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Impact of dietary *Aloe vera* juice on commercial traits of the Nistari silkworm, *Bombyx mori* L.

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Abstract
Silk farmers often add supplements to mulberry leaves in the hope of producing more silk, but most tests use commercial hybrid silkworms and few checks whether faster-growing larvae actually spin more silk. We tested a commercial *Aloe vera* juice on Nistari, the indigenous silkworm race of Bangladesh. Mulberry leaves were dipped in juice diluted to 5, 10, 15, 20 or 25 mL per 100 mL of water and fed from the third larval stage until spinning, alongside untreated leaves, in 18 rearing trays of 60 larvae each (three trays per group). Nineteen traits of the larvae, cocoons, pupae and eggs were measured. *Aloe vera* changed the speed of growth rather than its end point: treated larvae gained 0.14 g more than untreated larvae between the first and fourth recording days and were 14% heavier on day 4, but the untreated larvae caught up, and final weight and total weight gain did not differ. Egg hatchability rose from 92.6% to 94.9% and was higher at every concentration. Cocoon shell weight and shell ratio, which determine how much silk is produced, did not change detectably, although the experiment could only detect changes of about 15% or more. *Aloe vera* juice therefore helped Nistari larvae grow faster and improved hatching, which matters for producing silkworm eggs, but did not increase silk. This fits the idea that a silkworm stops growing at a fixed body size, so extra nutrition shortens growth rather than enlarging the larva. Supplements should be judged by their effect on silk itself, not on larval weight alone.

Highlights
- Mulberry leaves dipped in *Aloe vera* juice were fed to the indigenous Nistari silkworm of Bangladesh.
- Treated larvae grew faster in the middle of the feeding period and were 14% heavier on day 4.
- Untreated larvae caught up, and final larval weight and total weight gain were the same.
- Egg hatchability improved at every concentration tested.
- Cocoon shell weight, and therefore silk yield, did not change detectably.

Keywords: Sericulture mulberry leaf fortification, *Aloe vera*, larval growth, egg hatchability, cocoon shell ratio, Nistari, Bangladesh

Introduction
The mulberry silkworm, *Bombyx mori* L., feeds only on mulberry leaves, and the nutritional quality of those leaves governs how fast the larvae grow and how much silk they eventually spin (Ruth *et al*. 2019) [20]. Silkworm rearers and researchers have therefore tried for decades to improve the leaf by adding supplements before feeding. Vitamins (Rajabi Kanafi *et al*. 2007) [17], minerals (Kaliwal and Chakraborty 2006; Rahman *et al*. 2025) [8, 16], amino acids (Muzamil *et al*. 2023) [13], animal products such as milk (Konala *et al*. 2013) [11], seed powders (Alfazairy *et al*. 2024) [1] and plant extracts (Fouad and Ahmed 2025) [4] have all been reported to change larval growth or cocoon traits, and several reviews summarise this growing literature (Shahzadi *et al*. 2022) [22].

Aloe vera is among the plant supplements tested. Its leaf gel contains polysaccharides such as acemannan together with anthraquinones such as aloin and aloe-emodin, and it is widely used in food and medicine (Sánchez *et al*. 2020) [24]. Recent work has reported that fortifying mulberry leaves with *Aloe vera* and other medicinal plant extracts improved silkworm growth and silk characters (Fouad and Ahmed 2025) [4]. Almost all such studies, however, have used improved bivoltine or hybrid lines bred for high silk yield.

Indigenous multivoltine races remain important in tropical sericulture. In Bangladesh, sericulture began with the indigenous multivoltine race Nistari, which is valued for its hardiness, and improved races were introduced only later (International Sericultural Commission n.d.). Silk production in the country now falls far short of demand, and low productivity is one of the main barriers the industry faces (Jui *et al*. 2023) [7]. Simple, low-cost supplements that work with local races would therefore be useful, and a calcium supplement has recently been shown to improve larval, egg and cocoon traits in a race derived from Nistari stock (Rahman *et al*. 2025) [16].

A second gap concerns how results are judged. Studies usually report larval weight at one or two time points, and a heavier larva is often taken to mean more silk. The two need not go together: feeding bovine milk raised larval weight by 82.5% but cocoon weight by only 8% (Konala *et al*. 2013) [11]. In moths and butterflies the larva stops growing once it reaches a species-specific critical weight, which triggers the hormones that end feeding and start metamorphosis (Nijhout *et al*. 2006; Riddiford 2011) [14, 19]. In the silkworm this

point is reached around the fourth day of the final instar (Keshan *et al*. 2015) [10], and body weight peaks soon afterwards (Saito *et al*. 2024) [21]. A supplement that speeds up growth could therefore bring larvae to the same final size sooner without making them, or their cocoons, any larger.

We therefore fed mulberry leaves treated with a commercial *Aloe vera* juice at five concentrations to Nistari larvae and measured 19 traits covering larval growth, cocoons, pupae and eggs. We asked three questions: does *Aloe vera* change the course of larval growth; does any change in growth carry through to cocoon and silk traits; and does it affect egg quality?

Materials and Methods

1. Study site

The experiment was carried out in 2016–2017 in the silkworm rearing laboratory of the Department of Zoology, University of Rajshahi, Bangladesh, a 7.0 × 5.0 m room fitted with netted windows.

