

RESEARCH ARTICLE



Effect of Formulated Artificial Diets on Growth Performance and Silk Production in the Silkworm (*Bombyx mori* L.)

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**Abstract**

Nutrition is a critical determinant of growth and silk productivity in the silkworm (*Bombyx mori* L.), yet reliance on mulberry leaves creates seasonal constraints, particularly in Bangladesh. This study assessed the effects of two formulated artificial diets, based on mulberry leaf powder, soy flour, and corn flour, compared with mulberry-fed controls in the BN/M multivoltine race. Key growth, cocoon, pupal, and silk fibre traits were measured, and multivariate analyses (PCA, correlation, and clustering) were applied to reveal the interrelationships among these traits. Results showed that mulberry leaves ensured superior larval growth and silk gland development, but artificial diets provided trait-specific benefits. Diet 1 enhanced the shell ratio (14.57%) and fibre length (401 m), while Diet 2 yielded heavier male (0.869 g) and female pupae (1.268 g). PCA separated growth/biomass traits from silk productivity traits, highlighting trade-offs between larval development and fibre yield. Strong correlations, such as between fifth-instar weight and silk fibre output, confirmed the biological linkage between nutrient intake and cocoon productivity. Clustering further distinguished artificial diet treatments from mulberry-fed controls, indicating distinct phenotypic responses. This is the first systematic evaluation of artificial diets for the BN/M race under Bangladeshi sericulture conditions. The findings demonstrate that while mulberry leaves remain indispensable, artificial diets can supplement rearing during lean seasons by enhancing specific economic traits. A cost analysis revealed that although artificial diets are slightly more expensive, they help offset seasonal shortages and sustain cocoon yields, thereby supporting sericulture resilience in regions with limited mulberry availability.

Keywords: Artificial diet, *Bombyx mori*, Cocoon traits, Larval growth, Silk gland.



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Introduction

The domesticated silkworm (*Bombyx mori* L.), belonging to the family Bombycidae, is the most crucial insect for commercial silk production. It originated from its wild progenitor, *B. mandarina*, which is distributed across northern India, China, Japan, and Korea, and was domesticated in China more than 5,000 years ago (Jolly et al. 1979). Over centuries of selective breeding, *B. mori* has lost traits such as flight, pigmentation, and the ability to survive without human assistance (Krishnaswami et al. 1973). Globally, silk production is dominated by a few countries: China and India together produce ~95% of raw silk, while the top six producers account for ~99.2% of global output in 2022 (Popescu et al. 2024, Dutta et al. 2025). In Bangladesh, sericulture is concentrated in Rajshahi and Nawabganj, providing meaningful rural income opportunities (Kamruzzaman et al. 2023). Mulberry leaves are the sole natural diet of *B. mori*, and leaf quantity and quality strongly affect larval growth, survival, cocoon weight, and silk yield (Islam et al. 2004). However, leaf nutritional value fluctuates seasonally and under different management practices, resulting in variations in cocoon yield and shell ratio (Mehraj et al. 2023, Karur et al. 2024). Reliance on fresh leaves requires year-round plantation maintenance, labor-intensive harvesting, and transport to rearing houses, thereby increasing production costs and reducing efficiency, particularly for smallholder farmers.

Artificial diets have been explored as a solution to these challenges. Early attempts in Japan during the late 1970s demonstrated the feasibility of diet rearing (Shinbo and Yanagaw 1994), and subsequent research has refined formulations and adaptation mechanisms (Li et al. 2023, Wang et al. 2024, Zhang et al. 2025). Diets typically combine mulberry leaf powder with cereal meals, vitamins, and antimicrobial agents (Cappelozza et al. 2005, Bhattacharyya et al. 2016), providing year-round, balanced nutrition under controlled conditions. Nutritional

studies confirm that protein and carbohydrate levels influence larval growth, silk gland development, and cocoon traits (Moon 1973, Hori 1995, Shifa 2016, Yungen 2000). Yet, large-scale adoption remains limited by high costs and the need for silkworm strains acclimatized to diet feeding through multi-generation selection (Hori and Watanabe 1983, Etebari et al. 2002, Saviane et al. 2014, Li et al. 2023). For Bangladesh, where climatic and resource constraints often reduce the availability of high-quality mulberry leaves during lean seasons (Nahiduzzaman et al. 2023), artificial diets could enable consistent cocoon production, lower costs, and enhance sustainability. The present study was therefore designed to develop and evaluate cost-effective artificial feed formulations for silkworm rearing, and to assess their effects on larval growth, pupal development, and silk production, with the aim of supporting year-round sericulture in Bangladesh and other regions with limited mulberry availability.

Materials and Methods

Study site and silkworm strain

The experiment was conducted under controlled laboratory conditions at the Bangladesh Sericulture Research and Training Institute (BSRTI), Rajshahi, Bangladesh. Disease-free layings (DFLs) of the BN/M race of *Bombyx mori*L., a high-yielding multivoltine variety producing spindle-shaped yellow cocoons, were obtained from the BSRTI germplasm bank for use in this study (Fig. 1).

