

Effect of Antibiotics on the Haemocyte Count and Rearing Performance of Silkworm *Bombyx mori* L.

**Iqra Rafiq^{1*}, Z. I. Buhroo¹, K. A. Sahaf¹, N. A. Ganie¹, M. F. Baqual¹, S. A. Mir²,
Shahina A. Nagoo¹ and Sabiha Ashraf Kirmani¹**

¹College of Temperate Sericulture, Sher-e-Kashmir University of Agricultural Sciences and
Technology of Kashmir Srinagar, Jammu and Kashmir -190025, India.

²Division of Agriculture Statistics, Faculty of Horticulture, Sher-e-Kashmir University of Agricultural
Sciences and Technology of Kashmir Srinagar, Jammu and Kashmir -190025, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author IR carried out laboratory work and contributed to the protocol (writing of the manuscript). Authors ZIB, KAS and MFB conceptualized the study. Author NAG contributed to the experimental protocol. Author SAM contributed the experimental design and performed the statistical analysis. Authors SAN and SAK contributed in the revision of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2020/v39i4831274

Editor(s):

(1) Dr. Chen Chin Chang, Hunan Women's University, China.

Reviewers:

(1) Yong Wang, Shenyang Agricultural University, China.

(2) Alberto Aragón Muriel, Universidad del Valle, Colombia.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/66480>

Original Research Article

Received 15 November 2020

Accepted 29 December 2020

Published 31 December 2020

ABSTRACT

Background: Antibiotic agents have been used in recent years to increase the growth of silkworm larvae and to improve production. In this context, the present investigation reported the effects of ceftiofur sodium, oxytetracycline and enrofloxacin on total haemocyte count (cells/mm³), survival percentage and economic characteristics in silkworm *Bombyx mori* L. at various concentration levels. The study regarding the effect of antibiotics was conducted on the silkworm breed APS-45. Three concentrations (0.05%, 0.10% and 0.15%) were prepared for each antibiotic. The worms were reared upto 3rd instar without any treatment. After third moult silkworms were supplemented with antibiotic fortified mulberry leaves.

Results: The experimental results showed that the effect of different antibiotics on economic parameters like larval weight, larval duration, cocoon weight, shell ratio and raw silk percentage

*Corresponding author: E-mail: iqrabadi88@gmail.com;

were significantly increased in the treated lines in comparison to the control. Antibiotics also increased survival percentage and the most effective result was obtained at 0.15% of ceftiofur sodium followed by oxytetracycline and enrofloxacin. In the present study highest THC value of 9157.33 mm/cm³ was recorded in treatment ceftiofur sodium treated batch at concentration C₃ 0.15%. An increment in the total haemocyte count was observed in other treatments as well and the cell count increased with an increase in concentration of antibiotic dosage and the effectiveness of the antibiotics followed the order ceftiofur sodium, oxytetracycline and enrofloxacin.

Conclusion: This research outlines that there is a significant improvement in the total haemocyte count and economic traits in silkworm with the supplementation of antibiotic fortified mulberry leaves. The antibiotic treatment has significantly improved the raw silk percentage and has been found to be effective in reducing the mortality of silkworms. So, this antibiotic fortification can be further exploited for higher raw silk production.

Keywords: Antibiotics; *Bombyx mori*; haemocytes; rearing performance; silkworm.

1. INTRODUCTION

Silkworm, *Bombyx mori* L. is an important lepidopteron insect of economic importance that has been under domestication from 5,000 years now. Silk is "the sovereign of strands" since it is a smooth, sparkling, astonishing and exceptional fibre spun by silkworms. Successful silk production and quality attributes depend on the larval health growth and the required environmental conditions. Growth and development affect the productivity, which depends on the intricate physiological processes. In sericulture, the productivity and quality generally relies upon the rearing of disease free healthy silkworm larvae. As such, success of sericulture depends on proper management and protection of silkworm crop from diseases.

Silkworm is highly susceptible to infection caused by major pathogenic groups like bacteria, protozoa, fungi and virus. Mulberry silkworm is a monophagous insect, which feeds only on mulberry leaves. The low nutritional value of mulberry leaves will not be able to provide larvae sufficient quantities of essential prerequisites that are important for the development of antibacterial and anti-viral factors. So, various antimicrobial agents are being used for controlling silkworm diseases and to improve the production of silk and among those the most frequently advocated medications in modern scientific era are antibiotics which have shown promising results in controlling bacterial and viral diseases in farm animals including silkworm [1]. Antibiotic feed supplementation not only showed prophylactic steps to deter bacterial diseases, but the nourishment and economic parameters gets further upgraded in *B. mori* [2]. Antibiotic administration has shown attractive outcomes in rearing superior crop harvest and controlling the

silkworm disease [3]. Antibiotic supplement to silkworms has been reported to boost the oxygen absorption of the silkworm gut, which also has a beneficial effect on the regulation of the larval intestinal flora [4].