2. Silkworms and rearing

Disease-free eggs of the indigenous multivoltine race Nistari were obtained from the germplasm bank of the Bangladesh Sericulture Research and Training Institute, Rajshahi. Rearing appliances and the room were disinfected with formaldehyde solution before use, and larvae were reared at 26 ± 2 °C and 70 ± 10% relative humidity following standard methods. Fresh leaves of *Morus alba* L. were picked each morning from the mulberry garden of the Department of Zoology and fed four times a day, at 5 a.m., 10 a.m., 4 p.m. and 10 p.m. Young larvae received finely chopped tender leaves, and larvae from the fourth instar onward received whole mature leaves.

3. Treatments

A commercial *Aloe vera* juice (Nature Cellco Ltd., Korea) was used as supplied. Measured volumes were diluted separately in 100 mL of distilled water to give five concentrations: 5, 10, 15, 20 and 25 mL per 100 mL. Fresh leaves were dipped in each solution, dried by fanning and fed from the first day of the third instar until the larvae began to spin; control larvae received untreated leaves, and all trays received the same quantity of leaf. After the second moult, larvae were divided into six groups of three trays, each tray holding 60 larvae, giving 18 trays and 1080 larvae in total (Figure 1). The composition of the juice was not analysed, and the amount of leaf eaten was not measured.

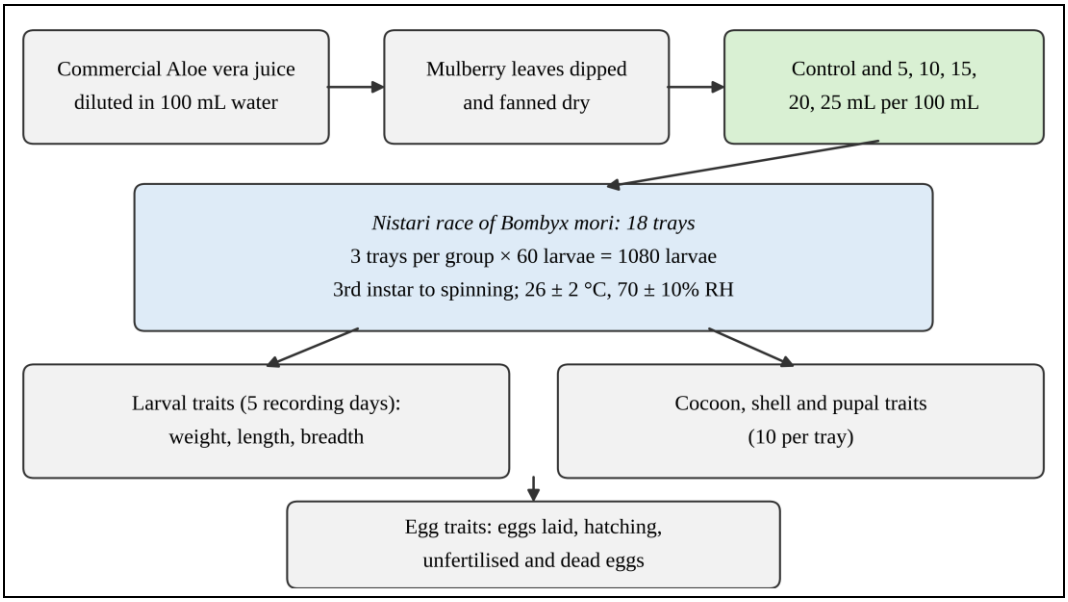


Fig 1: Design of the feeding experiment. Eighteen trays of 60 Nistari larvae received mulberry leaves dipped in *Aloe vera* juice at one of five concentrations, or untreated leaves, from the third instar until spinning.

4. Traits measured

Larval weight was recorded on five successive days, and larval length and breadth once, each as the mean of 10 larvae per tray. Ten cocoons per tray were weighed and measured for length and breadth, then cut open to obtain the shell weight and the weight, length and breadth of the pupa. Shell ratio, the proportion of the cocoon made of silk, was calculated as shell weight divided by cocoon weight × 100. For egg traits, 10 egg layings per tray were used to record the number of eggs laid per female and the percentages of eggs that hatched, were unfertilised or died.

5. Data quality control

Every tray value was checked for internal consistency before analysis. Three decisions resulted. First, one pupal weight (0.103 g, 20 mL group) was physically impossible, because the cocoon and shell of the same tray weighed 1.129 g and 0.108 g; this value was left out of the main analysis and the analysis was repeated with it included. Second, in one control tray the recorded cocoon weight (0.780 g) was lower than the combined pupal and shell weight of that tray (1.017 g); the tray was kept in the main analysis, and the traits concerned were reanalysed without it. Third, survival was not analysed, because some tray values exceeded 100%, which is impossible for counts of 60 larvae. Shell ratio was calculated from the shell and cocoon weights of each tray.

6. Statistical analysis

The rearing tray was the unit of replication (three trays per group; each tray value is the mean of 10 individuals or layings). Differences among the six groups were tested by one-way analysis of variance and confirmed by a permutation test with 5000 randomisations. Each *Aloe vera*

concentration was compared with the control by Dunnett's test (Dunnett 1955) [3]. The overall effect of *Aloe vera* was estimated by comparing the average of the five treated groups with the control, expressed as a percentage of the control mean with a 95% confidence interval. Because 19 traits were tested, P values were adjusted by the false discovery rate method of Benjamini and Hochberg (1995) [2], and an effect is called clear only when the adjusted value (q) was below 0.05. For each trait we also calculated the smallest change from the control that this design could reliably detect (5% significance, 80% power), so that results showing no effect can be interpreted correctly.

