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Studies on the Impact of Ruminant Microflora on Mulberry Leaf Digestion, Growth and Economic Characteristics of *Bombyx mori* (Linnaeus)

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Abstract: *Bombyx mori* is a commercially important monophagous lepidopteran insect feeding on mulberry leaf. Dietary supplementation increased the economic characteristics of the silkworms. The silkworm feeds mulberry leaves containing cellulose. Cellulose is digested by the enzyme cellulase synthesized by symbiotic microbes such as *Butyrivibrio fibrisolvens* and *Ruminococcus albus* which is likely to improve the energy parameters and cocoon characters of fourth and fifth instar caterpillars.

Keywords: Silkworm, Mulberry leaves, Cellulose digesting microbes, *Butyrivibrio fibrisolvens*, *Ruminococcus albus*

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Introduction

A vast, untapped reservoir of potentially beneficial substances for contemporary medicine exists in insects. Insects make up over 80% of all animal species on Earth. A significant amount of our genetic makeup is shared by all living things on Earth, including the most basic worms. One well-known beneficial lepidopteran insect, the silkworm (*Bombyx mori*), is responsible for producing the silky, sensual fibre that is sometimes referred to as the Queen of textiles (Soumya *et al.*, 2017).

In the past two decades, India has ranked next to China in total raw silk production (Pande *et al.*, 2014). The influence of micronutrients on larval development and cocoon characteristics of silkworms was studied by Shilpashree *et al.* (2015). Mathavan *et al.* (1984) proved that the single-cell algae *Spirulina fugiformis* increased the economic characteristics of silkworms.

Fields *et al.* (1998) reported the role of ruminal carboxymethyl celluloses in the degradation of glucans from cereal grains. Miron

Table 1: Energy parameters of silkworm *B. mori* fed with *B. fibrisolvans*

Energy parameters	IV instar			V instar		
	Control	10 ⁻¹ dilution	10 ⁻³ dilution	Control	10 ⁻¹ dilution	10 ⁻³ dilution
Food consumption (mg)	384.90 ± 0.94	395.90±0.93 (2.85)	385.30±0.93 (0.10)	1110.60± 1.38	122.60 ± 1.40 (-88.92)*	1196.60 ± 1.41 (7.74)
Assimilation (mg)	273.00 ± 0.92	282.70 ± 0.89 (3.55)	278.20 ± 0.89 (1.90)	383.10 ± 1.15	705.20 ± 1.29 (84.06)	549.20 ± 1.25 (43.25)
Tissue growth (mg)	60.00 ± 0.04	76.60 ± 0.04 (27.55)	111.200 ± 0.01 (84.99)	248.50 ± 0.02	695.50 ± 0.01 (179.60)	450.40± 0.30 (81.16)
Approximate digestibility (%)	70.92 ± 0.87	71.40 ± 0.93 (0.67)	72.20 ± 0.92 (1.80)	34.49 ± 0.52	57.52 ± 0.51 (66.78)	45.89 ± 0.35 (33.06)
Tissue growth efficiency (%)	21.97 ± 0.46	27.09 ± 0.05 (23.29)	39.97 ± 0.03 (81.90)	64.86±0.07	98.62±0.21 (52.02)	82.01 ± 0.11 (26.42)
Ecological growth efficiency (%)	15.58 ± 0.03	19.34 ± 0.02 (24.13)	28.86 ± 0.05 (85.23)	22.37± 0.04	56.72 ± 0.07 (153.50)	37.63 ± 0.08 (68.21)
						n = 10

Per cent deviation over control values in parentheses; *t-test values significant at $P \leq 0.05$

et al. (1990) investigated some aspects of the cellobiose effect on bacterial cell surface structures involved in lucerne cell wall utilisation by fresh isolates of rumen bacteria. Bacteria are considered to be the major plant cell wall degrading agents.

The silkworm feeds mulberry leaves containing cellulose. Cellulose is digested by the enzyme cellulase (1-4-B-D-Glucan4-glucanohydrolase) synthesized only by symbiotic microbes. Insect guts provide unique ecosystems for microbial colonization, and particularly gut bacteria may serve their hosts with a variety of useful functions. The degree to which insects for essential activities require gut bacteria varies greatly. In response to changes in the availability of nutrients and environmental factors, the composition as well as the structure of the gut bacteria will alter and have an impact on the production and quality of cocoons (Jang and Kikuchi, 2020).