The silkworm model is technically convenient, ethically acceptable and fast as larval period is short, and can be used on a wider scale in the study of pathogens and drugs [5]. Further more silkworms have been utilized to study pathogenic bacterial toxin [6]; evaluate the target specificity of antibacterial agents [7]; identify novel *S. aureus* virulence genes [8]; and identify novel probiotic bacteria that promote survival during *P. aeruginosa* infection [9]. By utilizing the silkworm infection model, novel antimicrobial agents- Lysocin E [10], nosokomycins [11] and ASP2397 [12] has been successfully identified.

Insect blood or haemolymph is better defined as the circulating intracellular fluid filling the cavity of the body or haemocoel while bathing different tissues [13]. A thin permeable membrane lining the haemocoel physically separates the haemolymph from direct contact with the body tissues [14]. Insect haemolymph contains haemocytes that are suspended in the plasma. Haemocytes play multiple important roles during insect growth and development. Five types of haemocytes have been identified in the silkworm (*B. mori*) viz., prohemocyte, plasmatocyte, granulocyte, spherulocyte, and oenocytoid. Haemocytes have basic parts in different physiological activities [15]. Haemocytes in the haemolymph of the insects assume a significant function in the protection mechanism. Similarly haemolymph plays an important role in the inherent immunity response, which is induced when bacteria invade the body of the silkworm [16]. Insects possess an effective innate immune

system against foreign microorganisms. In addition, there is growing evidence that diverse classes of antibiotics have immunomodulatory effects, in addition to their antimicrobial activity [17]. These studies further added that the useful activity of the antibiotics can be ascribed to enhance action of anti-infection agents which diminishes fundamentally the occurrence of flacherie and grasserie. With this background, the present investigation is important to study the influence of antibiotics on the haemocyte count and rearing performance in silkworm *Bombyx mori* L.

2. MATERIALS AND METHODS

2.1 Silkworm Rearing

The present study was carried out during spring 2020 at silkworm rearing laboratory of Division of Silkworm Breeding and Genetics, College of Temperate Sericulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir Srinagar, (Mirgund) India. The silkworm rearing was conducted by following the recommended package of practices as outlined by [18]. The silkworm race APS-45 was selected to conduct experiment. Rearing was carried out under hygienic conditions. The laying's were incubated at $25\pm 1^{\circ}\text{C}$ temperature and relative humidity of 75-80% for about 10-12 days till hatching. The hatched larvae were brushed and reared upto 3rd moult by feeding on mulberry leaves without any treatment (Fig. 1a). After 3rd moult, three replications were maintained for each treatment with 100

silkworms of uniform age and size and each treatment received different concentration of antibiotics.

2.2 Preparation of Stock Solution

The three antibiotics were procured from a standard drug company (Table 1). For preparation of standard stock solution different concentration of three antibiotics viz. Ceftiofur sodium, Oxytetracycline and Enrofloxacin solution were prepared in distilled water. The concentrations of the antibiotics prepared were 0.05% (w/v), 0.10% (w/v), and 0.15% (w/v). For preparation of 0.05% stock solution, 0.5 g of antibiotic was dissolved in 100 ml of distilled water which is equivalent to 0.5% solution. From this solution 10 ml was taken and added to 90 ml of distilled water which is equivalent to 0.05% of stock solution. Similarly, for preparation of 0.10% stock solution, 0.5 g of antibiotic was dissolved in 100 ml of distilled water which is equivalent to 0.5% solution. From this solution 20 ml was taken and added to 80 ml of distilled water which is equivalent to 0.10% of stock solution. Likewise, for preparation of 0.15% stock solution, 0.5 g of antibiotic was dissolved in 100 ml of distilled water which is equivalent to 0.5% solution. From this solution 25 ml was taken and added to 75 ml of distilled water which is equivalent to 0.15% of stock solution. Fresh mulberry leaves were smeared by each treatment and were air dried for about 10 minutes prior to feeding. The feeding of antibiotics along with mulberry leaves was supplemented after 3rd moult once in a day.

