Effect of Antibiotics Administration on Food Ingesta, Digesta, Approximate Digestibility and Reference Ratio of Mulberry Silkworm, *Bombyx mori* L.

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Abstract- Silkworm *Bombyx mori* (L.) is one of the most unique holometabolous insects known for the production of the most versatile silk protein. The consumption rate pattern varies significantly from the first to the last day of the larval period when silkworm larvae are treated with different antibiotics. The current experiment was conducted to determine the exact quantum of food required by silkworm larvae in different instars, such as third, fourth, and fifth instars. For the quantification of food, various parameters, including ingestion, digestion, approximate digestibility, and the reference ratio of the larvae for different instars, were studied. The selection of the silkworm bivoltine hybrid FC1 × FC2 was made based on its practical demand in most of the bivoltine sericultural areas of the country. The maximum food consumption indices were observed in mulberry leaves reared on silkworms treated with ceftiofur sodium (0.10%), like food ingesta (283.01 g/20 larvae), food digesta (242.96 g/20 larvae), approximate digestibility (85.85%), and reference ratio (7.06). Therefore, the current experiment helps determine the exact quantity of feed required by silkworm larvae at different instars and the relationship between ingestion and digestion ratios.

Key words : Food consumption, ingesta, digesta, approximate digestibility and reference ratio

INTRODUCTION

The silk originates in the spittle of an insect, and is a natural fibrous substance obtained from pupal nests or cocoons spun by larvae known as silkworms. It consists of two main proteins, *viz*., sericin and fibroin, being the structural center of the silk. Silk is considered the most elegant textile in the world due to its unparalleled grandeur, natural sheen, and unique affinity for dyes. It has high absorbency, is lightweight, offers a soft touch, and boasts high durability. For these reasons, it is often referred to as the "Queen of Textiles" (Mala et al., 2017). Sericulture is an agro-based rural industry that plays an important role in the transformation of the Indian rural economy since it assures regular employment and periodic returns all year, improving the socio-economic status of farmers. With the development of new technologies in mulberry cultivation and silkworm rearing, sericulture has now emerged as a sustainable, indispensable profession and cash crop for the rural folk of the country (Gurjar *et al*., 2018). The antibiotics are highly specific, and the effect also varies from one silkworm breed to another. The beneficial action of the antibiotics has been attributed to their ability to condition the composition of intestinal flora, their potential role as possible growth factors, their biological efficiency in increased turnover of feed into body weight, and their potential disease control activity (Goldberg, 1959). The nutritional performance of the mulberry silkworm is evident in the digestion and assimilation of the nutritional materials present in mulberry leaves. Nutritional indices help to determine the quantity of leaf required to produce the silk. It also helps to study the amount of food consumed and digested, as the larval resource directly affects its body structure. It is glaring that ingesta, digesta, and excreta parameters of larvae enlarge at some point of the fifth instar development, and at once affect the larval weight, cocoon weight, and shell weight that are the

crucial aspects measured for the productivity of silkworm rearing (Horie and Inokuchi, 1978). The amount of food consumed and the quantity digested by the silkworms have direct effects on their performance, mating success, and reproduction. Deficiency of certain nutrients or a nutritionally imbalanced diet affects the digestibility and metabolic activity of larvae. The conversion rate of ingested food by the silkworm, so that we can analyze the distribution rate of mulberry intake in silkworm for various factors like growth rate, silk production, and faecal matter. The consumption indices were computed on a fresh weight basis by the gravimetric method (Waldbauer, 1968).