Supplementing the diet of silkworms with beneficial microbes are likely to improve the energy parameters and cocoon characters of fourth and fifth instar caterpillars. Keeping this in view, the present study was undertaken to elucidate the impact of ruminant microflora on mulberry leaf digestion, growth and economic characters of *Bombyx mori* (Linnaeus).

Materials and Methods

Disease free layerings (DFL's) of *Bombyx mori* (PM xNB₄D₂) were incubated at $27 \pm 1^\circ\text{C}$ and 70 - 80% RH. The present investigation was designed to supply important highly efficient bacteria such as *R. albus* and *B. fibrisolvans* which were isolated from the rumen of goat and used as feed supplements.

Various biochemical tests were adopted for isolation of bacteria. They are catalase test, methyl red, gelatine liquefaction, and nitrate-reduction-tests. The energy parameters of silkworm caterpillars were carried out based on

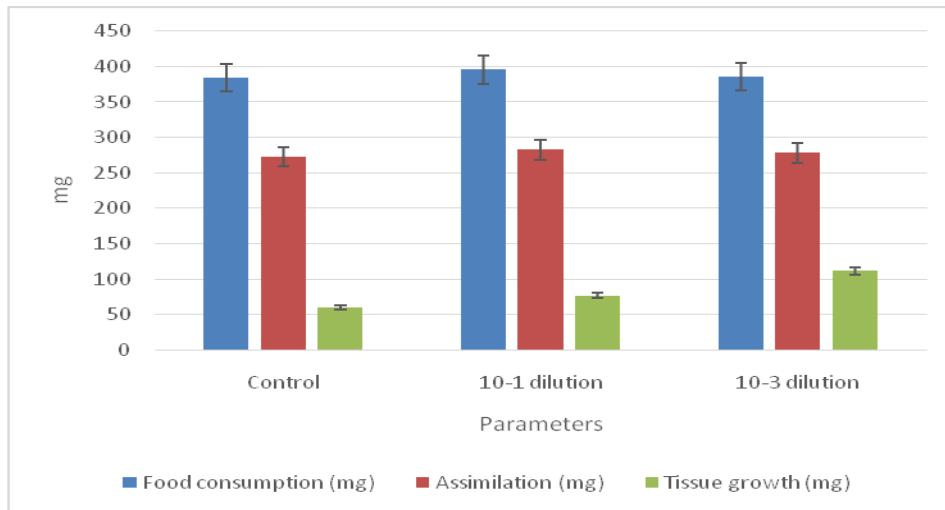


Fig. 1: Energy parameters of silkworm *B. mori* fed with *B. fibrisolvans* (IV Instar).

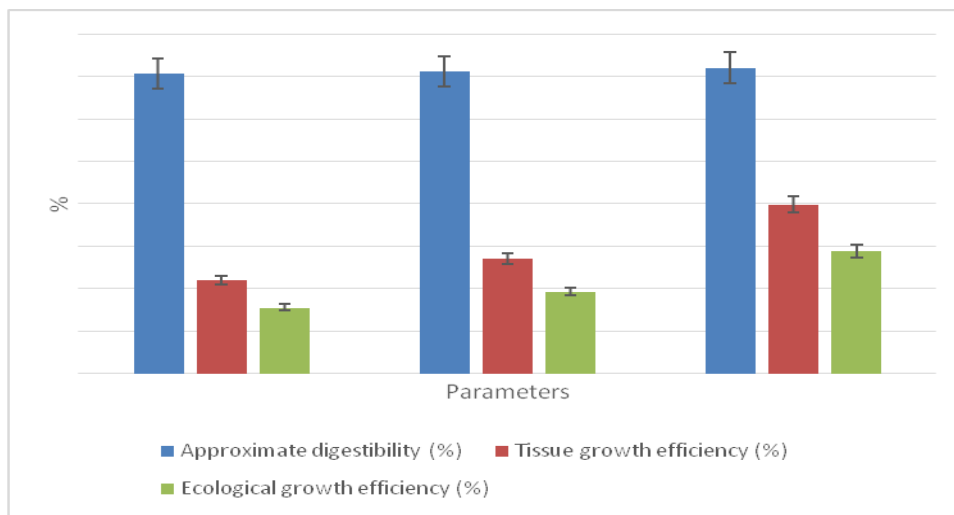


Fig. 2: Energy parameters of silkworm *B. mori* fed with *B. fibrisolvans* (IV Instar).