Table 1. Details of antibiotics used in the study

Antibiotics	Description	Trade name	Company/Source
Ceftiofur sodium	<ul style="list-style-type: none"> Sodium salt of ceftiofur. Broad spectrum antibiotic active against both gram positive and gram negative bacteria. $\text{C}_{19}\text{H}_{17}\text{N}_5\text{O}_7\text{S}_3$ 	Xyrofur -1	INTAS Pharmaceuticals Ltd.
Enrofloxacin	<ul style="list-style-type: none"> Fluroquinone antibiotic. Broad spectrum antibiotic active against both gram positive and gram negative bacteria. $\text{C}_{19}\text{H}_{22}\text{FN}_3\text{O}_3$ 	Floxidin	Intervet India Pvt. Ltd.
Oxytetracycline	<ul style="list-style-type: none"> Broad spectrum antibiotic active against both gram positive and gram negative bacteria. $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_9$ 	Steclin-LA	Zydus Animal Health

2.3 Mulberry Variety

The mulberry leaves that were utilized as feed for silkworm in the present study were harvested from the established mulberry farm (Germplasm) maintained by the Division of Mulberry Genetics and Breeding, CoTS, SKUAST-K Mirgund. The popular mulberry variety viz, Gosheorami was utilized as feed for all the silkworm larval instars during entire period of the study.

2.4 Haemolymph Collection

Haemolymph was obtained by puncturing the abdominal legs with sanitized needle/edge. The haemolymph consequently drained and was collected in pre-cooled tubes containing crystals of phenyl thiourea at 1 mg / sample (Fig. 1b). Phenyl thiourea was used to evade the action of prophenol oxidase followed by melanization of the haemolymph samples [19]. The samples were kept at -20°C till further use. Further to study the total haemocyte count (THC), the haemolymph was drawn into Thoma white blood cell pipette upto 0.5 mark and was diluted upto 11 mark with a diluting fluid (sodium acetate 3.0 g and distilled water 100 ml). The number of circulating haemocytes per cubic millimetre (mm^3) in Neubauer ruling haemocytometer (Fig. 1c) was calculated using the following formula adopted by [20]:

$$\frac{(\text{Haemocytes in five } 1 \text{ mm}^2 \text{ squares} \times \text{Dilution} \times \text{depth factor of the chamber})}{\text{Number of squares counted}}$$

Where,

$$\begin{aligned} \text{Dilution} &= 20 \text{ times} \\ \text{Depth factor of the chamber} &= 10 \text{ (constant)} \\ \text{Number of squares counted} &= 05 \end{aligned}$$

2.5 Experimental Groups

The worms in the C_1 batch received the mulberry leaves sprayed with 0.05% of antibiotic solution. The larvae in the C_2 batch received the leaves smeared with 0.10% of antibiotic solution and the larvae in C_3 batch received the mulberry leaves sprayed with 0.15% of an antibiotic solution. However, the C_4 batch served as a control as the larvae received the mulberry leaves sprayed with only distilled water. The treatment combination details are given here under in Table 2.

2.6 Statistical Analysis

The design of the experiment followed was completely randomized design (CRD). Data was analysed by two way analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

3.1 Effects of Antibiotic on the Total Haemocyte Count in silkworm *B. mori* L.

The circulating cells in the insect haemolymph are called haemocytes. These are morphologically distinct cell types. These circulating insect haemocytes play important roles in the insect immune system and fight against pathogens. Antibiotics have therapeutic effects on silkworms infected with the pathogens by inhibiting the proliferation of pathogens in the body fluid. In the present study highest THC value of 9157.33 mm/cm^3 was recorded in treatment A_1 (Ceftiofur sodium) treated batch at concentration C_3 0.15%. An increment in the total haemocyte count was observed in other treatments as well and the cell count increased with an increase in concentration of antibiotic

Table 2. Treatment combination details of the antibiotics used in the study

Treatment	Antibiotics	Concentration (%)
A_1	Ceftiofur Sodium	$C_1= 0.05$
		$C_2=0.10$
		$C_3=0.15$
		$C_4=0.00$
A_2	Enrofloxacin	$C_1= 0.05$
		$C_2=0.10$
		$C_3=0.15$
		$C_4=0.00$
A_3	Oxytetracycline	$C_1= 0.05$
		$C_2=0.10$
		$C_3=0.15$
		$C_4=0.00$

dosage (Table 3). It has also been reported that biochemical parameters could be elevated by antibiotic supplementation in healthy silkworm larvae [21]. In case of concentration means the results revealed that the treatments were statistically significant at different concentration levels. The highest THC value of 9009.00 cells/mm³ was recorded at concentration C₃ (0.15%), followed by concentrations C₂ (0.10%) and C₁ (0.05%) with total haemocyte cell count of 8599.33 and 8361.22 cells/mm³ respectively. However, the lowest THC of 8360.11 cells/mm³ was recorded at concentration C₄ (0.0%) where only distilled water was given as treatment (Table 3). The total haemocyte count was increased in the haemolymph of silkworm infected with *Beauveria bassiana* when supplemented with a systemic fungicide (Bayleton 25% WP) [22]. Total haemocyte count and specific haemocyte count indicates the tolerance status of the insect to diseases [23]. Physiology also has a part in insect immune response system. The number of haemocytes is greatly affected by age, stage and physiological status of the insect [24]. The increase in the haemocyte count may be attributed to the antibiotics as it improves feed consumption and growth by stimulating metabolic processes within the silkworm as well as reduce the occurrence of diseases. It has been revealed that feeding efficiency of the larvae increases the haemocyte count in insects [25].

