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The effects of amoxicillin, oxytetracyclin and doxycyclin on the growth and development of silkworm, *Bombyx mori* L.

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Abstract
The present investigation reported the effects of amoxocillin, oxytetracyclin and doxycyclin on physiological parameters, growth and development of the larva and pupa, mortality percentage and different cocoon characteristics of silkworm, *Bombyx mori* L. at various concentrations. The experimental results showed that the effect of different antibiotics on physiological parameters like food consumption, food utilization and food digestibility of 5th instar larvae were increased in the treated lines in comparison to the control. The highest food consumption, food utilization and food digestibility of 5th instar larvae was recorded at 5% doses. Weight of larva and pupa were significantly increased due to different antibiotics application in mulberry leaves and the most effective result was found at 5% doses of amoxicillin, followed the order oxytetracyclin and doxycyclin. In case of larval and pupal duration, it was observed that the duration of larval and pupal period of silkworm, *B. mori* was significantly decreased and the lowest period has been recorded at 5% doses of amoxicillin. Antibiotics also reduced the larval and pupal mortality percentage at different concentrations. The cocoon characters e.g. cocoon weight, shell weight and shell ratio were significantly increased at different antibiotic concentrations like 2% and 5%. The effectiveness of different antibiotics followed the order amoxicillin > oxytetracyclin > doxycyclin.

Keywords: *Bombyx mori* L., antibiotics, amoxicillin, oxytetracyclin, doxycyclin

Introduction

Agro-climate condition of Bangladesh is favorable for the development of sericulture. Bangladesh can offer a wide range of areas to support the production of quality silk to satisfy domestic demand. Sericulture can be an attractive source of income for its rural people, and it requires low investment with potential for relatively higher returns. In sericulture, the productivity and quality largely depends on rearing of disease free healthy silkworm larvae. Silkworms are affected by a number of diseases due to various biological, chemical, physical, nutritional and environmental causes [1].

Mulberry leaves of poor nutritive value will not be able to provide sufficient quantity of essential requirement to the larvae to produce anti-bacterial and anti-viral factor. It resulted in higher rate of multiplication of infectious bacteria and development of flacherie disease [2]. Antibiotics used for clinical purpose have therapeutic effects on silkworms infected with the pathogens. Ganciclovir, foscarnet, vidarabine and ribavirin (antiviral agents) inhibit the proliferation of baculovirus in silkworm body fluid and had therapeutic effects [3].

The effect of antibiotics supplementation with mulberry leaves on growth and development of silkworm have been reported by many researchers [4-7]. Oral administration of antibiotics along with mulberry leaves to healthy silkworm boosts the growth, fecundity and silk contents [8] as well as reduced the incidences of diseases [5]. The antibiotics such as penicillin, ampicillin and streptomycin were found to be effective in reducing the mortality of silkworms by 23-25% without affecting the cocoon parameters [5]. The antibiotics such as amoxicillin, tetramycine, streptomycin, gentamycin, and kanamycin supplemented through mulberry leaves resulted in significant reduction in the occurrence in both grasserie and flacherie diseases [6, 7]. Shyamala *et al*. [9] have reported that chlormycetin supplementation enhance the oxygen uptake of the gut of the silkworm. It was thought that these antibiotics exerted a beneficial influence in controlling the intestinal flora of the caterpillars.

Feeding of antibiotics enhanced the nutrition and economic parameters in *B. mori* as well as

showed a prophylactic measure to prevent bacterial infection. Thilagavathi *et al.* [10] reported that feeding of amoxicilin increased the larval weight, growth, fecundity and silk content. Santha *et al.* [6] also reported that fecundity was found to be more after ampicilin, cloxacillin and chlormphenicol treatments.