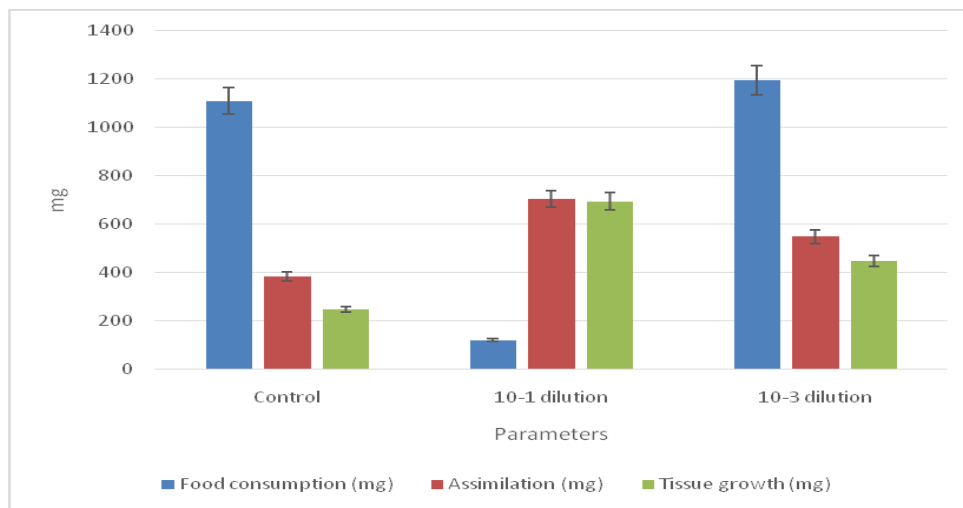


Fig. 3: Energy parameters of silkworm *B. mori* fed with *B. fibrisolvans* (V Instar).

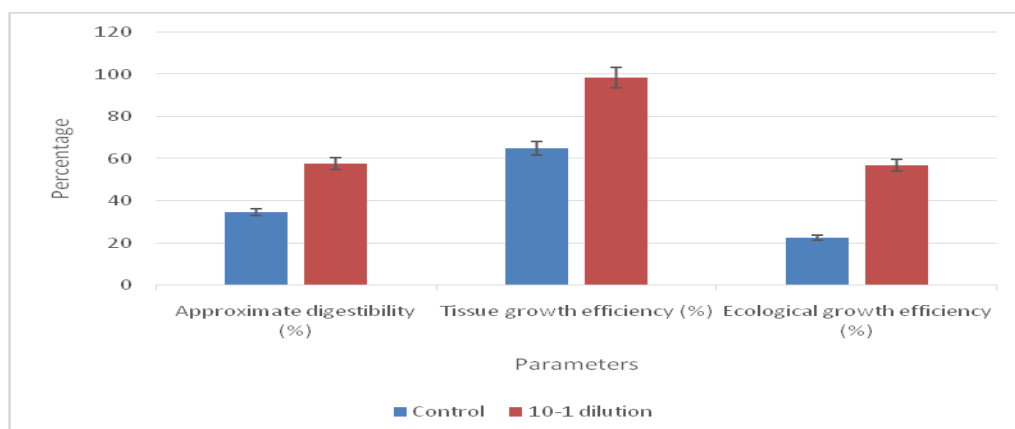


Fig. 4: Energy parameters of silkworm *B. mori* fed with *B. fibrisolvens* (V Instar).