3.2 Effects of Antibiotic on the Growth and Development of *B. mori* L.

Larval weight is one of the important parameter which determines not only the health of the larvae, but also the quality of the cocoons spun [26]. Present study revealed that there was significant variation in larval weight in different antibiotic treatments. With respect to treatment A₁ (Ceftiofur sodium) significant differences among the different treatment combinations were observed. The highest larval weight of 43.60g was recorded at concentration C₃ (0.15%). It has been revealed that in case of treatment A₂ (Enrofloxacin) the treatments were statistically significant at different concentration levels. Highest larval weight of 40.38g was recorded at concentration C₃ (0.15%) (Table 4). The results obtained from treatment A₃ (Oxytetracycline) revealed that there were significant differences among the treatment combinations. It was observed that all the treatments have recorded significant increase in larval weight as compared to control. The highest larval weight of 41.87g

was recorded at concentration C₃ (0.15%). It has been reported that the oral supplementation of the antibiotics to 4th instar larvae resulted in significant increase in the larval weight [27].

In the current study, a decreasing trend in larval duration was observed among all the treated groups, with respect to treatment A₁ (Ceftiofur sodium), significant differences were observed among the different treatment combinations (Table 4). The lowest 5th age larval duration of 172.80 hrs was recorded at concentration C₃ (0.15%), in case of treatment A₂ (Enrofloxacin) minimum 5th age larval duration of 193.68 hrs was recorded at concentration C₃ and with respect to treatment A₃ (Oxytetracycline), the minimum 5th age larval duration of 191.76 hrs was recorded at concentration C₃ (0.15%).

The results from treatment A₁ (Ceftiofur sodium) revealed that the lowest larval duration of 650.76 hrs was recorded at concentration C₃ (0.15%), in treatment A₂ (Enrofloxacin) the minimum larval duration of 660.00 hrs was recorded at concentration C₃ (0.15%) and in treatment A₃ (Oxytetracycline), the minimum larval duration of 651.39 hrs was recorded at concentration C₃ (0.15%) (Table 4). These findings are in agreement with results that have reported the larval and pupal duration of silkworm *B. mori* are significantly decreased due to different antibiotic application on mulberry leaves [28]. The present findings corroborate with the findings that reported the larval duration in silkworm has significantly decreased as the minimum larval period was recorded at 5% dosage of amoxicillin [29].

3.3 Effects of Antibiotic on the Cocoon Characters of *B. mori* L.

Cocoon weight, shell ratio and filament length are highly heritable traits and are significantly important as these determine the quality, quantity and efficiency of the reeling process. All the treatments showed significant increase in cocoon weight than control and the better performance were recorded with the increase of antibiotic concentrations (Table 5). Treatment A₁ (Ceftiofur sodium) resulted that the highest cocoon weight of 1.87g was obtained at concentration C₃ (0.15%), in case of treatment A₂ (Enrofloxacin) the highest cocoon weight of 1.79g was obtained at concentration C₃ (0.15%) and with respect to treatment A₃ (Oxytetracycline), the results revealed that the highest cocoon weight of 1.86g

was obtained at concentration C₃ (0.15%). The results of the present finding were in conformity with the findings that have reported significant increase in cocoon parameters (length, width and weight respectively) with supplementation of

Amoxicillin (5% concentration) [28]. Significant increase in cocoon weight, have been recorded while using Chloramphenicol as feed supplementation to silkworm *B. mori* [3].

Table 3. Influence of antibiotics at different concentration levels on total haemocyte count (cells/mm³) in silkworm *B. mori* L.

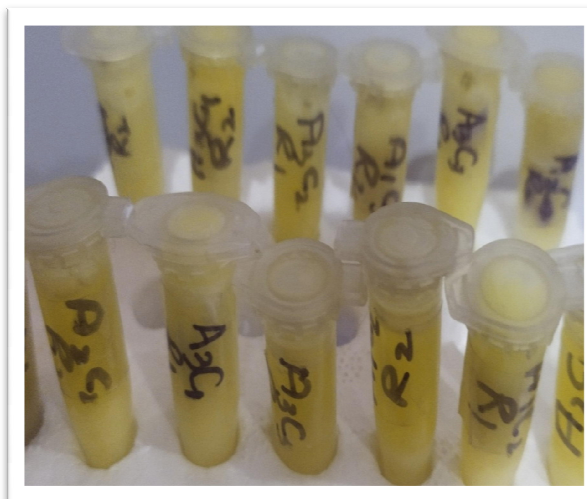
Antibiotics	Concentration (%)	C ₁ : 0.05	C ₂ : 0.10	C ₃ : 0.15	C ₄ : 0.00	Antibiotic mean
A ₁ (Ceftiofur sodium)		8666.66	8990.00	9157.33	8023.33	8709.33 ^a
A ₂ (Enrofloxacin)		8174.00	8192.00	8875.66	8049.00	8322.66 ^a
A ₃ (Oxytetracycline)		8243.00	8616.00	8994.00	8114.00	8491.75 ^a
Concentration Mean		8361.22 ^c	8599.33 ^b	9009.00 ^a	8062.11 ^d	

