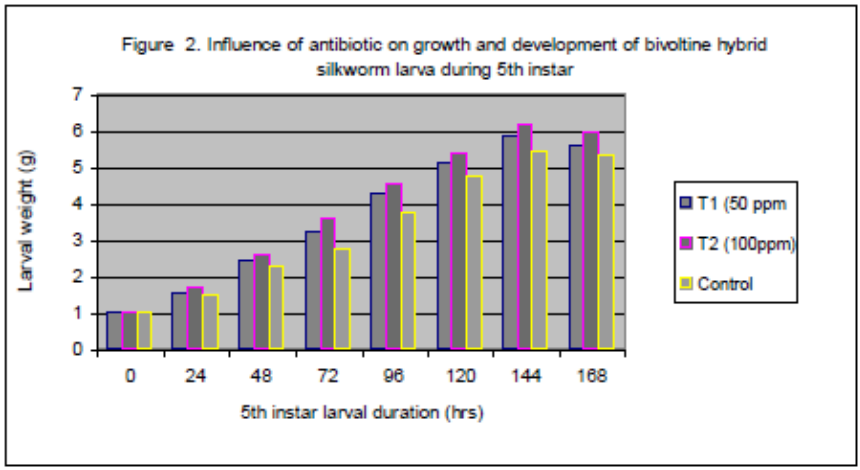
* Significant at 5% level, ** Significant at 1% level, NS- Non significant, SE±=Standard error

Table 4. Influence of antibiotic on growth index of silkworm larva during different hours.

		Growth index of Larva in different hours					
Treatments		24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs
T1 (50ppm)	Bivoltine hybrid	57.20	146.95	226.06	332.04	413.40	493.30
	Cross breed hybrid	54.80	142.78	210.09	335.09	402.40	458.89
T2 (100ppm)	Bivoltine hybrid	70.38	160.24	260.64	356.38	447.66	526.77
	Cross breed hybrid	79.50	163.85	234.13	374.75	419.23	479.32
Control	Bivoltine hybrid	48.40	126.40	177.69	280.32	374.40	439.55
	Cross breed hybrid	51.26	125.69	185.71	300.24	350.72	380.76

Table 5. Influence of antibiotic on growth index of silk gland during different hours.

		Growth index of silk gland in different hours					
Treatments		24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs
T1 (50ppm)	Bivoltine hybrid	97.18	267.60	451.64	586.79	719.33	789.15
	Cross breed hybrid	115.25	267.20	429.94	609.65	696.59	740.90
T2 (100ppm)	Bivoltine hybrid	119.33	293.86	510.32	625.94	775.15	839.15
	Cross breed hybrid	132.76	282.38	473.86	66.47	727.27	775.56
Control	Bivoltine hybrid	91.98	241.98	374.17	520.75	652.35	697.16
	Cross breed hybrid	96.61	240.90	385.87	543.00	613.63	660.79



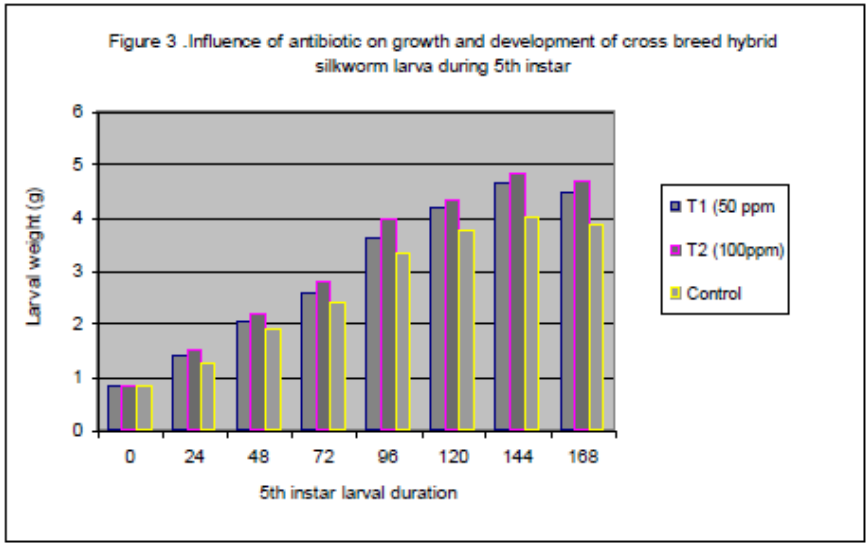


Figure 4. Influence of antibiotic on growth and development of silkgland of bivoltine silkworm hybrid during 5th instar

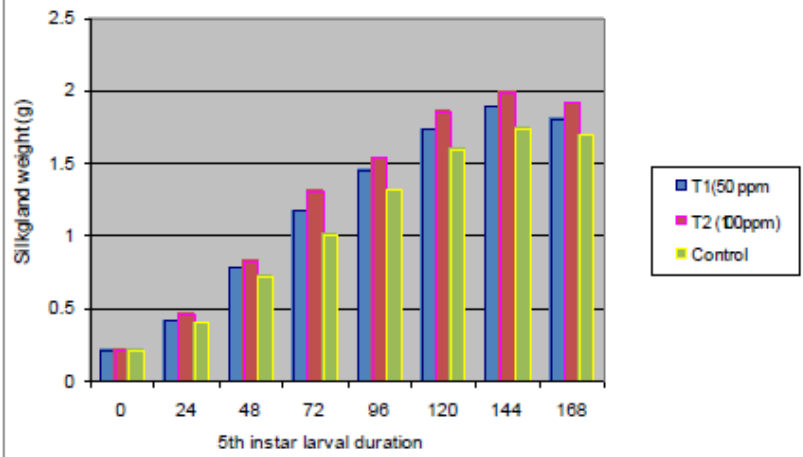


Figure 5. Influence of antibiotic on growth and development of silkgland of cross breed hybrid silkworm during 5th instar