MATERIAL AND METHODS

The present study titled "Effect of Antibiotics Administration on the Growth and Development of Silkworm, *Bombyx mori* L." was conducted at the Agricultural Research Station in Hagari, Ballari district, under the University of Agricultural Sciences, Raichur, during the 2023-24 period. Standard stock solutions of various concentrations of six antibiotics were prepared: Ceftiofur Sodium, enrofloxacin, oxytetracycline, chloramphenicol, streptomycin, and ampicillin. Two concentrations were created: 0.05% and 0.10%. The 0.05% concentration was prepared by dissolving 0.05 g of the antibiotic in 100 ml of distilled water, while the 0.10% concentration was prepared by dissolving 0.10 g of the antibiotic in 100 ml of distilled water. Fresh mulberry leaves were dipped in each antibiotic treatment and allowed to air-dry for about ten minutes before being fed to the silkworm larvae. The antibiotics were administered alongside mulberry leaves only once during the third, fourth, and fifth instars of the larvae.

The observations taken are given below

Food ingesta (g)

Ingesta (g) = Weight of fresh leaves offered to larvae (g) – Weight of fresh remnants (g)

Food digesta (g)

Digesta (g) = Weight of fresh food ingested (g) – Weight of fresh excreta produced (g)

Approximate digestibility (%)

$$AD\ (\%) = \frac{\text{Weight of food ingested (g)} - \text{Weight of faeces (g)}}{\text{Weight of food ingested (g)}} \times 100$$

Reference ratio (RR)

$$RR = \frac{\text{Ingesta (g)}}{\text{Faecal matter (g)}}$$

RESULTS AND DISCUSSION

Two antibiotic concentrations were tested: 0.05% and 0.10%. The results indicated that various food consumption indices, such as food ingesta, digesta, approximate digestibility, and reference ratio of mulberry silkworms, were significantly increased at the 0.10% antibiotic concentration. At this concentration (0.10%), food consumption indices for silkworms in the third, fourth, and fifth instars, when fed mulberry leaves supplemented with 0.10% ceftifour sodium, recorded the highest values.

Specifically, the food ingesta was 22.09 g/20 larvae, 48.38 g/20 larvae, and 283.01 g/20 larvae; the food digesta was 17.46 g/20 larvae, 40.17 g/20 larvae, and 242.96 g/20 larvae; approximate digestibility percentages were 79.04%, 83.03%, and 85.85%; and the reference ratios were 4.77, 5.89, and 7.06 when compared to the control group.

The gut microbiome, a complex system essential for digestion and nutrient assimilation, is profoundly affected by antibiotics. The increased food intake directly impacts the synthesis of total DNA and RNA, which are crucial for the cellular processes involved in silk production. Antibiotics may selectively alter the gut microbiota, potentially by eliminating intestinal pathogens, and thus improve larval health. Moreover, the increase in food intake across different treatments can be attributed to the activation of biochemical reactions catalyzed by enzymes in the silkworm's midgut. These findings align with Moustafa and Soliman (2019), who reported that silkworms

supplemented with a combination of probiotics Lactobacillus rhamnosus and Bifidobacterium bifidum at a six percent concentration yielded the highest food ingesta. The rate of digestion in silkworms increases with advancing instars, reaching about 75% in the fifth instar. Furthermore, antibiotics may directly influence digestive enzyme activity, stimulate appetite, and promote faster digestion, thereby enhancing the digestive physiology of the silkworm and benefiting its overall health.

These results are consistent with Kumar et al. (2019), who found that the supplementation of mulberry leaves with Spirulina at 300 ppm resulted in the highest food digesta at 1.195 g. The approximate digestibility in silkworms measures the proportion of ingested food that is digested and absorbed. These findings corroborate the results of Mahdi et al. (2017), which reported the highest food digestibility rate of 92.65% at a five percent dose of amoxicillin.

Higher reference ratio values indicate a greater rate of digestion and food absorption, supporting the findings of Sharma et al. (2022), who observed the highest reference ratio of 2.486 when silkworm larvae were fed protinex (10% enriched) leaves.

Table 1: Effect of antibiotics administration on food ingesta of mulberry silkworm, *Bombyx mori* L.