C.D (p≤0.05)

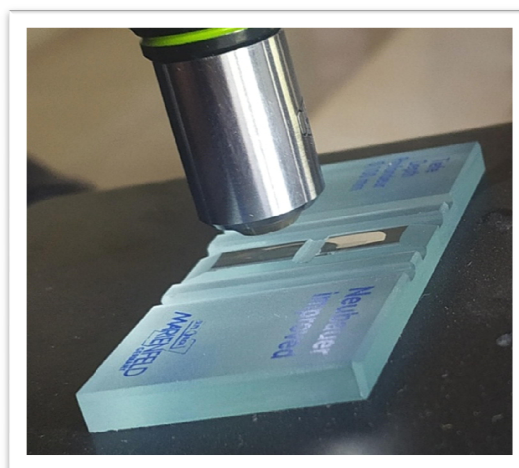
Antibiotics (A): 74.393; Concentration (C): 85.902; A × C: 148.787; (a,b,c,d): Ranking given to the different means based on standard competition ranking; (C.D): Critical Difference



(a) Rearing of silkworms



(b) Haemolymph collection



(c) Haemocyte count

Fig. 1. Silkworm rearing and haemolymph collection from silkworm

Table 4. Influence of antibiotics at different concentration levels on larval weight, 5th age larval duration and total larval duration (hrs) in silkworm *B. mori*

Antibiotics	Concentration (%)	Larval weight (g)	5 th age larval duration (hrs)	Total larval duration (hrs)
A ₁ (Ceftiofur sodium)	C ₁ (0.05)	40.93	196.08	653.44
	C ₂ (0.10)	41.72	197.28	652.28
	C ₃ (0.15)	43.94	193.68	650.76
	C ₄ (control)	38.87	196.56	678.02
Mean		41.38	190.02	658.62
A ₂ (Enrofloxacin)	C ₁ (0.05)	39.58	195.60	677.28
	C ₂ (0.10)	40.33	194.64	673.65
	C ₃ (0.15)	40.38	191.76	660.00
	C ₄ (control)	37.63	196.80	679.73
Mean		39.48	195.90	672.67
A ₃ (Oxytetracycline)	C ₁ (0.05)	39.18	195.60	672.72
	C ₂ (0.10)	39.78	194.64	664.89
	C ₃ (0.15)	41.87	191.76	651.39
	C ₄ (control)	38.72	196.80	679.56
Mean		39.89	194.70	667.14
C.D (p<0.05) (Antibiotic Mean)		0.626	1.17	0.490

(C.D= Critical difference)
(N.S= Non Significant)

Cocoon shell weight is an important character in determining the silk weight. The results from the present study revealed that among all the treated groups, the highest shell weight of 0.39g was recorded in treatment A₁ (Ceftiofur sodium) at concentration C₃ (0.15%). The improvement in shell weight was also recorded in the other treatments. The findings of present study are in agreement with the findings that have reported administration of antibiotic Norfloxacin results in significant increase in the shell weight [30]. While accessing the oral antibiotic administration to silkworm with different concentrations there is a significant increase in the single shell weight and shell ratio [1]. The results of present study showed that antibiotic supplementation have shown significant effect towards the shell ratio in all the treatments. The present findings are well supported by the findings that have reported that the impact of antibiotic administration Norfloxacin have shown improved results in shell ratio percentage [30].

Higher values of effective rate of rearing are indicative of higher silk productivity and a good cocoon crop. With respect to treatment A₁ (Ceftiofur sodium), significant differences were observed among the different treatment combinations (Table 5). The highest cocoon yield of 8740.0 was recorded at concentration C₃ (0.15%), among the different treatment combinations A₂ (Enrofloxacin) recorded highest

cocoon yield by number 8164.0 at concentration C₃ (0.15%) and in treatment A₃ (Oxytetracycline), the highest cocoon yield by number 8674.3 was recorded at concentration C₃ (0.15%).