Growth over the five recording days was analysed with a linear mixed model of larval weight on treatment and day, with tray as a random effect, testing whether the treatments changed the shape of the growth curve. Weight gain per tray over days 1–4, days 4–5 and days 1–5 was analysed as a simpler check. Relations among traits were examined with Spearman correlations on tray means and with a principal component analysis of 15 standardised traits. Analyses were run in Python 3 (statsmodels and SciPy) with a fixed random seed.

Results

1. Growth: faster, but to the same end

Aloe vera changed the shape of the growth curve but not where it ended (Figure 2). The treatment × day interaction was significant in the mixed model (likelihood ratio = 50.7, df = 20, P < 0.001), and so was the simpler comparison of all treated groups with the control over time (likelihood ratio = 18.4, df = 4, P = 0.001).

On day 1 the groups did not differ (-0.0% (95% CI -7.7 to +7.6; P = 0.992, q = 0.992)), and on day 2 the treated larvae were slightly lighter (-9.8% (95% CI -20.3 to +0.7; P = 0.065, q = 0.124)). From day 3 the treated larvae pulled ahead (+7.8% (95% CI -1.7 to +17.3; P = 0.100, q = 0.158)), and on day 4 they were clearly heavier: 0.990 g in the control against 1.131 g with *Aloe vera* (+14.3% (95% CI +8.7 to +20.0; P < 0.001, q = 0.001)). On day 5 the difference had disappeared (-1.1% (95% CI -10.9 to +8.7; P = 0.805, q = 0.990)). The weight gains tell the same story: between days 1 and 4 the treated larvae gained 0.782 g against 0.640 g in the control (P = 0.001); between days 4 and 5 the control gained more (0.284 g against 0.127 g; P = 0.041); and over the whole period the gains were the same (0.924 g and 0.910 g; P = 0.518). Larvae fed *Aloe vera* were also broader (+6.6% (95% CI +3.7 to +9.4; P < 0.001, q = 0.002)) but not longer (-0.2% (95% CI -2.9 to +2.4; P = 0.841, q = 0.990)).

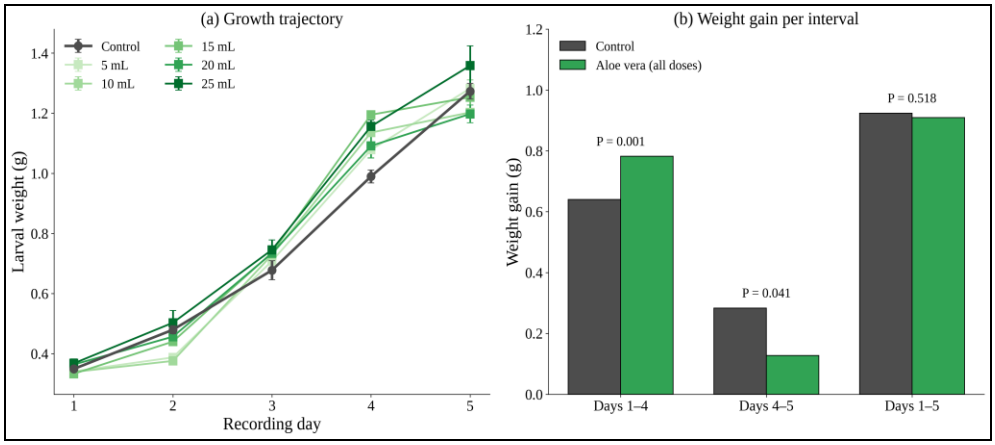


Fig 2: *Aloe vera* juice changed the timing of growth but not its end point. (a) Larval weight (mean ± SE of three trays) on five recording days; the control is shown in dark grey. (b) Weight gain over three intervals for the control and for all *Aloe vera* groups together, with the P value from analysis of variance across all six groups.

Table 1: Mean ± standard error of every trait (three trays per group; each tray value is the mean of 10 individuals or layings). Concentrations are mL of *Aloe vera* juice per 100 mL of water.