Table 2: Economic characters of *B. mori* fed with *B. fibrisolvens*

Cocoon characters	Control	Bacterial dilution 10 ⁻¹	Bacterial dilution 10 ⁻³
cocoon weight (mg)	1039.80±0.14	1290.80±0.12 (24.09)	1288.50±0.09 (23.87)
Shell weight (mg)	197.20 ± 0.03	253.90 ± 0.02 (28.74)	251.50 ± 0.053 (27.53)
Shell ratio (%)	18.96 ± 0.02	19.67 ± 0.07 (3.74)	19.52 ± 0.06 (2.95)
Pupal weight (mg)	842.60 ± 0.07	1036.90 ± 0.06 (23.04)	1037.00 ± 0.04 (23.06)
			n = 10

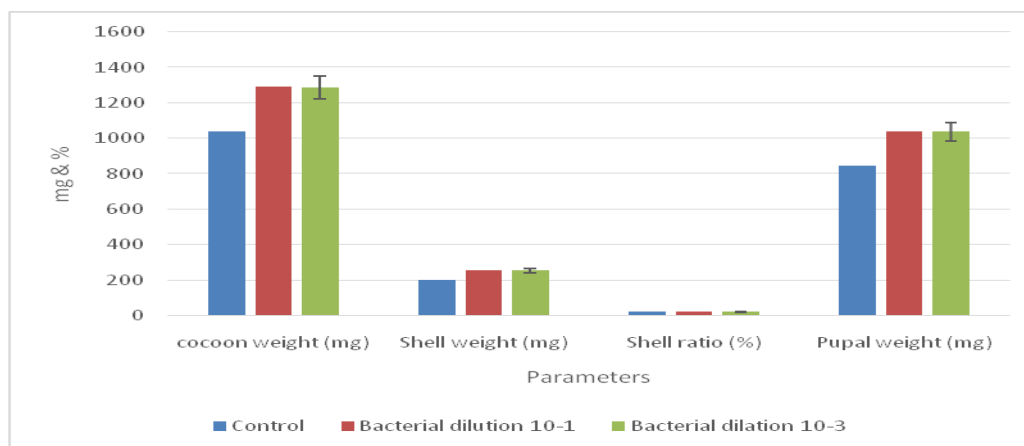


Fig. 5: Economic characters of *B. mori* fed with *B. fibrisolvens*.

the methods developed by Kam and Degen (1997). The cocoons were harvested on the fourth day, and cocoon characters were recorded.

Results and Discussion

The present study exemplified the impact of ruminant microflora on mulberry leaf digestion,

growth and economic characteristics of *Bombyx mori* (Linnaeus). The growth and silk quality of *B. mori* larvae significantly increased when the worms were supplemented with the microbes. Table 3 shows that food consumption was increased (25.43 percent) at 10⁻¹ dilution at the fourth instar when fed with *R. albus* and only 7.74

Table 3: Energy parameters of silkworm *B. mori* fed with *R. albus*

Energy parameters	IV instar			V instar		
	Control	10 ⁻¹ dilution	10 ⁻³ dilution	Control	10 ⁻¹ dilution	10 ⁻³ dilution
Food consumption (mg)	552.80±0.86	693.50±0.93 (25.43)	562.30±0.18 (1.71)	114.49±1.33	124.03±1.36 (8.33)	117.17±1.35 (2.34)
Assimilation (mg)	402.00±0.81	413.20±0.90 (2.78)	420.00 ± 0.75 (4.45)	793.20 ± 1.25	917.20 ± 1.27 (15.62)	849.00±1.26 (7.03)
Tissue growth (mg)	265.60 ± 0.03	348.60 ± 0.01 (31.30)	81.50 ± 0.01 (5.99)	564.90 ± 0.05	821.80 ± 0.17 (45.47)	630.30 ± 0.12 (11.57)
Approximate digestibility (%)	72.72 ± 0.94	80.15 ± 0.96 (10.17)	74.56 ± 0.94 (2.52)	69.28 ± 0.94	73.94±0.93 (6.71)	72.45±0.93 (4.56)
Tissue growth efficiency (%)	66.06 ± 0.03	84.36 ± 0.01 (27.63)	82.42 ± 0.02 (24.28)	71.21±0.03	89.59 ± 0.13 (25.73)	74.24 ± 0.10 (4.24)
Ecological growth efficiency (%)	48.04±0.03	50.26±0.01 (4.61)	49.97 ± 0.02 (4.01)	49.34 ± 0.04	66.25 ± 0.13 (34.15)	53.79±0.09 (8.98)
						n = 10

Per cent deviation over control values in parentheses; t-test values not significant at $P \leq 0.05$