However, with respect to treatment A₁ (Ceftiofur sodium), significant differences were observed among the different treatment combinations. The highest cocoon yield by weight (16.67 kg) was recorded at concentration C₃ (0.15%), the different treatment combinations A₂ (Enrofloxacin) recorded highest cocoon yield by weight (14.32 kg) at concentration C₃ (0.15%) and in treatment A₃ (Oxytetracycline). Wherein, the highest the cocoon yield by weight (16.02 kg) was recorded at concentration C₃ (0.15%) (Table 5). In support of the findings of present study similar results have been obtained with the oral administration of chloramphenicol that acts as a growth promoter and significantly improved the cocoon yield by weight [31]. Similar types of result have been reported that are in conformity with the results of present study [32].

3.4 Effects of Antibiotic on the Survival Rate and Raw Silk Percentage of *B. mori* L.

The present results revealed that treatment A₁ (Ceftiofur sodium) at concentration C₃ (0.15%) recorded maximum survival rate of 91.20%, even in the other treatments there was significant

increase in the survival rate as compared to control (Table 6). Taking all treatments into consideration it was found that supplement antibiotics with mulberry leaves at different concentrations has recorded minimum mortality rate as oral feeding of antibiotics along with mulberry leaves have significantly reduced the incidence of diseases. Hence, present findings

are in conformity with the reports that revealed 0.12% concentrations of antibiotics viz, ofloxacin, acyclovir and griesovin along with feed were found to be most effective not only in increasing the survival rate but also preventing the occurrence of grasserie, flacherie and muscardine [33]. It also been reported that there is decrease in grasserie incidence due to

Table 5. Influence of antibiotics at different concentration levels on cocoon weight, shell weight, shell ratio and cocoon yield in silkworm *B. mori*

Antibiotics	Concentration (%)	Single cocoon weight (g)	Single shell weight (g)	Shell ratio (%)	ERR by number	ERR by weight (kg)
A ₁ (Ceftiofursodium)	C ₁ (0.05)	1.78	0.31	19.67	8511.0	15.26
	C ₂ (0.10)	1.82	0.36	20.51	8605.3	15.57
	C ₃ (0.15)	1.87	0.39	21.27	8740.0	16.67
	C ₄ (control)	1.71	0.31	18.63	7920.0	13.37
	Mean		1.80	0.35	20.02	8444.1
A ₂ (Enrofloxacin)	C ₁ (0.05)	1.74	0.33	18.28	7935.0	13.94
	C ₂ (0.10)	1.77	0.33	18.65	7963.0	14.01
	C ₃ (0.15)	1.79	0.33	19.16	8164.0	14.32
	C ₄ (control)	1.69	0.31	18.25	7951.3	13.65
	Mean		1.75	0.33	18.59	8003.3
A ₃ (Oxytetracycline)	C ₁ (0.05)	1.80	0.35	19.77	8040.0	14.69
	C ₂ (0.10)	1.83	0.36	19.96	8196.3	15.03
	C ₃ (0.15)	1.86	0.37	20.38	8674.3	16.02
	C ₄ (control)	1.75	0.32	18.54	7945.0	13.68
	Mean		1.81	0.35	19.67	8214.0
C.D (p≤0.05) (Antibiotic Mean)		1.010	0.008	NS	88.42	0.200

(C.D= Critical difference)
(N.S= Non Significant)

Table 6. Influence of antibiotics at different concentration levels on survival rate and raw silk percentage in silkworm *B. mori*

Antibiotics	Concentration (%)	Survival rate (%)	Raw silk (%)
A ₁ (Ceftiofur sodium)	C ₁ (0.05)	85.33	15.32
	C ₂ (0.10)	91.00	15.81
	C ₃ (0.15)	91.20	15.85
	C ₄ (control)	82.00	15.01
	Mean		87.38
A ₂ (Enrofloxacin)	C ₁ (0.05)	82.00	15.06
	C ₂ (0.10)	83.00	15.21
	C ₃ (0.15)	84.00	15.69
	C ₄ (control)	80.60	14.63
	Mean		82.40
A ₃ (Oxytetracycline)	C ₁ (0.05)	86.33	15.15
	C ₂ (0.10)	85.00	15.49
	C ₃ (0.15)	86.37	15.75
	C ₄ (control)	82.10	14.59
	Mean		84.95
C.D (p≤0.05) (Antibiotic Mean)		1.16	0.084

C.D= Critical Difference

antibiotics like enterocycline, penicillin, erythromycin and streptomycin [1]. Thus from these studies the increase in survival rate has been attributed to antibiotics, as antibiotics play a significant role in conferring immunity to silkworms thereby increasing the effective rate of rearing.

Treatment A₁ (Ceftiofur sodium) resulted the highest raw silk of 15.85% at concentration C₃ (0.15%), in case of treatment A₂ (Enrofloxacin) highest raw silk of 15.69% was recorded at concentration C₃ (0.15%) and with respect to treatment A₃ (Oxytetracycline) the highest raw silk of 15.75% was recorded at concentration C₃ (0.15%). Moreover, the raw silk percentage evaluated was relatively higher in all the treatments as compared to control (Table 6). These results are in line with the observations that have reported that antibiotic Norfloxacin and its increase in concentration significantly improved the raw silk percentage [30]. In light of the present findings it has also been reported that chloromycetin supplementation individually and in combination with glycine yielded about 6% more silk than the control [34].