When antibiotics were administered to the silkworm, there is a shift in nitrogen metabolism in favour of increasing the body weight and increase output of silk [11]. Studies on auromycin and chlormycetin showed that their addition to the mulberry diet resulted in heavier caterpillars with increased nitrogen metabolism [4]. Diet supplementation along with mulberry leaves is an effective method to build up the body, sustain life, spin cocoons and egg production of silkworm [12,-14]. Different researchers have been reported such nutritional requirements in food consumption and showed the effect of different antibiotics supplementation on larval and cocoons weight, amount of silk production, pupation and reproductive traits in silkworm, *B. mori* [4, 9, 15].

Some selected group of antibiotics were used to digest their food more efficiently, get maximum benefit from it and allow them to develop into strong and healthy individuals. This was achieved by destroying or inhibiting undesirable bacteria in the gut which prevent optimum absorption of food [16]. Different antibiotics as growth stimulating factors are extensively used to enrich the nutrition of farm and other animals for their increased productivity [17]. Broad spectrum antibiotics *viz*, penicillin, streptomycin, tetracycline and chloramphenicol were already tried on silkworm and found successful [18]. Amoxicillin, oxytetracyclin and doxycyclin are moderate-spectrum bacteriolytic β-lactum antibiotic. However, reports on the use of amoxicillin, oxytetracyclin and doxycyclin for mulberry silkworm are negligible. Therefore, this study has been carried out to know the impact of these antibiotics on *B. mori*.

2. Materials and methods

2.1. Experimental animal

The animal of the present experiment was an improved multivoltine Bangladeshi variety of silkworm, *B. mori* L., popularly known as Urboshi-1. Host plants of silkworm, i.e. mulberry leaves of *Morus alba* species were used through the experiment. To serve the purpose of the present experiment, the silkworms were reared following the scientific rearing techniques [19, 20] in the Sericulture Research Laboratory, Department of Zoology, University Rajshahi, Bangladesh.

2.1. Preparation of doses of antibiotics

Three antibiotics selected for food supplementation, which were amoxicillin, oxytetracyclin and doxycyclin. Various concentrations of the antibiotics were prepared by mixing the requisite amounts of antibiotics in distilled water. The concentrations of the antibiotics were 1%, 3% and 5%. Fresh mulberry leaves were treated by dipping in these solutions and after that these were dried by fanning.

2.3. Experimental design

In the present investigation, after disinfection and incubation of collected eggs, the newly hatched larvae were brushed in the rearing trays (25 × 40 cm) and reared up to second instar on fresh mulberry leaves. After the second moult, the 3rd to 5th instar larvae were fed with mulberry leaves treated with different concentrations (1%, 3% and 5%) of antibiotics. A control batch was also maintained where larvae were reared by mulberry leaves dipped in distilled water only. There were three replications per concentration and control batch, and in each replication 60 healthy larvae were selected at the beginning of 3rd instar.

2.4. Experimental data

The characters considered for the study were weight of mature larvae (LW), pupal weight (PW), larval duration (LD), pupal duration (PD), larval mortality % (LM), pupal mortality % (PM), cocoon weight (CW), shell weight (SW) and shell ratio (SR %).

2.5. Statistical analyses

Results on growth and development for different characters were presented as mean ± standard error (SE). The feed efficacy parameters like food consumption rate, food utilization rate, digestibility rate were calculated [21] by using the following formula.

- Food consumption rate (FCR) = Dry weight of leaves offered - Dry weight of residual leaves.
- Food utilization rate (FUR) = Weight of food consumed - Weight of faecal matter
- Food digestibility rate (FDR):

$$\text{FDR} = \frac{\text{Dry weight of food eaten} - \text{Dry weight of faecal produced}}{\text{Total No. of cocoon harvested}}$$

In case of larval mortality when control batch had any mortality, the mortality percentage was corrected using Abbott's formula [22] as follows.

$$Pt = \frac{Po - Pc}{100 - Pc} \times 100$$

Where, Pt = Corrected mortality % Po = observed mortality % and Pc = Control mortality %.

For testing the significance of different parameter on the effect of food supplementation, the collected data were statistically analyzed (ANOVA) using Microsoft Excel.