Trait	Control	5 mL	10 mL	15 mL	20 mL	25 mL
Larval weight day 1 (g)	0.349 ± 0.002	0.341 ± 0.006	0.339 ± 0.017	0.333 ± 0.013	0.364 ± 0.007	0.368 ± 0.015
Larval weight day 2 (g)	0.480 ± 0.014	0.388 ± 0.010	0.376 ± 0.007	0.440 ± 0.011	0.457 ± 0.023	0.503 ± 0.041
Larval weight day 3 (g)	0.678 ± 0.032	0.708 ± 0.041	0.729 ± 0.018	0.736 ± 0.009	0.734 ± 0.015	0.746 ± 0.033
Larval weight day 4 (g)	0.990 ± 0.022	1.079 ± 0.026	1.136 ± 0.010	1.195 ± 0.005	1.091 ± 0.040	1.156 ± 0.020
Larval weight day 5 (g)	1.273 ± 0.026	1.282 ± 0.084	1.203 ± 0.010	1.253 ± 0.058	1.198 ± 0.029	1.358 ± 0.066
Larval length (cm)	5.43 ± 0.088	5.37 ± 0.033	5.37 ± 0.033	5.47 ± 0.067	5.40 ± 0.058	5.50 ± 0.058
Larval breadth (cm)	2.53 ± 0.033	2.63 ± 0.033	2.67 ± 0.033	2.67 ± 0.033	2.80 ± 0.000	2.73 ± 0.033
Cocoon weight (g)	0.977 ± 0.100	1.095 ± 0.034	1.103 ± 0.041	1.124 ± 0.045	1.190 ± 0.038	1.153 ± 0.005
Cocoon length (cm)	3.33 ± 0.033	3.27 ± 0.033	3.40 ± 0.058	3.40 ± 0.000	3.43 ± 0.033	3.47 ± 0.033
Cocoon breadth (cm)	4.27 ± 0.033	4.37 ± 0.033	4.40 ± 0.058	4.37 ± 0.033	4.30 ± 0.058	4.30 ± 0.000
Shell weight (g)	0.107 ± 0.001	0.107 ± 0.003	0.119 ± 0.008	0.109 ± 0.000	0.110 ± 0.001	0.111 ± 0.001
Shell ratio (%)	11.15 ± 1.164	9.80 ± 0.041	10.80 ± 0.732	9.70 ± 0.418	9.26 ± 0.195	9.60 ± 0.067
Pupal weight (g)	0.938 ± 0.022	0.959 ± 0.059	0.973 ± 0.025	0.858 ± 0.122	0.941 ± 0.070	1.011 ± 0.014
Pupal length (cm)	2.23 ± 0.033	2.33 ± 0.033	2.40 ± 0.058	2.37 ± 0.033	2.30 ± 0.000	2.33 ± 0.033
Pupal breadth (cm)	3.03 ± 0.088	3.07 ± 0.033	3.17 ± 0.033	3.23 ± 0.033	3.10 ± 0.058	3.23 ± 0.033
Eggs per female (no.)	511 ± 4.842	496 ± 15.567	510 ± 4.726	510 ± 8.090	514 ± 4.256	527 ± 6.658

Egg hatching (%)	92.56 ± 0.170	95.61 ± 0.352	94.83 ± 0.442	94.70 ± 0.276	94.61 ± 0.175	94.69 ± 0.157
Unfertilised eggs (%)	2.97 ± 0.199	2.10 ± 0.123	2.39 ± 0.277	2.31 ± 0.246	2.55 ± 0.224	2.12 ± 0.265
Dead eggs (%)	2.07 ± 0.372	1.54 ± 0.021	1.41 ± 0.085	1.54 ± 0.150	1.59 ± 0.033	1.70 ± 0.114

2. Overview of all traits

Of the 19 traits, three showed a clear effect of *Aloe vera* after correction for multiple testing: day-4 larval weight, larval breadth and egg hatchability (Figure 3; Table 2). Several others moved in the same direction but did not pass the correction. The remaining traits, including final larval weight, cocoon shell weight and the number of eggs laid, showed no detectable change.

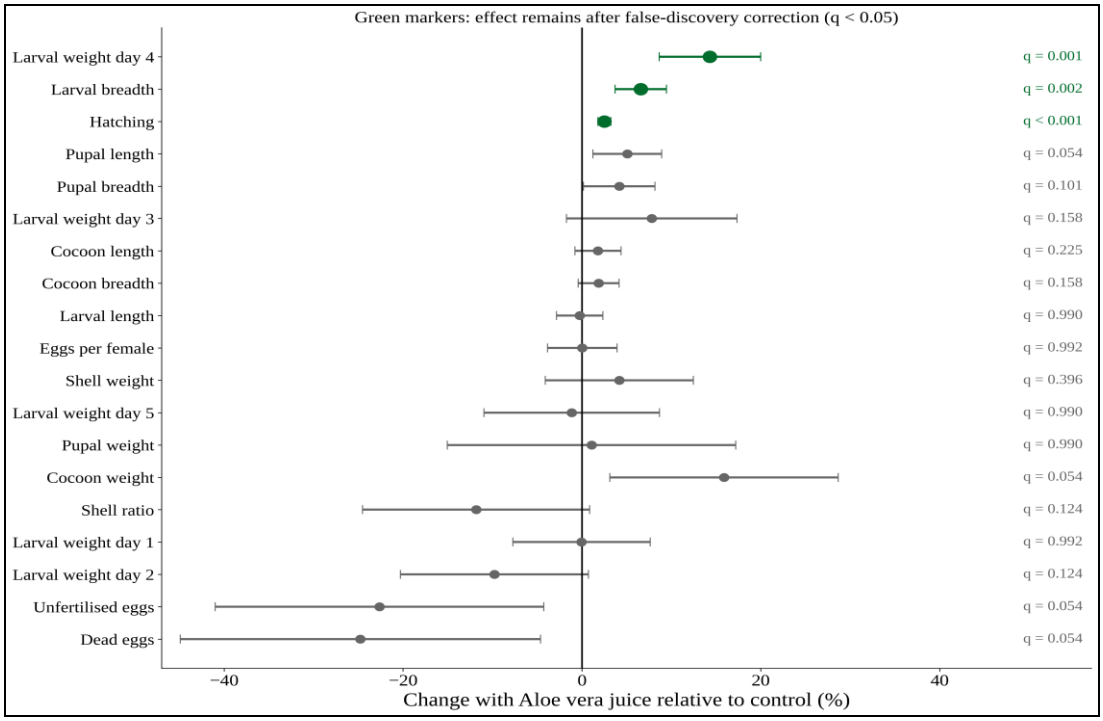


Fig 3: Change in each trait with *Aloe vera*, as a percentage of the control mean with its 95% confidence interval. Green markers show effects that remain after correction for testing 19 traits (q < 0.05); q values are given on the right.