Treatments	Food ingesta (g/20 larvae)					
	Third instar		Fourth instar		Fifth instar	
	C1 (0.05%)	C2 (0.10%)	C1 (0.05%)	C2 (0.10%)	C1 (0.05%)	C2 (0.10%)
T_1: Ceftiofur sodium	20.24^{a}	22.09^{a}	46.21^{a}	48.38^{a}	267.32^{a}	283.01^{a}
T_2: Enrofloxacin	17.34^{b}	20.41^{b}	41.46^{b}	43.26^{b}	250.74^{b}	253.75^{b}
T_3: Oxytetracycline	13.76^{e}	14.97^{e}	33.56^{e}	35.87^{e}	209.23^{e}	219.17^{e}
T_4: Chloramphenicol	14.89^{d}	15.69^{d}	36.85^{d}	38.36^{d}	220.46^{d}	229.45^{d}
T_5: Streptomycin	12.11^{f}	13.24^{f}	32.47^{f}	34.28^{f}	187.21^{f}	204.81^{f}
T_6: Ampicillin	16.61^{c}	17.98^{c}	40.48^{c}	42.34^{c}	236.76^{c}	242.73^{c}
T_7: Water spray	7.82^{g}	8.01^{g}	26.39^{g}	26.71^{g}	152.27^{g}	152.36^{g}
T_8: Control	7.02^{h}	7.11^{h}	26.02^{g}	26.54^{g}	147.85^{h}	147.87^{h}
S. Em (±)	0.032	0.028	0.043	0.049	0.105	0.137
CD (0.01)	0.135	0.116	0.178	0.202	0.435	0.566
CV (%)	1.530	1.263	1.256	1.395	1.267	1.595

Different letters designate significant difference within column by Duncan's Multiple Range Test at P= 0.01

Table 2: Effect of antibiotics administration on food digesta of mulberry silkworm, *Bombyx mori* L.

Treatments	Food digesta (g/20 larvae)					
	Third instar		Fourth instar		Fifth instar	
	C1 (0.05%)	C2 (0.10%)	C1 (0.05%)	C2 (0.10%)	C1 (0.05%)	C2 (0.10%)
T_1: Ceftiofur sodium	15.72^{a}	17.46^{a}	38.05^{a}	40.17^{a}	227.88^{a}	242.96^{a}
T_2: Enrofloxacin	12.95^{b}	15.89^{b}	33.34^{b}	35.09^{b}	211.61^{b}	214.73^{b}
T_3: Oxytetracycline	9.50^{d}	10.60^{e}	25.60^{e}	27.86^{e}	172.23^{e}	182.17^{e}
T_4: Chloramphenicol	10.57^{c}	11.30^{d}	28.84^{d}	30.33^{d}	182.76^{d}	191.21^{d}
T_5: Streptomycin	8.14^{e}	9.23^{f}	24.55^{f}	26.32^{f}	150.49^{f}	166.92^{f}
T_6: Ampicillin	12.25^{b}	13.49^{c}	32.39^{c}	34.20^{c}	197.76^{c}	203.73^{c}
T_7: Water spray	4.98^{f}	5.20^{g}	19.64^{g}	19.96^{g}	117.27^{g}	117.26^{g}
T_8: Control	4.31^{f}	4.50^{h}	19.30^{g}	19.81^{g}	114.84^{h}	114.86^{h}
S. Em (±)	0.028	0.022	0.039	0.033	0.107	0.113
CD (0.01)	0.118	0.094	0.163	0.139	0.442	0.470
CV (%)	1.571	1.194	1.298	1.080	1.422	1.454

Different letters designate significant difference within column by Duncan's Multiple Range Test at P= 0.01

Table 3: Effect of antibiotics administration on approximate digestibility of mulberry silkworm, *Bombyx mori* L.