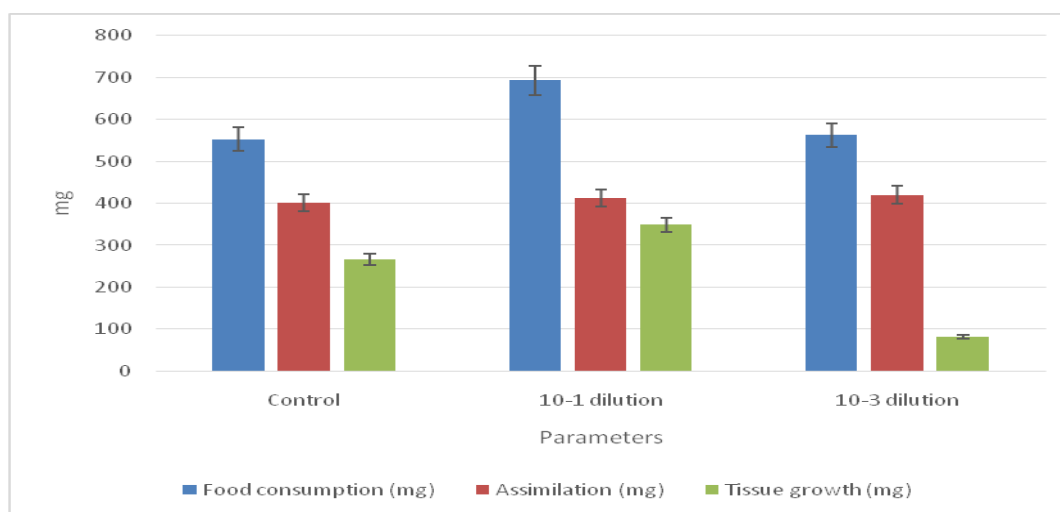


Fig. 6: Energy parameters of silkworm *B. mori* fed with *R. albus* (IV Instar).

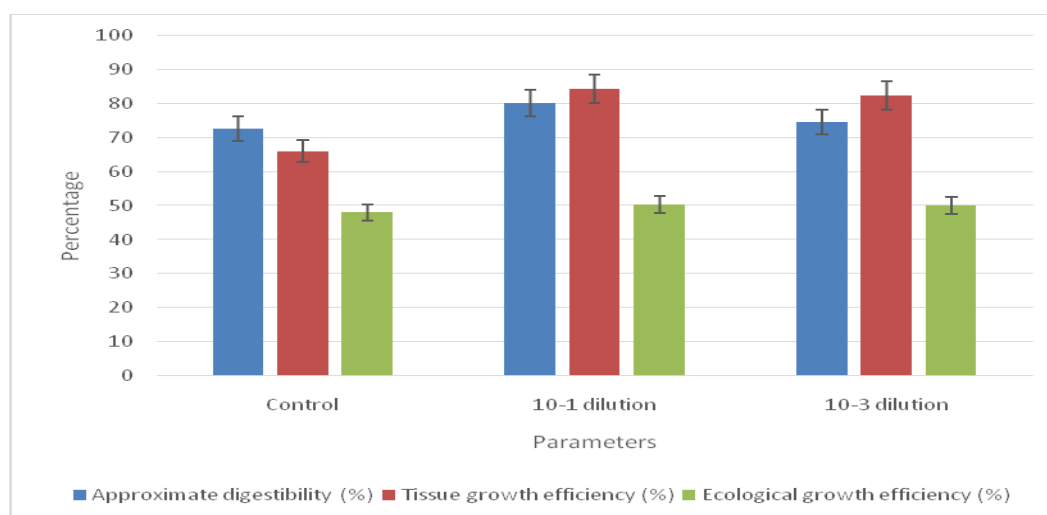


Fig. 7: Energy parameters of silkworm *B. mori* fed with *R. albus* (IV Instar).