Table 2: Effect of *Aloe vera* juice on each trait. F and P are from one-way analysis of variance across the six groups, confirmed by a permutation test. The change compares the average of the five *Aloe vera* groups with the control; q is the value adjusted for testing 19 traits. "Smallest detectable change" is the difference from the control that this design could reliably detect.

Trait	F	P	P (permutation)	Change (%)	95% CI (%)	q	Smallest detectable change (%)	Verdict
Larval weight day 1	1.56	0.245	0.239	-0.0	-7.7 to +7.6	0.992	14	No detectable effect
Larval weight day 2	5.67	0.007	0.002	-9.8	-20.3 to +0.7	0.124	19	No detectable effect
Larval weight day 3	0.85	0.543	0.552	+7.8	-1.7 to +17.3	0.158	17	No detectable effect
Larval weight day 4	9.36	< 0.001	0.002	+14.3	+8.7 to +20.0	0.001	10	Clear effect
Larval weight day 5	1.28	0.334	0.322	-1.1	-10.9 to +8.7	0.990	18	No detectable effect
Larval length	0.84	0.545	0.517	-0.2	-2.9 to +2.4	0.990	5	No detectable effect
Larval breadth	8.84	0.001	0.003	+6.6	+3.7 to +9.4	0.002	5	Clear effect
Cocoon weight	1.92	0.165	0.106	+15.9	+3.1 to +28.7	0.054	23	Possible effect
Cocoon length	4.03	0.022	0.030	+1.8	-0.8 to +4.4	0.225	5	No detectable effect
Cocoon breadth	1.60	0.234	0.274	+1.9	-0.4 to +4.2	0.158	4	No detectable effect
Shell weight	1.47	0.270	0.201	+4.2	-4.1 to +12.5	0.396	15	No detectable effect
Shell ratio	1.59	0.236	0.150	-11.8	-24.5 to +0.9	0.124	23	No detectable effect
Pupal weight	0.67	0.656	0.688	+1.1	-15.0 to +17.2	0.990	29	No detectable effect
Pupal length	2.54	0.086	0.109	+5.1	+1.2 to +8.9	0.054	7	Possible effect
Pupal breadth	2.81	0.066	0.069	+4.2	+0.2 to +8.2	0.101	7	Possible effect
Eggs per female	1.42	0.286	0.276	+0.0	-3.8 to +3.9	0.992	7	No detectable effect
Egg hatching	12.96	< 0.001	0.002	+2.5	+1.8 to +3.2	< 0.001	1	Clear effect
Unfertilised eggs	1.98	0.154	0.160	-22.6	-41.0 to -4.3	0.054	33	Possible effect
Dead eggs	1.73	0.202	0.172	-24.8	-44.9 to -4.6	0.054	36	Possible effect

3. Egg traits

Egg hatchability was the most consistent response (Figure 4a). It rose from 92.6% in the control to 94.9% with *Aloe vera* (+2.5% (95% CI +1.8 to +3.2; P < 0.001, q < 0.001)), and every concentration was higher than the control (Dunnett < 0.001 to 0.001). The highest value was at the lowest concentration, 5 mL (95.6%). Unfertilised eggs (-22.6% (95% CI -41.0 to -4.3; P = 0.020, q = 0.054)) and dead eggs (-24.8% (95% CI -44.9 to -4.6; P = 0.020, q = 0.054)) were fewer with *Aloe vera*, although neither effect passed the correction for multiple testing. The number of eggs laid per female did not change (+0.0% (95% CI -3.8 to

+3.9; P = 0.983, q = 0.992)). Across the 18 trays, hatchability was closely and negatively related to the percentage of unfertilised eggs (Spearman r = -0.76, P < 0.001).

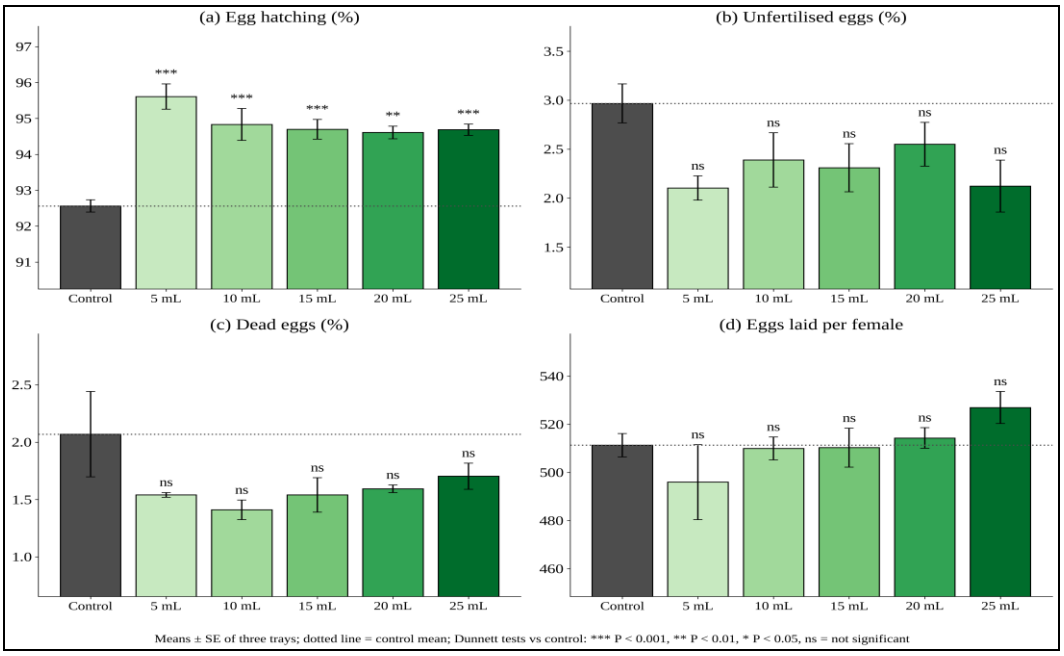


Fig 4: Egg traits at each concentration. (a) Hatching, (b) unfertilised eggs, (c) dead eggs and (d) eggs laid per female. Bars are means ± SE of three trays; the dotted line is the control mean; symbols are Dunnett comparisons with the control.