3. Results

3.1. Effects of Antibiotic on the physiological parameters of *B. mori* L.

The results of the supplementation of different antibiotics on food consumption rate (FCR), food utilization rate (FUR) and food digestibility rate (FDR) of *B. mori* at different concentrations, viz. 1%, 3% and 5% is shown in Table 1. Antibiotic increased the FCR, FUR and FDR at different doses compared to control. The most effective dose of the antibiotic was found to be 5% followed by 3% and 1%. Results of analyses of variance are also presented in Table 1. Most of the items showed highly significant differences at the 1% level of significance. The effectiveness of different antibiotics on FCR, FUR and FDR followed the order amoxocillin > oxytetracyclin > doxycyclin.

Table 1: Effect of antibiotics on FCR (%), FUR (%) and FDR (%) of silkworm, *B. mori* L.

Antibiotics	Doses (D)	Treatment (Mean±SE)		
		FCR (%)	FUR (%)	FDR (%)
Amoxocillin	Control	50.96±0.19	46.14±0.47	86.22±0.20
	1%	48.35±0.06	44.46±0.06	87.65±0.26
	3%	52.11±0.14	48.44±0.33	89.43±0.26
	5%	55.70±0.26	52.01±0.21	92.65±0.49
Oxytetracyclin	Control	50.96±0.19	46.14±0.47	86.22±0.20
	1%	47.86±0.29	45.06±0.07	87.11±0.51
	3%	51.05±0.18	47.86±0.48	88.57±0.04
	5%	53.72±0.54	50.23±0.56	92.21±0.13
Doxycyclin	Control	50.96±0.19	46.14±0.47	86.22±0.20
	1%	47.60±0.15	44.08±0.25	86.75±0.22
	3%	50.74±1.19	46.16±0.03	88.24±0.23
	5%	52.84±0.24	50.21±0.43	91.41±0.31

ANOVA

Items	df	F		
		FCR (%)	FUR (%)	FDR (%)
A= Antibiotic	2	90.82**	46.60**	12.54**
R= Replication	2	2.72^{ns}	4.28*	0.30^{ns}
D= Dose	5	375.37**	427.19**	215.57*
DA= Dose × Antibiotic	10	7.68**	9.67**	1.02^{ns}

** = 1% Significant, * = 5% Significant, ns = not significant,

3.2. Effects of Antibiotic on the growth and development of *B. mori* L.

As shown in **Table 2** the mature larval weight (LW) and pupal weight (PW) were increased and, larval duration (LD) and pupal duration (PD) of *B. mori* were decreased at different doses, viz. 1%, 3% and 5%. The maximum LW and PW were observed at 5% dose of different antibiotics. But in case of LD and PD the results were reversed. Results of analyses of variance showed that most of the items except replication and D×A showed highly significant differences at the 1% level of significance (Table 2). The effectiveness of different antibiotics followed the order amoxocillin > oxytetracyclin > doxycyclin.

Table 2: Effect of antibiotics on LW (gm), PW (gm), LD (day) and PD (day) of silkworm, *B. mori* L.

Antibiotics	Doses (D)	Treatment (Mean±SE)			
		LW (gm)	PW (gm)	LD (day)	PD (day)
Amoxocillin	Control	3.11±0.01	1.22±0.01	22.94±0.06	7.32±0.01
	1%	3.05±0.02	1.16±0.01	22.30±0.01	6.88±0.01
	3%	3.23±0.03	1.39±0.01	21.88±0.02	6.70±0.01
	5%	3.25±0.06	1.54±0.00	21.09±0.02	6.61±0.02
Oxytetracyclin	Control	3.11±0.01	1.22±0.01	22.94±0.06	7.32±0.01
	1%	2.99±0.01	1.09±0.05	22.27±0.05	6.79±0.01
	3%	3.13±0.03	1.24±0.03	21.95±0.13	6.71±0.01
	5%	3.24±0.01	1.44±0.01	21.78±0.10	6.64±0.01
Doxycyclin	Control	3.11±0.01	1.22±0.01	22.94±0.06	7.32±0.01
	1%	2.89±0.04	1.22±0.03	22.25±0.09	7.01±0.05
	3%	3.04±0.01	1.36±0.00	21.52±0.40	6.75±1.05
	5%	3.16±0.03	1.38±0.01	22.13±0.10	6.70±0.01