Treatments	Approximate digestibility (%)					
	Third instar		Fourth instar		Fifth instar	
	C1 (0.05%)	C2 (0.10%)	C1 (0.05%)	C2 (0.10%)	C1 (0.05%)	C2 (0.10%)
T_1: Ceftiofur sodium	77.668	79.040	82.341	83.030	85.246	85.849

	(61.80)[a]	(62.75)[a]	(65.15)[a]	(65.67)[a]	(67.41)[a]	(67.90)[a]
T_2: Enrofloxacin	74.683 (59.79)[b]	77.854 (61.93)[b]	80.415 (63.73)[b]	81.114 (64.24)[b]	84.394 (66.73)[b]	84.623 (66.91)[b]
T_3: Oxytetracycline	69.041 (56.19)[e]	70.808 (57.30)[e]	76.281 (60.85)[d]	77.669 (61.80)[e]	82.316 (65.13)[d]	83.118 (65.74)[c]
T_4: Chloramphenicol	70.987 (57.41)[d]	72.020 (58.06)[d]	78.263 (62.21)[c]	79.067 (62.77)[d]	82.899 (65.57)[d]	83.334 (65.91)[c]
T_5: Streptomycin	67.217 (55.07)[f]	69.713 (56.61)[f]	75.608 (60.40)[e]	76.779 (61.19)[f]	80.386 (63.71)[e]	81.500 (64.53)[d]
T_6: Ampicillin	73.751 (59.18)[c]	75.028 (60.02)[c]	80.015 (63.45)[b]	80.775 (63.99)[c]	83.528 (66.06)[c]	83.933 (66.37)[c]
T_7: Water spray	63.683 (52.94)[g]	64.919 (53.68)[g]	74.422 (59.62)[f]	74.729 (59.82)[g]	78.015 (62.04)[f]	78.032 (62.05)[e]
T_8: Control	61.396 (51.59)[h]	63.291 (52.71)[h]	74.174 (59.46)[f]	74.642 (59.76)[g]	77.670 (61.80)[g]	77.683 (61.81)[f]
S. Em (±)	0.068	0.061	0.061	0.060	0.072	0.059
CD (0.01)	0.281	0.253	0.252	0.248	0.298	0.243
CV (%)	1.409	1.251	1.195	1.172	1.382	1.120

*Figures in parentheses are arcsine transformed value

Different letters designate significant difference within column by Duncan's Multiple Range Test at P= 0.01

Table 4 : Effect of antibiotics administration on reference ratio of mulberry silkworm, *Bombyx mori* L.

Treatments	Reference ratio					
	Third instar		Fourth instar		Fifth instar	
	C1 (0.05%)	C2 (0.10%)	C1 (0.05%)	C2 (0.10%)	C1 (0.05%)	C2 (0.10%)
T_1: Ceftiofur sodium	4.48[a]	4.77[a]	5.66[a]	5.89[a]	6.77[a]	7.06[a]
T_2: Enrofloxacin	3.95[b]	4.52[b]	5.11[b]	5.29[b]	6.40[b]	6.50[b]
T_3: Oxytetracycline	3.23[d]	3.43[d]	4.22[d]	4.48[c]	5.65[d]	5.92[d]
T_4: Chloramphenicol	3.45[c]	3.57[d]	4.60[c]	4.78[c]	5.84[d]	6.00[d]
T_5: Streptomycin	3.05[e]	3.30[e]	4.10[d]	4.31[d]	5.09[e]	5.40[e]
T_6: Ampicillin	3.81[b]	4.00[c]	5.00[b]	5.20[b]	6.07[c]	6.22[c]
T_7: Water spray	2.75[f]	2.85[f]	3.91[e]	3.96[e]	4.48[f]	4.88[f]
T_8: Control	2.59[f]	2.72[f]	3.87[e]	3.94[e]	4.35[f]	4.86[f]
S. Em (±)	0.011	0.014	0.013	0.017	0.017	0.018
CD (0.01)	0.047	0.061	0.055	0.073	0.072	0.078
CV (%)	1.000	1.263	1.034	1.345	1.226	1.301

Different letters designate significant difference within column by Duncan's Multiple Range Test at P= 0.01

CONCLUSION

The growth of larvae is influenced by various physiological processes, which are crucial for sericulture's economic success. This success relies heavily on the metabolic regulation and molecular components of the silkworm. The study concluded that administration of antibiotics showed better performance in food ingesta, digesta, approximate digestibility, and reference ratio.

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