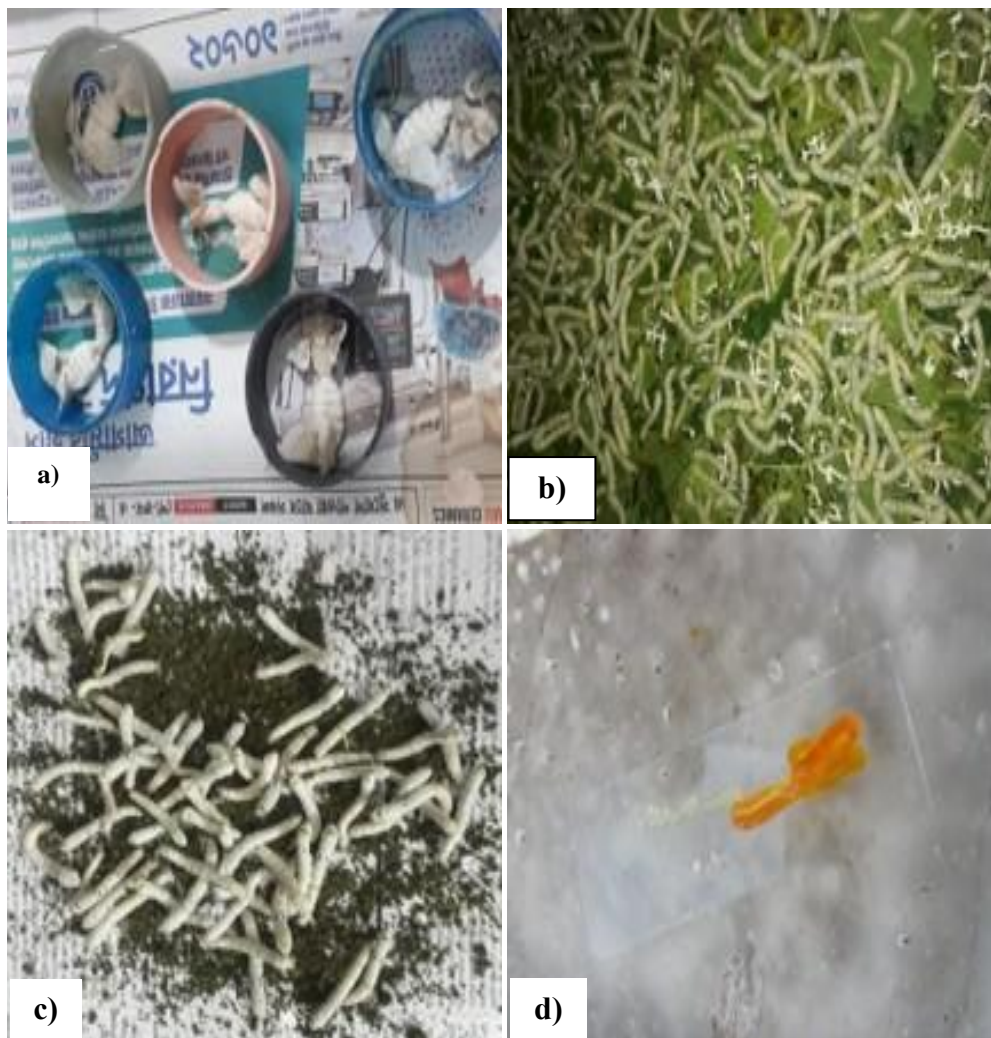


Fig. 1(a-d): Morphological features of the BN/M silkworm and its silk gland. (a) Adult moth of the BN/M race, (b) larva reared on mulberry leaves, (c) larva reared on an artificial diet, and (d) dissected middle silk gland.

Rearing conditions and equipment

Silkworm rearing was conducted in a dedicated rearing house maintained at $26 \pm 2^\circ\text{C}$ and $75 \pm 10\%$ relative humidity. Adequate aeration was provided by a high ceiling and air conditioning units. Larvae were reared in wooden almirahs ($1.5 \times 0.7 \times 2.5 \text{ m}^3$) designed to protect them from predators such as flies, lizards, and rats. The almirahs were placed on cemented ant wells half-filled with water and kerosene to prevent ant infestation. Standard rearing trays ($40 \times 25 \times 5 \text{ cm}^3$) were used to house the larvae, with polythene sheets and wet foam pads placed beneath the trays to maintain bed moisture. Temperature and relative humidity were monitored daily using wet-bulb and dry-bulb thermometers and hygrometers. Mulberry leaves, the primary feed source, were chopped on wooden boards with sharp knives, and a simple wooden leaf chamber was used to keep harvested leaves fresh. Handling of delicate larvae was performed hygienically using bamboo chopsticks (17-22 cm) and soft feathers to minimize injury during brushing and bed cleaning.

Experimental design and diet preparation

A Completely Randomized Design (CRD) was employed with three treatments: two artificial diets and a mulberry leaf control (Table 1). Each treatment included 100 larvae per replication, with three replications, for a total of 900 larvae. Fresh mulberry leaves (*Morus alba* L.) were harvested, washed, dried in a forced air dryer, and ground into fine powder. The powder was mixed with soya flour, corn flour (for Diet 1 only), and water to form a homogeneous paste. The mixture was microwaved for 3-8 minutes to eliminate microbial contaminants, cooled under aseptic conditions, refrigerated until firm, and cut into uniform pieces for feeding.

Table 1: Treatments and composition of artificial diets for silkworm rearing.

Genotype	Treatment	Composition	Diet composition (per 100 ml water)
BN/M (Multivoltine)	T ₁	Artificial diet 1 + mulberry leaves	25 g mulberry powder + 22.5 g soya flour + 2.5 g Corn flour
	T ₂	Artificial diet 2 + mulberry leaves	25 g mulberry powder + 25 g soya flour (no corn)
	Control	Mulberry leaves only	-

Feeding, mounting, and grainage procedures

Larvae were reared on fresh mulberry leaves until the completion of the second instar. From the onset of the third instar, larvae were divided into treatment groups as described in Table 1. Diet 1 and Diet 2 were supplied twice daily and supplemented with mulberry leaves at six-hour intervals (5:00, 11:00, 17:00, and 23:00), while the control group was maintained exclusively on mulberry leaves. Daily bed cleaning was performed to remove leftover leaves, excrement, and dead larvae, thereby maintaining hygienic conditions. During molting, feeding was suspended until 90-95% of the larvae had completed ecdysis, ensuring dry beds and reducing the risk of disease.

When the larvae reached maturity, as indicated by a translucent body and decreased feeding activity, they were transferred to bamboo mountages ("chandrakis") to facilitate cocoon spinning. Cocoons were harvested five days after completion of spinning and were used for the assessment of cocoon and pupal parameters. For grainage, only healthy, uniformly aged cocoons were selected, and defective cocoons were discarded. Male and female cocoons were separated and placed in grainage almirahs until moth emergence, which occurred between 06:00 and 08:00 h. Freshly emerged, healthy moths were allowed to mate for 3-6 hours under semi-dark conditions. Following mating, pairs were carefully separated, and female moths were transferred to egg-laying cards. After oviposition, eggs were examined microscopically, and layings from diseased moths were eliminated using the procedure described by Jolly (1983). The remaining disease-free layings were preserved for subsequent rearing cycles.

Data collection

Data were recorded from ten randomly selected individuals in each replication for all measured parameters. Larval weight was measured using an electronic balance, and larval length was determined with a compass and a centimeter scale. The middle silk gland was carefully dissected and weighed immediately. Cocoons were randomly sampled, and individual cocoon weights were recorded; pupae were then extracted by cutting open the cocoons, and their weights were measured. Shell weight was obtained by weighing the empty cocoons after pupal removal, and shell ratio was calculated as $(\text{Shell Weight}/\text{Cocoon Weight}) \times 100$. For reeling traits, silk fibre was reeled from 10 cocoons per replication, and both silk fibre weight and filament length were measured; mean values were used for statistical analysis.