ANOVA

Items	df	F			
		LW (gm)	PW (gm)	LD (day)	PD (day)
A= Antibiotic	2	19.33**	22.74**	12.86**	10.60*
R= Replication	2	3.28^{ns}	4.87*	0.32^{ns}	2.48^{ns}
D= Dose	5	48.55**	173.25**	43.07**	1052.57**
DA= Dose × Antibiotic	10	3.27**	11.25**	4.16**	9.64**

** = 1% Significant, * = 5% Significant, ns = not significant,

3.3. Effects of Antibiotic on the mortality of *B. mori* L.

The effect of antibiotics on larval (LM) and pupal (PM) mortality percentage of *B. mori* is shown in Table 3. Antibiotics decreased both the mortality at different doses compared to control. In different antibiotics the lowest mean values of LM and PM were observed at 5% dose. The most effective dose of the antibiotics was in order 5%, 3% and 1%. ANOVA results indicated that the decrement were significant.

Table 3: Effect of antibiotics on LM (%) and PM (%) of silkworm, *B. mori* L.

Antibiotics	Doses (D)	Treatment (Mean±SE)	
		LM (%)	PM (%)
Amoxocillin	Control	15.00±1.73	12.00±0.00
	1%	6.00±1.15	6.00±1.15
	3%	4.00±0.00	4.00±1.15
	5%	2.00±1.15	0.00±0.00
Oxytetracyclin	Control	15.00±1. 73	12.00±0.00
	1%	10.00±1.15	8.00±2.31
	3%	5.00±0.58	6.00±1.15
	5%	2.00±0.00	4.00±2.31
Doxycyclin	Control	15.00±1.73	12.00±0.00
	1%	8.00±0.03	2.00±0.00
	3%	6.00±1.15	4.00±2.31
	5%	7.00±0.58	0.00±0.00

ANOVA

Items	df	F	
		LM (%)	PM (%)
A= Antibiotic	2	14.07*	12.00*
R= Replication	2	3.76^{ns}	3.57^{ns}
D= Dose	5	37.85**	34.40**
DA= Dose × Antibiotic	10	1.36^{ns}	1.28^{ns}

** = 1% Significant, * = 5% Significant, ns = not significant,

3.4. Effects of Antibiotic on the cocoon characters of *B. mori* L.

The cocoon characters like cocoon weight (CW), shell weight (SW) and shell ratio (SR%) were significantly increased when the worms were reared on mulberry leaves enriched with antibiotics (Table 4). In amoxocillin, oxytetracyclin and doxycyclin the highest and the lowest mean value for CW, SW and SR% were observed at 5% and at 1% respectively. Results of analyses of variance are presented in Table 4. It indicates that most of the items except replication showed highly significant differences at the 1% level of significance.

Table 4: Effect of antibiotics on CW (gm), SW (gm) and SR (%) of silkworm, *B. mori* L.

Antibiotics	Doses (D)	Treatment (Mean±SE)		
		CW (gm)	SW (gm)	SR (%)
Amoxocillin	Control	1.38±0.01	0.12±0.01	11.50±0.58
	1%	1.24±0.01	0.10±0.00	10.20±0.23
	3%	1.42±0.03	0.16±0.00	15.60±0.17
	5%	1.75±0.01	0.17±0.00	16.80±0.40
Oxytetracyclin	Control	1.38±0.01	0.12±0.01	11.50±0.58
	1%	1.21±0.03	0.11±0.00	10.50±0.17
	3%	1.41±0.02	0.15±0.00	15.00±0.17
	5%	1.59±0.04	0.16±0.01	16.20±0.29
Doxycyclin	Control	1.38±0.01	0.12±0.01	11.50±0.58
	1%	1.35±0.00	0.09±0.01	8.80±0.92
	3%	1.43±0.00	0.12±0.00	12.00±0.12
	5%	1.55±0.00	0.15±0.00	14.80±0.35