4. CONCLUSION

The antibiotic treatment (Ceftiofur sodium, Enrofloxacin, Oxytetracycline) has significantly improved the raw silk percentage and has been found to be effective in reducing the mortality of silkworms. Total haemocyte count was significantly increased in the Ceftiofur sodium fortified batches and this may be due to higher feed conversion efficiency or physiological action of the antibiotics. Also the oral administration of antibiotics along with mulberry leaves enhanced silk production. So, this antibiotic fortification can be further exploited for higher raw silk production.

ACKNOWLEDGEMENTS

The first author sincerely acknowledges the support provided by Associate Dean, CoTS, SKUAST-K, Mirgund and Head Division of Silk Product Sciences, CoTS, SKUAST-K, Mirgund for providing the required facilities in pursuit of this research program. Thanks are also due to Division of Mulberry Genetics and Breeding, CoTS, SKUAST-K Mirgund for providing mulberry leaves which were utilized as feed to the silkworm larvae during entire period of the investigation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Radha NV, Natarajan T, Muthukrishnan TS, Oblisami G. Effect of antibiotics on mulberry silkworm. Proceedings in Sericulture Symposium. Coimbatore. 1980;173-177.
2. Sheebha A, Quraiza Fatima, Thilsath M, Das Manohar, Sam S, Bai Ramani M. Effect of prophylactic antibiotic treatment on the growth and cocoon characteristics of *Bombyx mori* L., Journal of Basic and Applied Biology. 2008;2(1): 19-22.
3. Tayade DS, Jawale MD, Unchegaonkar PK. Effect of antibiotic on the growth of silkworm *Bombyx mori* L., Indian Journal of Sericulture. 1988;27(2):69-72.
4. Shyamala MB, Bhat JV. On the relationship between panthothenate levels and the growth response of the silkworm to chloromycetin. International Journal of Current Research. 1961;20:333-335.
5. Iyengar MNS. Silkworm larvae as a surrogate animal model for infections pathogenic to humans and for drug screening. Indian Silk. 2008;17.
6. Hossain MS, Hamamoto H, Matsumoto Y, Razanajatovo IM, Larranaga J, Kaito C, et al. Use of silkworm larvae to study pathogenic bacterial toxins. J. Biochem. 2006;140:439-444.
7. Kurokawa K, Hamamoto H, Matsuo M, Nishida S, Yamane N, Lee BL, et al. Evaluation of target specificity of antibacterial agents using *Staphylococcus aureus* *ddlA* mutants and D-cycloserine in a silkworm infection model. Antimicrob. Agents Chemother. 2009;53: 4025-4027.
8. Miyazaki S, Matsumoto Y, Sekimizu K, Kaito C. Evaluation of *Staphylococcus aureus* virulence factors using a silkworm model. FEMS Microbiol. Lett. 2012;326: 116-124.
9. Nishida S, Ono Y, Sekimizu K. Lactic acid bacteria activating innate immunity improve survival in bacterial infection model of silkworm. Drug Discov. Ther. 2016;10:49-56.
10. Hamamoto H, Urai M, Ishii K, Yasukawa J, Paudel A, Murai M, et al. Lysocin E is a new antibiotic that targets menaquinone in