Cost analysis

Each treatment was applied to 100 larvae in each cohort for one rearing cycle under standard rearing conditions. Input costs were estimated based on local market prices for land use, irrigation, labour, and feed ingredients (Table 2). Biological performance indicators included larval growth, silk gland development, pupal weight, shell ratio, and filament length. Practical feasibility was also evaluated in the context of seasonal mulberry leaf availability.

Table 2: Comparative cost-benefit analysis of mulberry leaves versus artificial diets for *Bombyx mori* rearing in Bangladesh.

Feeding regime	Estimated cost (per 100 larvae / 1 rearing cycle)*	Key advantages	Key limitations	Practical scenario
Mulberry leaves only (Control)	~USD 3.0–3.5 (land, irrigation, labour, leaf transport)	Superior larval growth, the highest silk gland weight, and traditional farmer familiarity	Seasonal fluctuations; labour- and land-intensive	Best during peak mulberry season (spring)
Artificial diet 1 + mulberry	~USD 4.0–4.5 (mulberry powder, soy, corn, preparation)	Higher shell ratio (14.6%), longest fibre (401 m); stable supply	Slightly higher input cost; requires preparation	Lean seasons (autumn–winter) to maintain fibre length and shell ratio
Artificial diet 2 + mulberry	~USD 4.5–5.0 (mulberry powder, soy; no corn)	Heavier male (0.869 g) and female pupae (1.268 g); supports biomass gain	More costly than mulberry; moderate fibre yield	Supplement during low leaf supply; improves pupal biomass for breeding

*Costs are approximate, based on local market prices of mulberry leaf production (land, irrigation, transport) versus artificial ingredients (soy flour, corn flour, processing). The values are illustrative and should be validated through field-based economic surveys.

Statistical analysis

All experiments were conducted with three independent biological replicates, and results are presented as mean \pm standard error (SE). Statistical significance was assessed using one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) at $p < 0.05$, performed with IBM SPSS Statistics (version 20.0; IBM Corp., USA). Multivariate analyses, including principal component analysis (PCA), PCA trait contribution, correlation matrix heatmaps, and hierarchical clustering dendrogram. Dendrograms and PCA plots were generated using OriginPro 9.1 (OriginLab Corp., USA) and Python (version 3.13.7-amd64.exe) with the appropriate libraries (pandas and matplotlib), ensuring reproducibility and robustness. Heatmaps were constructed to illustrate relative

expression profiles and parameter associations across treatments. Additional graphical representations, such as bar charts, line graphs, and scatter plots, were prepared using OriginPro 9.1. Meanwhile, Python-based scripts were utilized to validate and cross-check the multivariate analyses.

Results

The effects of formulated artificial diets on larval growth, silk gland development, cocoon traits, and silk fibre characteristics in *Bombyx mori* are presented below:

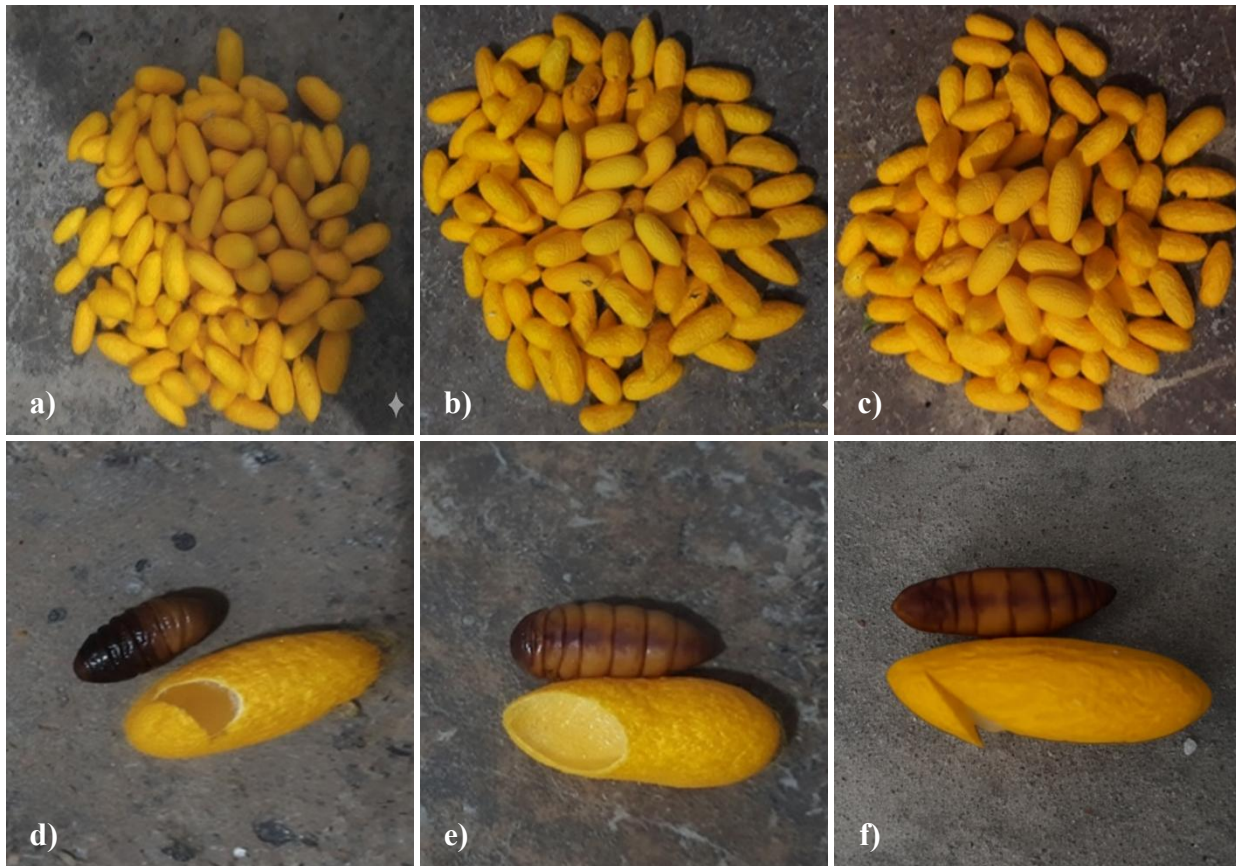


Fig. 2: Cocoon, pupal, and fiber characteristics of *Bombyx mori* under different treatments. (a) Cocoon from T₁, (b) Cocoon from T₂, (c) Cocoon from T₃ (control), (d) shell and pupa from T₁, (e) shell and pupa from T₂, (f) shell and pupa from T₃.