4. Cocoon, shell and pupal traits

Shell weight, the trait that determines how much silk is produced, did not change detectably (+4.2% (95% CI -4.1 to +12.5; P = 0.292, q = 0.396)), and neither did shell ratio (-11.8% (95% CI -24.5 to +0.9; P = 0.065, q = 0.124)). With three trays per group, the smallest changes this design could detect were 15% for shell weight and 23% for shell ratio (Figure 5). Cocoon weight appeared higher with *Aloe vera* (+15.9% (95% CI +3.1 to +28.7; P = 0.019, q = 0.054)), but this depended on the single control tray whose cocoon weight was inconsistent with its own pupal and shell weights; without that tray the difference was +5.3% (P = 0.237), so we do not treat cocoon weight as having responded (Figure 5d). Pupal weight was unchanged (+1.1% (95% CI -15.0 to +17.2; P = 0.885, q = 0.990)), and including the impossible value of 0.103 g did not alter that conclusion (-4.9%, P = 0.751).

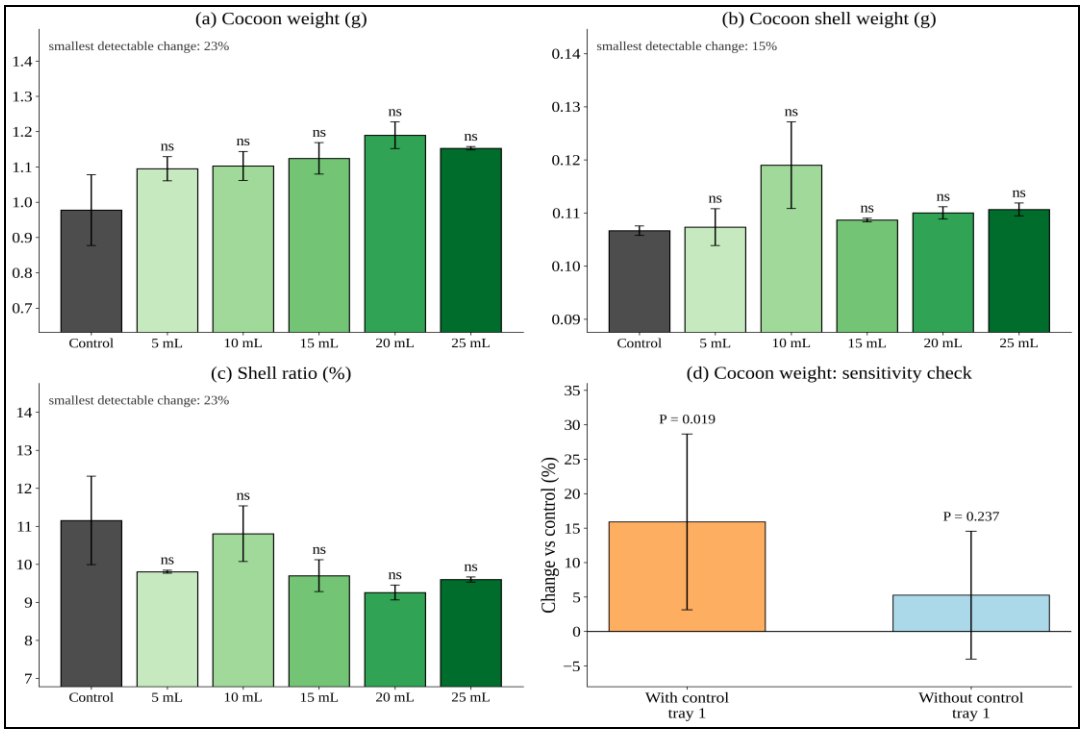


Fig 5: Cocoon and silk traits did not change detectably. (a) Cocoon weight, (b) cocoon shell weight and (c) shell ratio at each concentration, each with the smallest change the design could detect. (d) The apparent gain in cocoon weight depends on one control tray whose recorded cocoon weight was lower than its own pupal plus shell weight.

5. Growth and dose

Table 3: Change from the control at each concentration (% of the control mean) for seven key traits, with the Dunnett P value in brackets.