- the bacterial membrane. *Nat. Chem. Biol.* 2015;11:127–133.
11. Uchida R, Iwatsuki M, Kim YP, Ohte S, Omura S, Tomoda H. Nosokomycins, new antibiotics discovered in an in vivo-mimic infection model using silkworm larvae. I: fermentation, isolation and biological properties. *Journal of Antibiotics.* 2010;63:151–155.
 12. Nakamura I, Kanasaki R, Yoshikawa K, Furukawa S, Fujie A, Hamamoto H, et al. Discovery of a new antifungal agent ASP2397 using a silkworm model of *Aspergillus fumigatus* infection. *J. Antibiot.* 2017a;70:41–44.
 13. Jones JC. Pathways and pitfall in the classification and study of insect haemocyte. *Insect Hemocyte: Development Forms, Function and Techniques.* [Ed. A.P. Gupta], Cambridge University Press. 1979;279-300.
 14. Ashhurst DE. Haemocytes and connective tissue: A critical assessment. *Insect Hemocytes.* [Ed. A.P. Gupta]. Cambridge University press, Cambridge. 1979;319-330.
 15. Wigglesworth VB. Insect blood cells. *Annual Review of Entomology.* 1959;4: 1- 16.
 16. Hou Y, Zou Y, Wang F, Gong J, Zhong X, Xia Q, et al. Comparative analysis of proteome maps of silkworm haemolymph during different developmental stages. *Proteome Science.* 2010;8:45.
 17. Pasquale TR, Tan JS. Non-antimicrobial effect of antimicrobial agents. *Clinical Infectious Diseases.* 2005;40:127–135.
 18. Krishnaswamy S. New technology of silkworm rearing, *Bulletin, CSR and TI, Mysore, India.* 1978;2:1-24.
 19. Takeda H, Kawakuchi Y, Ohsika T, Maekawa H, Tsuchida K. Impaired yolk protein uptake by oocytes of a *Bombyx mori* mutant. *Insect Biochemistry and Molecular Biology.* 1996;26:607-616.
 20. Ganie NA, Kamili AS, Baqual MF, Sharma RK, Dar KA, Bashir M. Studies on the total and differential haemocyte count in some breeds of silkworm, *Bombyx mori* L. *International Journal of Advanced Biological Research.* 2015;5(1):58-61.
 21. Savithri G, Murali Mohan P. Pathogenicity of the bacterium *Bacillus coagulans* in silkworm *Bombyx mori* L. *Indian Journal of Sericulture.* 2003; 42(1):4-8.
 22. Mallikarjuna M, Balavenkatasubbaiah M, Nataraju B, Thiagarajan V. Effect of systemic fungicide on the total haemocyte count and haemolymph biochemical changes in silkworm, *Bombyx mori* L. infected with *Beauveria bassiana*. *International Journal of industrial Entomology.* 2002;5(2):189-194.
 23. Balavenkatasubbaiah M, Nataraju B, Thiagarajan V, Datta RK. Haemocyte counts in different breeds of silkworm, *Bombyx mori* L. and their changes during the progressive infection of BmNPV. *Indian Journal of Sericulture.* 2001;40(2): 158-162.
 24. Kerenhap W, Balasingh J, Thiagarajan V, Kumar V. Studies on the influence of feeding frequency on the total and differential haemocyte count in *Bombyx mori* L. *Indian Journal of Sericulture.* 2005;44(1):113-117.
 25. Paul DC, Subba Rao G, DEB DC. Impact of dietary moisture on nutritional indices and growth of *Bombyx mori* and concomitant larval duration. *Journal of Insect Physiology.* 1992;38(3): 229.
 26. Ito T. *Silkworm nutrition in the silkworm an important laboratory tool.* Tazima Y. (ed), Ko Ltd., Tokyo. 1978;121-157.
 27. Ahmed CAA, Chandrakala MV, Maribashetty VG, Raghuraman R. Impact of azithromycin and ciprofloxacin administration on nutritional efficiency in a multivoltine race of silkworm, *Bombyx mori*. *Journal of Entomology.* 2001; 26(1):1- 9.
 28. Thilagavathi G, Selvisabhanayakam, Ganesh Prabhu. Studies on the impact of amoxicillin on growth rate and economic parameters of silkworm *Bombyx mori* (L). *International Journal of Current Research.* 2013;10:3232-3237.
 29. Mahdi HAS, Rokonzaman MD, Aftab Uddin, Kamrul AMD. The effects of amoxicillin, oxytetracyclin and doxycyclin on the growth and development of silkworm, *Bombyx mori* L. *Journal of Entomology and Zoology Studies.* 2017; 5(6):1316-1321.
 30. Rahmathulla VK, Nayak Padmanav, Vindya GS, Himantharaj MT, Rajan RK. Effect of antibiotic (Norfloxacin) administration on commercial characters of new bivoltine and cross breed hybrid

- silkworm (*Bombyx mori*). International Journal of Industrial Entomology. 2003; 7(2):191-195.
31. Krishnaswami SS, Kamala singh, Raghuraman R, Geethadevi RG, Benchamin KV. Chloramphenicol as a growth Promoter in silkworm *Bombyx mori* L. Proceedings of sericulture symposium and seminar. TNAU Coimbatore. 1981; 1-28.
32. Baig M, Nataraju B, Samson MV, Studies on the effect of antibiotics on rearing performance and loss due to diseases in silkworm *Bombyx mori* L., Indian Journal of Sericulture. 1990;29(1): 54-58.
33. Amit srivastava, Venkatesh kumar. Efficacy of three antibiotics on reduction of mortality rate in mulberry silkworm (*Bombyx mori* L.) in the monsoon season of lucknow. Trends in Biosciences. 2009;2(2):33-35.
34. Sharada K, Shroff LT, Shyamala MB, Iyengar KS, Bhat JV. Large-scale experiments on the effect of chloromycetin and glycine in the nutrition of silkworm *Bombyx mori* L, Indian Institute of Science, Bangalore. 1956;3.

© 2020 Rafiq et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/66480>