Larval growth

Growth responses varied across larval instars under different dietary treatments (Fig. 3). In the third instar, larval weight ranged from 0.176 g in T₁ to 0.250 g in the control, while larval length ranged from 2.30 cm in T₁ to 2.49 cm in the control. Differences were not statistically significant. By the fourth instar, diets exerted a stronger influence. Larval weight was significantly higher in the control (0.555 g) compared with T₁ (0.504 g) and T₂ (0.491 g) ($p < 0.05$). Larval length also varied significantly ($p < 0.05$), with control-fed larvae averaging 3.70 cm, compared to 3.60 cm in T₁ and 3.69 cm in T₂. In the fifth instar, larval weight ranged from 2.621 g in T₂ to 2.767 g in T₁, while the control averaged 2.669 g. Larval length showed a slight but significant difference ($p < 0.05$), varying between 6.54 cm in T₁ and 6.61 cm in the control.

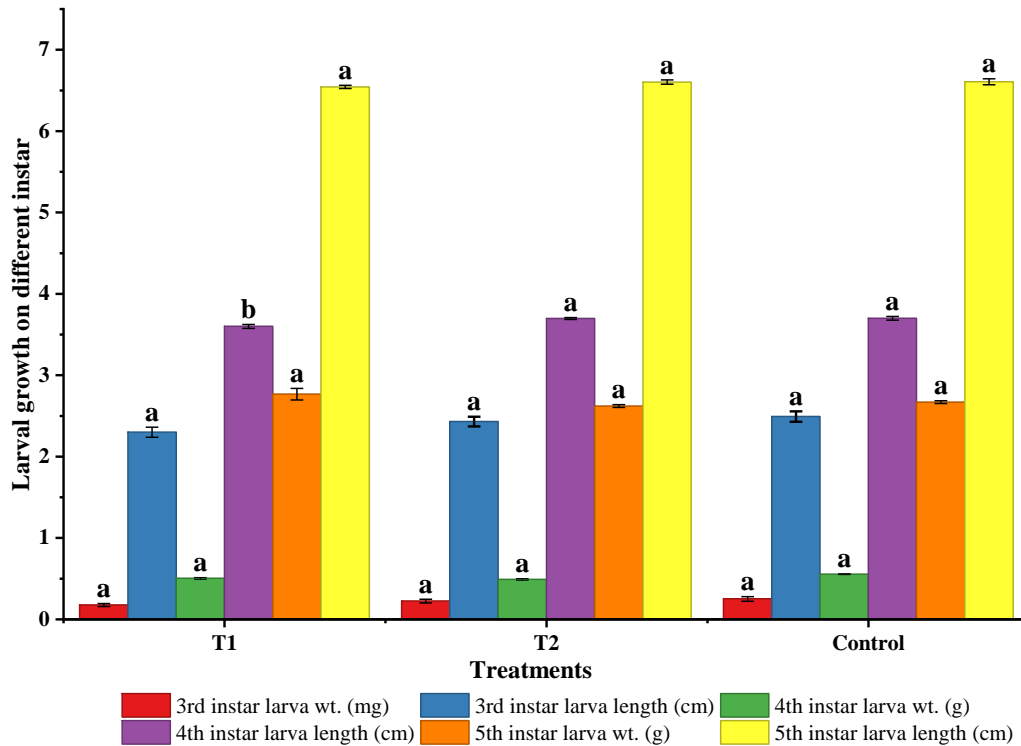


Fig. 3: Effects of formulated artificial diets on larval growth (3rd-5th instar weight and length) in *Bombyx mori* (BN/M multivoltine). Error bars indicate standard error (\pm SE). Means were compared using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$.

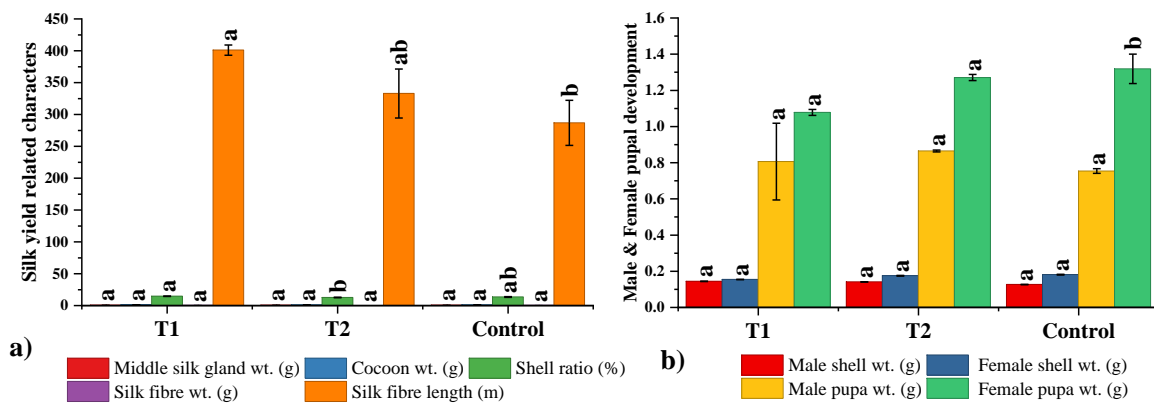


Fig. 4: Effects of formulated artificial diets on cocoon and silk production traits in *Bombyx mori* (BN/M multivoltine): a) Silk gland and Cocoon traits; b) Pupal and Sex-related traits. Error bars indicate standard error (\pm SE). Means were compared using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$.

Silk gland development

Middle silk gland weight was strongly affected by diet (Fig. 4a). The control group recorded the highest mean gland weight at 0.559 g, followed by 0.525 g in T₂, while the lowest was 0.501 g in T₁. The differences were highly significant ($p < 0.05$), underscoring the influence of diet composition on silk gland growth.