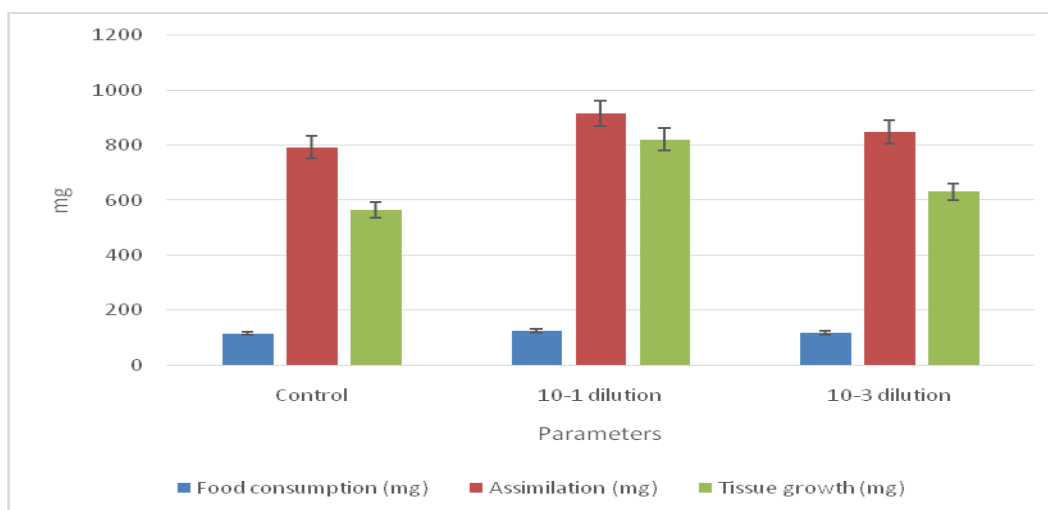


Fig. 8: Energy parameters of silkworm *B. mori* fed with *R. albus* (V Instar).

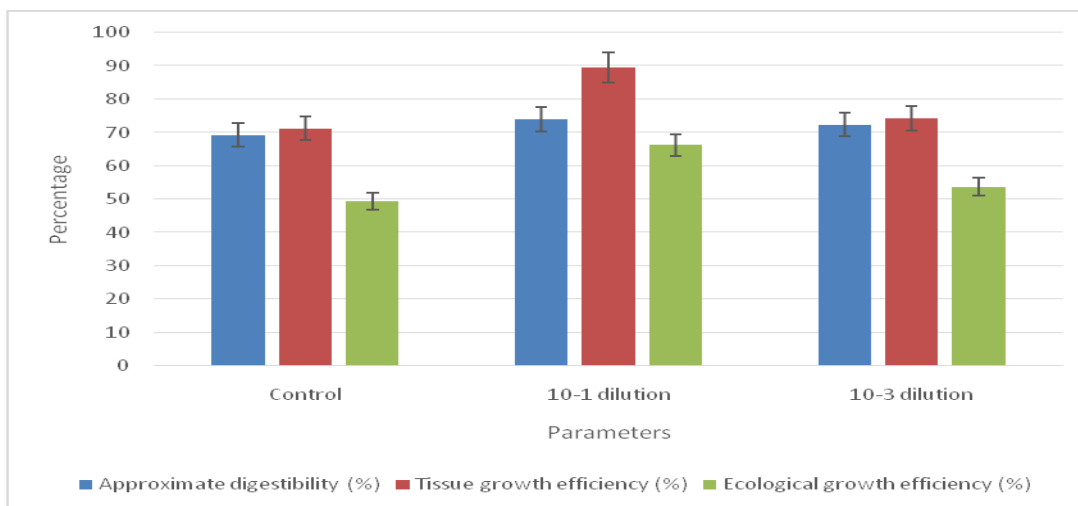


Fig. 9: Energy parameters of silkworm *B. mori* fed with *R. albus* (V Instar).