Trait	5 mL	10 mL	15 mL	20 mL	25 mL
Larval weight day 4	+9.0 (0.073)	+14.8 (0.004)	+20.7 (< 0.001)	+10.3 (0.038)	+16.8 (0.001)
Larval breadth	+3.9 (0.134)	+5.3 (0.035)	+5.3 (0.035)	+10.5 (< 0.001)	+7.9 (0.002)
Egg hatching	+3.3 (< 0.001)	+2.5 (< 0.001)	+2.3 (< 0.001)	+2.2 (0.001)	+2.3 (< 0.001)
Unfertilised eggs	-29.1 (0.074)	-19.4 (0.311)	-22.1 (0.214)	-14.0 (0.588)	-28.4 (0.083)
Shell weight	+0.6 (1.000)	+11.6 (0.127)	+1.9 (0.994)	+3.1 (0.948)	+3.8 (0.901)
Shell ratio	-12.1 (0.399)	-3.1 (0.991)	-13.0 (0.336)	-17.0 (0.150)	-13.9 (0.283)
Cocoon weight	+12.0 (0.409)	+12.8 (0.353)	+15.0 (0.231)	+21.7 (0.053)	+17.9 (0.124)

No trait showed a clear increase with concentration (Table 3). Day-4 larval weight was higher than the control at every concentration, and hatchability was highest at 5 mL and declined slightly at higher concentrations (linear trend across 5–25 mL, P = 0.047).

The number of eggs laid per female increased across the treated range (P = 0.021), from 496 at 5 mL to 527 at 25 mL, although no single concentration differed from the control.

6. All traits together

In the principal component analysis, the first two components explained 28% and 16% of the variation among trays, and trays fed *Aloe vera* separated clearly from control trays along the first component (P = 0.002; Figure 6a). Day-4 larval weight was only weakly related to cocoon weight (r = 0.34) and to shell weight (r = 0.27) across trays, whereas cocoon weight and shell weight were strongly related (r = 0.61; Figure 6b).

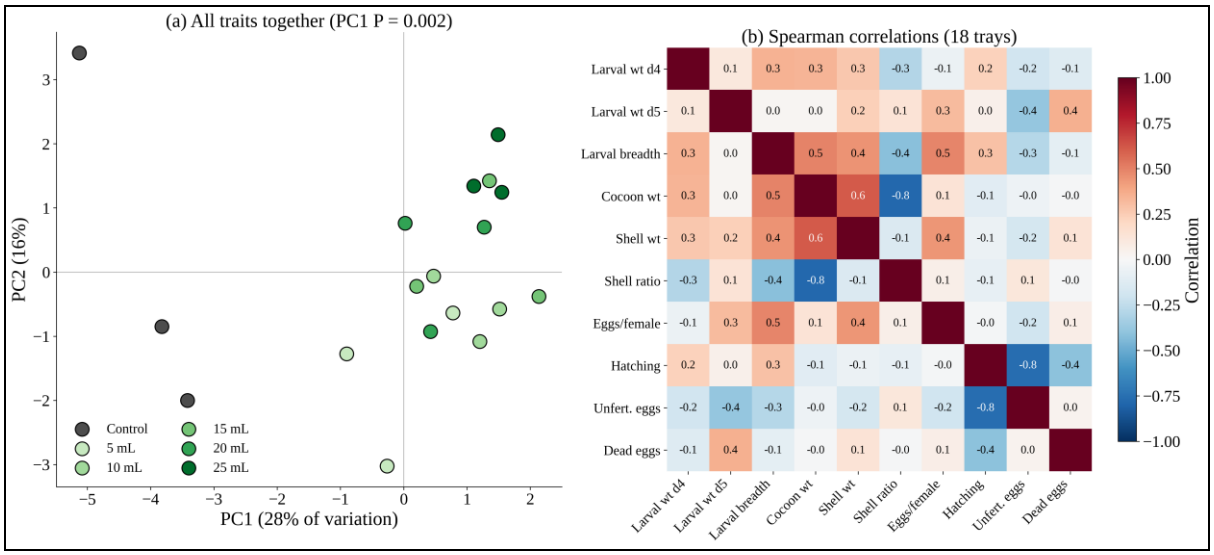


Fig 6: (a) Principal component analysis of 15 standardised traits; each point is one tray. (b) Spearman correlations among tray means for ten traits.

Discussion

1. Faster growth, same final size

The main result is that *Aloe vera* changed when the larvae grew, not how large they became. Treated larvae were 14% heavier than untreated larvae on day 4, yet both groups weighed the same on day 5 and gained the same weight overall. Treated larvae were broader but not longer, which suggests heavier larvae of the same length rather than larger larvae.

This pattern fits what is known about how insects control their final size. A moth or butterfly larva stops growing once it passes a critical weight, after which hormones bring feeding to an end largely regardless of further food (Nijhout *et al.* 2006; Riddiford 2011) [14, 19]. In the silkworm, larvae reach this critical weight around the fourth day of the final instar, when a small rise in the moulting hormone ecdysteroid appears (Keshan *et al.* 2015) [10], and body weight peaks on the fifth day (Saito *et al.* 2024) [21]. Growth before this point is driven by nutrient-sensing signals (Kawasaki *et al.* 2023) [9], so a better diet can speed it up; but once the critical weight is reached, the end point is largely fixed. Larvae fed *Aloe vera* appear to have reached the same final weight sooner, while untreated larvae caught up in the last day of feeding. The convergence of growth curves to a common end point is a well-known pattern in animals (Hector and Nakagawa 2012) [12]. Because hormone levels and development time were not measured, this explanation remains a hypothesis.

2. Why silk yield did not increase

If final larval size does not change, there is little reason to expect more silk. Our results agree: shell weight and shell ratio did not change, and day-4 weight was only weakly related to cocoon or shell weight across trays. This matches the earlier observation that milk raised larval weight by 82.5% but cocoon weight by only 8% (Konala *et al.* 2013) [11]. Supplements that do raise silk yield tend to act on the silk gland itself: sodium butyrate increased silk yield by promoting both larval growth and silk gland development (Ren *et al.* 2025) [18], and amino acid supplements raised cocoon and shell traits together (Muzamil *et al.* 2023) [13]. Resources can also be shifted between silk and

reproduction, since disabling the silk gland redirects nutrients toward egg production (Lye *et al*. 2024) [12]. Larval weight alone is therefore a poor guide to silk output, and studies of supplements should report shell weight and shell ratio directly.