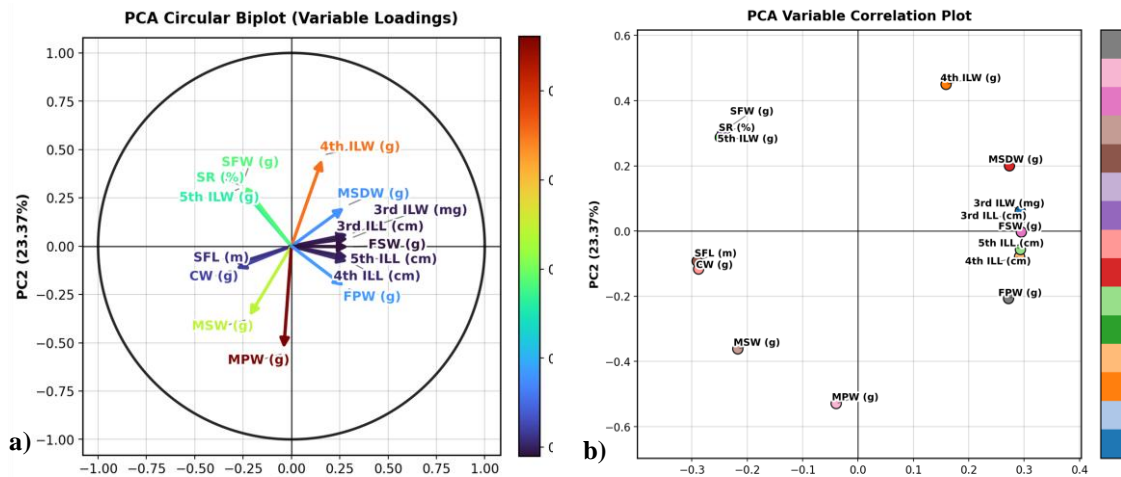


Fig. 5 (a-b): Principal component analysis (PCA) of 15 quantitative traits in silkworm: (a) PCA biplot showing trait loadings on the first two principal components (PC1 and PC2); and (b) Variable correlation plot displaying the clustering of traits based on their contributions to PC1 and PC2.

Cocoon traits

Cocoon weight showed no significant variation across treatments (Fig. 4a), with averages ranging from 1.27g in the control to 1.36 g in T₁. In contrast, the shell ratio differed significantly ($p < 0.05$). The highest ratio was recorded in T₁ (14.57%), followed by the control (13.35%), whereas T₂ exhibited the lowest ratio (12.70%). Shell and pupa weights also varied between sexes (Fig. 4b). Male shell weight was highest in T₁ (0.143 g) and lowest in the control (0.129 g), while female shell weight reached its maximum in the control (0.181 g) compared with 0.152 g in T₁. Male pupae attained maximum weight in T₂ (0.869 g), while the lowest was observed in the control (0.776 g). Female pupae were also heaviest in T₂ (1.268 g) compared with the lowest mean of 1.109 g in T₁.

Silk fiber characteristics

Although not statistically significant, differences in silk fiber weight and length were observed among treatments. Fiber weight was highest in T₁ (0.228 g), followed by the control (0.153 g), while T₂ recorded the lowest (0.108 g). Fiber length ranged from 401.07 m in T₁ to 286.87 m in the control, with T₂ producing an intermediate value of 333.00 m (Fig. 2 & 4a).

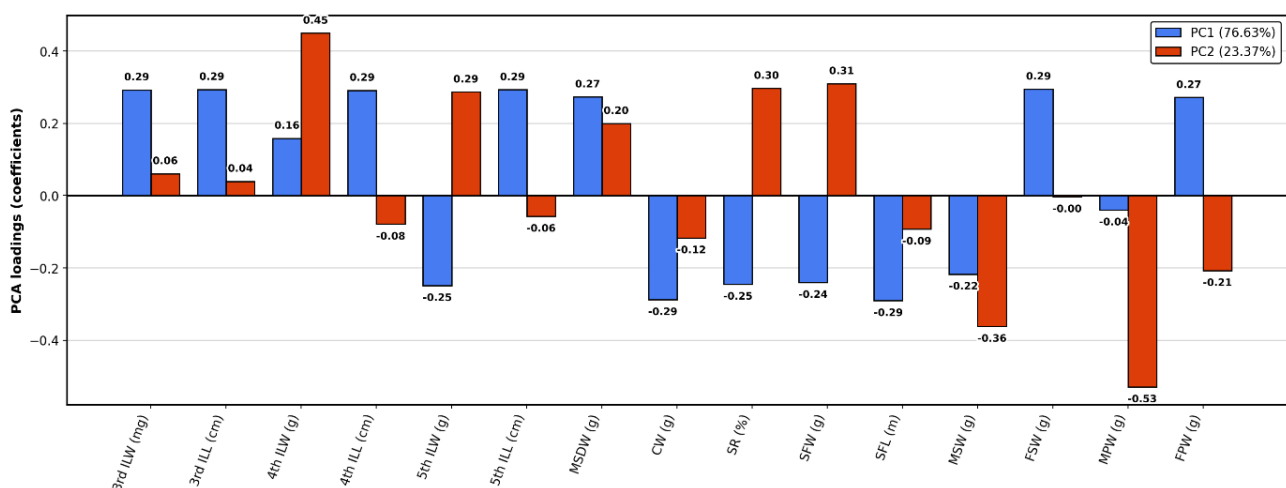


Fig. 6: Trait contributions of 15 silkworm characteristics to the first two principal components (PC1 and PC2). Bar plot showing PCA loadings of larval growth, cocoon, and silk traits on PC1 and PC2. Positive and negative values indicate the direction and strength of each trait's contribution to overall variance.

Artificial diets significantly influenced fourth instar larval weight and length, middle silk gland weight, and shell ratio, while effects on third and fifth instar growth, cocoon weight, and fiber traits were less pronounced. The control diet consistently produced the heaviest larvae and largest silk glands, highlighting the nutritional superiority of mulberry leaves. However, artificial diets demonstrated advantages in certain traits: T₁ enhanced cocoon shell ratio (14.57%) and fiber length (401.07 m), while T₂ supported heavier male (0.869 g) and female pupae (1.268 g). These findings suggest that although control feeding maximizes overall growth and gland development, selected artificial diets may offer targeted benefits for improving silk yield efficiency.