ANOVA

Items	df	F		
		CW (gm)	SW (gm)	SR (%)
A= Antibiotic	2	18.89**	27.83**	27.83**
R= Replication	2	2.16^{ns}	2.91^{ns}	2.91^{ns}
D= Dose	5	161.22**	109.09**	115.37**
DA= Dose × Antibiotic	10	11.48**	3.27*	3.41*

** = 1% Significant, * = 5% Significant, ns = not significant,

4. Discussion

Ciprofloxacin is a broad spectrum antibiotic, active against both gram positive and negative bacteria, which exerts a beneficial influence by killing the intestinal pathogens of silkworm larvae [23]. The absence of gut pathogens has resulted in improved food consumption, energy conversion and silk production. In chloramphenicol and other antibiotic treated silkworms the food assimilation rate and assimilation efficiency are higher due to the influence of the antibiotics on the physiology [24, 25]. In the present investigation the effects of amoxocillin, oxytetracyclin and doxycyclin on the physiological parameters of silkworm, *B. mori* L. at various concentrations were evaluated.

Physiological parameters like food consumption, food utilization and food digestibility of 5th instar larvae were increased in the treated lines in comparison to the control line. Several studies indicated that gut microflora is sensitive to the antibiotics and the loss of which cause adverse effects on the

physiological system of the insects. Chloromycetin administration cause general reduction in gut bacterial population of silkworms [26]. It was observed that population of endogenous gut bacteria *viz.* Micrococci, Streptococci and Flavobacteria were reduced in number with concomitant increases in the number of coliforms [9]. To develop strong and healthy silkworms, and get maximum production, a selected group of antibiotics was used. This was achieved by destroying or inhibiting undesirable bacteria in the gut which prevent optimum absorption of food [16].

Weight of the larva and pupa were significantly increased due to different antibiotics application in mulberry leaves, and the highest result has been coming on 5% doses. The oral supplementation of the antibiotics azithromycin, ciprofloxacin to 4th inster larvae resulted significant increase in larval weight [27]. Amoxicillin, tetracycline, streptomycin and penicillin treatments increased the larval weight of silkworm [6, 10].

Feeding of antibiotics along with mulberry leaves has increased the larval growth. The growth and development of silkworms and their economic characters are influenced to a great extent by the nutritional content of mulberry leaf [28]. Krishnaswami *et al.* [29] and Thilagavathi *et al.* [10] have also noticed a significant increase in larval weight due to antibiotics treatment. In case of larval and pupal duration it was observed that the duration of larval and pupal period of silkworm, *B. mori* was significantly decreased due to different antibiotics application in mulberry leaves. Larval and pupal mortality were also reduced in the treated lines. Radha *et al.* [30] observed that antibiotics administration with different concentration significantly improved the rearing and economic parameter like larval duration, larval weight, growth index, single cocoon weight, single shell weight, shell ratio, average filament length, non-breakable filament length, raw silk recovery percentage, denier, reelability and neatness and the better performance were recorded with the increase of antibiotics concentration.

The cocoon characters like cocoon weight, shell weight and shell ratio were significantly increased when the worms were reared on mulberry leaves enriched with different vitamins and antibiotics [18, 31]. The oral supplementation of the antibiotics tetracycline and sulphamethaxazole to 4th inster larvae resulted significant increase in cocoon weight, shell ratio [32].

5. Conclusion

After discussion, it is concluded that the antibiotics increased food consumption, food utilization and food digestibility of 5th instar larvae on silkworm. As a result, growth and development of silkworm, *B. mori* were significantly improved and enhance economic characters. The antibiotics were also found to be effective in reducing the mortality of silkworms. Therefore antibiotics such as, amoxocillin, oxytetracyclin and doxycyclin can be used to reduce the mortality rate and for better production of silk.

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