Earlier studies of *Aloe vera* and other plant extracts reported gains in cocoon and silk traits (Fouad and Ahmed 2025) [4], and supplements such as *Lepidium sativum* seed powder raised cocoon, pupal and shell weights (Alfazairy *et al*. 2024) [1]. Three differences may explain why we did not see such gains. Those studies mostly used improved hybrids bred for silk, whereas Nistari has a low shell ratio, about 10% in our trays; they used different preparations and doses; and a study with three trays per group can detect only fairly large changes, about 15% in shell weight here. Our result therefore shows that any effect on silk in this race was smaller than this, not that *Aloe vera* can never improve silk yield.

3. Better egg hatching

The improvement in hatchability, from 92.6% to about 94.9%, was small in absolute terms but appeared at every concentration and passed correction for multiple testing. Hatchability was closely linked to the proportion of unfertilised eggs, which suggests that *Aloe vera* improved fertilisation rather than the survival of developing embryos. Similar gains in egg traits have been reported for other supplements, including calcium carbonate in a race derived from Nistari stock (Rahman *et al*. 2025) [16], and reproductive traits in insects are known to vary with the condition and age of the female (Rahman *et al*. 2021) [15]. For a multivoltine race reared through several generations a year, better hatching means more usable eggs from each laying, which has direct value in silkworm egg production even when silk yield is unchanged. Because the best result came from the lowest concentration, there is no reason to use more juice.

4. The supplement itself

We used a commercial *Aloe vera* drink whose composition was not declared or analysed. *Aloe vera* gel contains polysaccharides, anthraquinones, vitamins and minerals with a wide range of biological activities (Sánchez *et al*. 2020) [24], so it is not possible to say which component affected the larvae. Minerals alone can change silkworm metabolism (Kaliwal and Chakraborty 2006) [8] and even developmental timing: calcium carbonate prolonged larval development by adding an extra moult (Sun *et al*. 2025) [23]. Future work should use a characterised *Aloe vera* preparation and measure how much the larvae actually eat.

5. What this means for sericulture in Bangladesh

Silk production in Bangladesh falls far short of demand (Jui *et al*. 2023) [7], and cheap supplements are attractive to small rearers. Our results suggest a realistic expectation for *Aloe vera* with the Nistari race: faster mid-instar growth and better egg hatching, but no more silk. That makes it potentially more useful to egg producers than to cocoon producers. If faster growth also shortens the larval period, it could reduce the time and leaf needed per crop, which is worth testing because development time was not recorded here.

6. Limitations

Several limitations apply. With three trays per group the design could detect only changes of roughly 10% to 36% depending on the trait, so results showing no effect mean no large effect. The composition of the juice was not known, the dose reflects the strength of the dipping solution rather than the amount eaten, and leaf consumption was not measured. Development time and post-cocoon silk traits such as filament length and denier were not recorded. Only one race and one rearing season were studied. Finally, a few recorded values were internally inconsistent; we handled them openly and showed that the conclusions do not depend on how they were treated.

7. Recommendations

Further work should use more trays per group, a chemically characterised *Aloe vera* preparation, measurement of leaf intake and development time, and post-cocoon traits such as filament length and reelability, and should compare Nistari directly with a commercial hybrid under the same supplement.

Table S1: (supplementary). Sensitivity of three traits to the data decisions described in Section 2.5.

Trait	Analysis	Change (%)	95% CI (%)	P
Cocoon weight	Main analysis	+15.9	+3.1 to +28.7	0.019
Cocoon weight	Pupal value 0.103 g included	+15.9	+3.1 to +28.7	0.019
Cocoon weight	Control tray 1 left out	+5.3	-4.0 to +14.6	0.237
Pupal weight	Main analysis	+1.1	-15.0 to +17.2	0.885
Pupal weight	Pupal value 0.103 g included	-4.9	-37.6 to +27.9	0.751
Pupal weight	Control tray 1 left out	-0.3	-20.3 to +19.7	0.974
Shell ratio	Main analysis	-11.8	-24.5 to +0.9	0.065
Shell ratio	Pupal value 0.103 g included	-11.8	-24.5 to +0.9	0.065
Shell ratio	Control tray 1 left out	-1.6	-12.4 to +9.1	0.743

Conclusions

Dipping mulberry leaves in *Aloe vera* juice made Nistari silkworm larvae grow faster in the middle of the feeding period and improved egg hatchability at every concentration tested, but final larval weight, cocoon shell weight and shell ratio did not change. Faster growth did not translate into more silk, which is consistent with the silkworm stopping growth at a fixed critical size. Supplements should therefore be judged by their effect on silk and on egg production directly, not by larval weight alone.

Declarations

Author contributions. Nazmul Hoque: investigation, data collection, formal analysis, writing of the original draft. Tandid Kamal: investigation, review and editing. Md. Ariful Hasan: conceptualization, methodology, supervision of the experiment, resources, review and editing. M. Habibur

Rahman: supervision, validation, review and editing, correspondence.

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Ethics. The study used insects only; no ethical approval was required.

Data availability. Tray-level data and analysis scripts are available from the corresponding author on request.

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