Comparative cost-benefit analysis

The control group (mulberry leaves only) showed superior larval growth, maximum silk gland weight, and strong farmer acceptance, with an estimated cost of USD 3.0-3.5 per cycle. However, this regime was constrained by seasonal leaf availability and labour intensity. Artificial Diet 1 (mulberry powder + soy + corn) increased the shell ratio (14.6%) and produced the longest filament (401 m). Although costs were slightly higher (USD 4.0-4.5), diet preparation ensured a stable food supply during lean mulberry seasons. Artificial Diet 2 (mulberry powder + soy) supported the heaviest pupae (males: 0.869 g; females: 1.268 g), enhancing biomass for breeding purposes. Costs were comparatively higher (USD 4.5-5.0), and filament yield was moderate (Table 2).

Multivariate analysis

Principal component analysis (PCA)

PCA was performed on 15 silkworm traits, including larval weights and lengths (3rd-5th instars), middle silk gland weight, cocoon weight, shell ratio, silk fibre weight and length, and male and female shell and pupa weights. The first two principal components, PC1 and PC2, explained 76.63% and 23.37% of the variance, respectively, and together accounted for nearly 100% of the total variation (Fig. 5a, b). PC1 showed strong positive loadings for third instar larva weight (0.293), third instar larva length (0.294), fourth instar larva length (0.292), fifth instar larva length (0.293), middle silk gland weight (0.274), and female shell weight (0.295). Conversely, it displayed negative loadings for fourth instar larva weight (-0.159), fifth instar larva weight (-0.249), cocoon weight (-0.288), shell ratio (-0.245), silk fibre weight (-0.240), silk fibre length (-0.291), and male shell weight (-0.217), indicating a clear contrast between larval growth traits and silk/cocoon productivity traits. PC2 was mainly influenced by positive loadings for fourth instar larva weight (0.450), fifth instar larva weight (0.287), shell ratio (0.297), and silk fibre weight (0.310). In contrast, strong negative loadings were observed for male pupa weight (-0.529), male shell weight (-0.362), female pupa weight (-0.207), and cocoon weight (-0.117). This suggests that PC2 reflects variation in late larval and pupal development, as well as sex-specific differences in biomass allocation. Taken together, the PCA differentiated growth-related traits (positive axis of PC1) from silk and cocoon traits (negative axis of PC1 and PC2), underscoring the trade-off between body growth and silk yield potential in silkworms (Fig. 5a, b).

The heatmap shows Pearson correlation coefficients among larval growth, cocoon, and silk-related traits. Positive correlations are indicated by shades of blue to green, while negative correlations are represented by shades of red to purple. Strong positive associations were observed between larval growth and cocoon productivity traits, as well as between cocoon weight and silk fiber length.

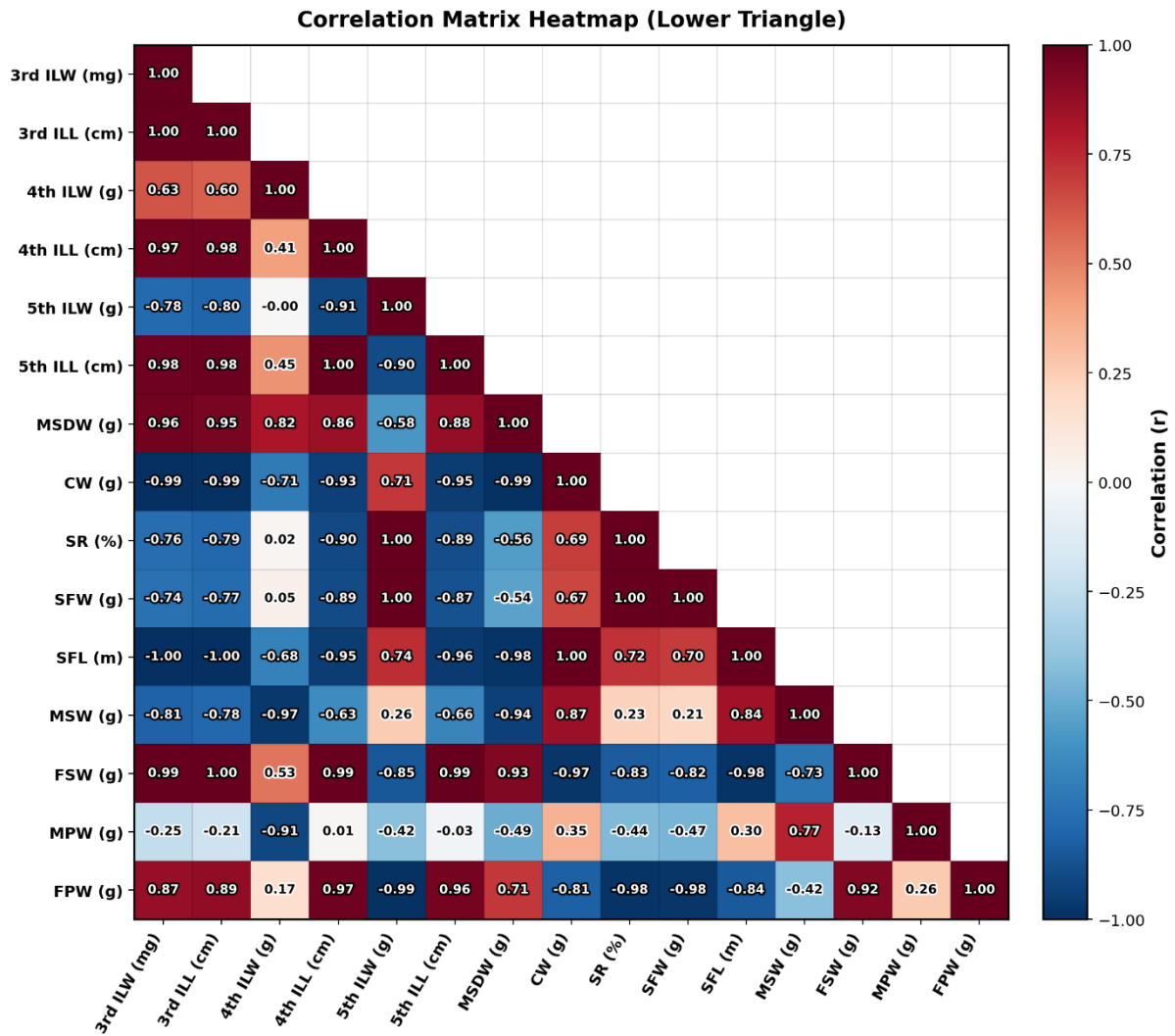


Fig. 7: Correlation matrix heatmap of 15 quantitative silkworm traits.