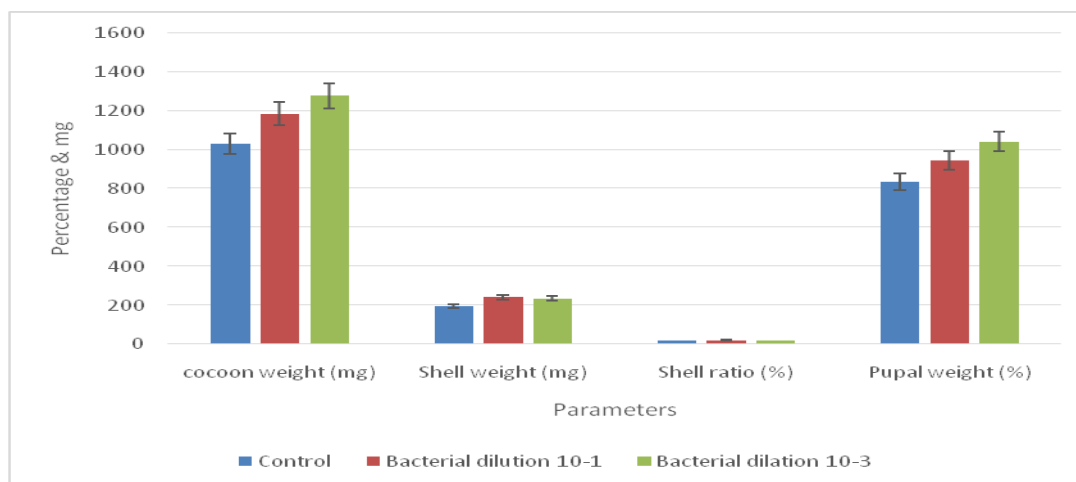
per cent was observed when fed with *B. fibrisolvans*. Table 1 and Figures 1 – 4 shows that assimilation was higher (84.06 per cent) at 10^{-1} dilution at the fifth instar level fed with *B. fibrisolvans* than *R. albus* (15.62 per cent) at 10^{-1} dilution at the fifth instar. The highest tissue growth (179.60 per cent) was seen in the fifth instar at the dilution of 10^{-1} fed with *B. fibrisolvans* than *R. albus* (45.47 per cent) (Table 1). Approximate digestibility also increased (66.78 per cent) when fed with *B. fibrisolvans* to the fifth instar larvae at 10^{-1} dilution.

Tissue growth efficiency was maximum (81.90 per cent) at 10^{-3} in the fourth instar larvae for *B. fibrisolvans* and the highest growth (153.50 percent) found at 10^{-1} dilution in the fifth instar larvae for ecological growth efficiency for *B. fibrisolvans* than *R. albus* (34.15 per cent) at 10^{-1} dilution at the fifth instar level (Table 3; Figs. 6–9).

The cocoon characters of *B. mori* varied following *B. fibrisolvans* and *R. albus* supplementation than control. The cocoon weight

Table 4: Effect of *R. albus* on the *economic* characters of *B.mori*

Cocoon characters	Control	Bacterial dilution 10 ⁻¹	Bacterial dilution 10 ⁻³
Cocoon weight (mg)	1032.80 ± 0.16	1186.10 ± 0.18 (14.84)	1278.50 ± 0.27 (23.78)
Shell weight (mg)	197.20 ± 0.03	243.20 ± 0.04 (23.32)	235.40 ± 0.05 (19.37)
Shell ratio (%)	18.40 ± 0.09	20.50 ± 0.11 (11.40)	18.41 ± 0.16 (0.05)
Pupal weight (%)	835.60 ± 0.08	943.90 ± 0.09 (12.95)	1043.10 ± 0.14 (24.81)
			n = 10

Fig. 10: Effect of *R. albus* on the *economic* characters of *B. mori*.

(mg), shell weight (mg), shell ratio (%) and pupal weight (mg) were more effective for *B. fibrisolvens* than *R. albus* at 10⁻¹ dilution (Tables 2, 4; Figs. 5, 10).

Improved growth and development of silkworm following supplementation have been recorded by several workers. Ahamed *et al.* (2001) studied the effect of chloramphenicol (antibiotic) in the fifth instar *B. mori* caterpillar and reported that the antibiotic enhances food utilization and dietary water intake. Rate of enzyme secretion and activity increased with increasing quantity of food consumed resulting in a more or less constant efficiency of assimilation (Bursell, 1970). El-Karakasy and Idriss (1990) observed improvement in the growth and development of *B. mori* larvae following ascorbic acid supplementation. Gylswyk and Toorn (1986) studied enumeration of *Bacteroides succinogenes*

in the rumen of sheep fed maize straw diets. The present revealed that the *B. fibrisolvens* was more efficient bacteria which increases the energy parameters at the 10⁻¹ dilution.

Conclusion

Economic characteristics and energy parameters of silkworms has been increased when they were given dietary supplements. The impact of *B. fibrisolvens* on *B. mori* is attributable to its stimulatory role on economic characteristics and energy parameters.

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