Trait contributions to PCA

The contribution analysis of 15 quantitative traits to the first two principal components (PC1 and PC2) is presented in Fig. 6. Together, PC1 and PC2 explained 76.63% and 23.37% of the total variation, respectively, accounting for the full phenotypic variance. PC1 was primarily driven by positive contributions from third instar larval weight and length, fourth instar larval length, fifth instar larval length, female shell weight, and middle silk gland weight, indicating that this axis essentially represents growth- and biomass-related variation. In contrast, traits such as cocoon weight, silk fibre length, and shell ratio contributed negatively, highlighting an inverse association between larval growth and silk/cocoon traits. PC2 showed strong positive contributions from fourth instar larval weight, shell ratio, and silk fiber weight, whereas male pupa weight, male shell weight, and, to a lesser extent, female pupa weight, loaded negatively. This indicates that PC2 primarily differentiates silk productivity traits from sex-specific pupal biomass allocation. Overall, the contribution plot clearly demonstrated that PC1 captured variation in larval growth, while PC2 separated silk yield traits from sex-dependent pupal traits, reinforcing the multivariate differentiation of silkworm phenotypes (Fig. 6).

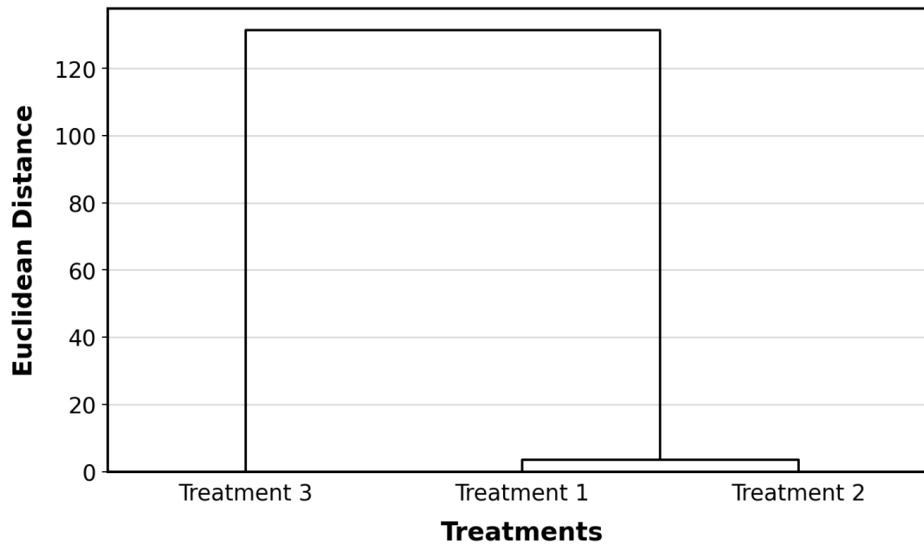


Fig. 8: Hierarchical clustering dendrogram of silkworm traits across treatments.

Correlation analysis

The correlation matrix revealed strong and significant associations among most silkworm traits (Fig. 7). Third instar larval weight and length were almost perfectly correlated ($r = 0.999$, $p < 0.001$) and also showed very high correlations with fourth instar larval length ($r > 0.96$, $p < 0.01$), reflecting coordinated growth patterns during early larval stages. Fifth instar larval weight exhibited strong positive correlations with both shell ratio ($r = 0.999$, $p < 0.001$) and silk fiber weight ($r = 0.999$, $p < 0.001$), suggesting that heavier larvae directly contribute to cocoon productivity. Cocoon weight and silk fiber length were nearly perfectly correlated ($r = 0.999$, $p < 0.001$), indicating that improved cocoon traits directly enhance filament length. Furthermore, female shell weight showed a strong positive association with female pupa weight ($r = 0.924$, $p < 0.01$), consistent with their biological relationship. Overall, the strong, mostly significant correlations ($p < 0.05$) highlight the tight interdependence among growth, cocoon, and silk traits in silkworms.

Hierarchical clustering of silkworm traits

Hierarchical clustering based on Euclidean distance grouped the treatments into two major clusters (Fig. 8). Treatment 1 and Treatment 2 were closely related, suggesting similar patterns of larval growth, cocoon, and silk-related traits under these conditions. In contrast, Treatment 3 (control) formed a distinct cluster, reflecting substantial divergence from the other two treatments. This separation highlights that Treatment 3 induced markedly different phenotypic responses compared to Treatments 1 and 2, indicating variability in growth and silk productivity traits under different experimental conditions. The dendrogram was constructed using Euclidean distance to assess treatment similarity. Treatments 1 and 2 formed a close cluster, while Treatment 3 separated distinctly, indicating greater divergence in trait performance.

Discussion

The present study demonstrated that artificial diets can significantly influence certain growth, cocoon, and silk fiber traits in *Bombyx mori*. However, mulberry leaves remain the most effective feed for overall larval development and silk gland growth. Early instar (third instar) traits were largely unaffected by diet, indicating a strong dependence on the nutritional composition of mulberry leaves at this stage. Similar results were reported by Cappellozza et al. (2005), who observed that vitamin C deprivation in artificial diets did not impair early survival but reduced subsequent cocoon yield. By the fourth instar, larval weight and length were significantly higher in the control, confirming the superior nutritional value of mulberry. This pattern agrees with Yin et al. (2023), who found that silkworms reared entirely on artificial diets exhibited reduced cocoon weight, cocoon shell rate, and silk gland index compared with mulberry-fed larvae. However, in our study, artificial diets demonstrated clear trait-specific advantages. Diet 1 increased the shell ratio (14.57%) and fiber length (401 m), while Diet 2 supported heavier male and female pupae. Comparable outcomes were reported in India by Trivedy et al. (2001 & 2003), who demonstrated that carefully formulated artificial diets could maintain cocoon yields. Additionally, Nakada and Murakami (1991) and Bhattacharyya et al. (2016) noted improved silk quality traits with protein supplementation. These findings

highlight that while mulberry leaves maximize growth and silk gland development, artificial diets can strategically enhance certain economic traits.

The effects of artificial diets on silk gland weight were also noteworthy. Although mulberry-fed larvae produced the heaviest glands, Diet 2 supported substantial gland development, suggesting that balanced formulations with mulberry powder, soy flour, and corn flour may partially substitute for mulberry. Similar improvements have been reported in Japan and China, where soy-based enrichment enhanced silk gland development and cocoon quality (Shinbo and Yanagaw 1994, Huang et al. 2013). Our results further align with those of Zhang et al. (2024), who demonstrated that rearing methods significantly influence cocoon silk strength, underscoring the importance of diet composition and rearing system in determining fiber properties.

Notably, this research bridges the gap between laboratory-based diet formulations developed in Japan, China, and India, and the regional context of Bangladesh's sericulture, where seasonal and climatic factors limit mulberry availability. The demonstration that artificial diets can partially sustain growth and improve trait-specific outcomes in the BN/M multivoltine race provides valuable region-specific insights for sustaining cocoon yield during lean mulberry seasons. Formulated artificial diets reshaped the multivariate phenotype of *Bombyx mori* by differentially influencing growth and silk productivity traits. The PCA clearly separated a growth/biomass axis (positive PC1: early instar weights and lengths, middle silk gland, female shell) from a silk/cocoon axis (negative PC1: cocoon weight, shell ratio, silk fibre traits) and a secondary axis contrasting late-instar and sex-specific biomass allocation (PC2). This pattern is consistent with earlier reports linking larval weight and silk gland development with cocoon yield (Horie 1980, Akai et al. 1985, Ghosh et al. 2019).

The correlation analysis (Fig. 7) revealed near-perfect positive associations among key economic traits, for example, fifth-instar larval weight with shell ratio and silk fiber weight, and cocoon weight with filament length, paralleling previous findings that heavier larvae contribute directly to cocoon and shell weights (Şahan et al. 2016, Yadav et al. 2025). These strong correlations are biologically plausible since improved nutrient intake enhances silk gland biosynthesis, thereby increasing shell deposition and filament length (Horie 1980). The dendrogram (Fig. 8) clustered Treatments 1 and 2 closely, while Treatment 3 (control) diverged, indicating greater phenotypic differences under the control diet. Similar clustering patterns were reported in studies where artificial diets enriched with protein or mineral supplements significantly improved cocoon weight, shell ratio, and silk filament length compared to feeding only on mulberry (Majumder 1982, Shi et al. 2022, Koul and Sethi 2023).

Sex-specific differences were also evident along PC2, with negative loadings for male pupae and shells and positive associations with silk productivity traits. This finding aligns with reports that male and female silkworms allocate biomass differently, with hormonal regulation influencing silk gland development and yield (Sutrisno et al. 2020, Wang et al. 2024). The results confirm mulberry leaves as the most economical and biologically effective option, consistent with earlier reports that highlight the superiority of fresh leaves for larval growth and silk gland development (Dong et al. 2017). However, dependence on mulberry is constrained by seasonal leaf shortages (Saviane et al. 2014). Supplementation with artificial diets improved cocoon traits: Diet 1 enhanced the shell ratio and fiber length, in agreement with prior findings that semi-synthetic diets improve silk quality (Gheorghe 2023). Diet 2 favoured pupal biomass, reflecting the role of protein sources, such as soybean meal, in enhancing pupal weight (Bhattacharyya et al. 2023). However, full artificial feeding across all instars may reduce silk gland performance and alter metabolism (Yin et al. 2023). Therefore, an integrated approach of mulberry leaves in peak seasons, supplemented with artificial diets during lean periods, appears to be the most practical for sustainable sericulture in Bangladesh.

Taken together, our findings align with and extend prior studies: (i) larval growth and silk gland size are strong predictors of cocoon performance; (ii) artificial diets, when balanced for macronutrients, can match or exceed mulberry feeding in supporting silk production; and (iii) sex-related variation is an important factor shaping trait expression. Practically, these results emphasize the need for diet formulations that optimize early instar growth without reducing silk gland allocation, and for sex-aware management strategies to maximize reeling yield. The novelty of this study lies in its first systematic evaluation of artificial diets for the BN/M race under Bangladeshi sericulture conditions, demonstrating not only the limitations but also the targeted advantages of diet-based rearing. These findings contribute practical knowledge for supplementing mulberry feeding and improving the resilience of silk production systems. While mulberry leaves remain the most economical option during peak production, artificial diets provide resilience during lean seasons. The illustrative cost-benefit analysis suggests that Diet 1 may be particularly useful for maintaining fiber length and shell ratio, whereas Diet 2 supports pupal biomass. Adoption of such diets during seasonal shortages could stabilize cocoon yields despite slightly higher input costs. The present investigation acknowledges certain limitations, including the absence of biochemical profiling of diets,

molecular markers of silk protein expression, and an economic feasibility analysis. However, these represent clear avenues for future research. Nutrient and amino acid profiling, digestive enzyme assays, and analysis of fibroin/sericin gene expression could help clarify mechanistic links between diet composition and silk productivity.

Taken together, the findings reaffirm mulberry leaves as the gold standard but demonstrate that artificial diets, particularly those supplemented with soy and corn flour, offer a promising supplementary strategy for stabilizing cocoon yields and improving specific silk quality traits. This positions artificial diets as a valuable complementary tool for the sustainable development of sericulture in Bangladesh and other regions facing similar agro-ecological challenges.

Conclusion

This study confirms that mulberry leaves remain the most effective feed for *Bombyx mori*, producing superior larval growth and silk gland development. However, artificial diets incorporating mulberry powder, soy flour, and corn flour demonstrated notable trait-specific benefits, including higher shell ratio, heavier pupae, and longer fiber length. This represents one of the first systematic evaluations of artificial diets for the BN/M race in Bangladesh, highlighting their potential as a practical supplement during lean seasons when mulberry availability declines. Although artificial diets cannot fully replace mulberry leaves, they provide a valuable tool for stabilizing cocoon yields and supporting sericulture sustainability. A cost analysis shows that artificial diets are slightly more expensive than mulberry leaves, but they help sustain production during leaf shortages. Future work should focus on refining nutrient composition and exploring the long-term adaptability of silkworm strains to diet-based rearing.

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Authors' contributions (CRediT Taxonomy)

Nadia Sarkar, Mst Papia Khatun and Rumana Ferdus Bint-A-Rahman: Methodology, Investigation, Data Curation, and visualization; Abdur Rahim and Md Shakil Rahman Shoagh: Formal Analysis, Visualization; Razaul Karim: Writing-Review & Editing, Visualization; Md Monzur Hossain: Conceptualization, Supervision, Writing-Review & Editing; Md Nasiruddin: Conceptualization, Supervision, Writing original draft-Review & Editing.

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