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Genotype × diet interaction in Uzbek breeds and hybrids of the silkworm (*Bombyx mori*) for enabling year-round sericulture

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Abstract. *Bakhtiyar M, Anvar M, Diloram I, Arzigul T, Ismoil R. 2025. Genotype × diet interaction in Uzbek breeds and hybrids of the silkworm (*Bombyx mori*) for enabling year-round sericulture. Asian J Agric 9: 808-817.* Sericulture in Uzbekistan faces seasonal limitations due to dependence on fresh mulberry leaves, limiting cocoon production to one or two cycles per year. This study evaluated the interaction between genotype and diet type in ten *Bombyx mori* genotypes—five pure breeds (*Ipakchi-5, UzNIISH-9, Ipakchi-1, Gulshan,* and *Nafis*) and five hybrids (*Zarafshon, Gulshan × Nafis, Oltin Vodiy, Kumush Tola,* and *Navruz*)—to assess adaptability to artificial feeding for year-round sericulture. Larvae were reared on four diets: freeze-dried mulberry leaves, convectively dried leaves, a Japanese standard artificial diet, and natural leaves as a control. Biological and technological traits were analyzed using two-way ANOVA (Diet × Genotype) and Tukey's HSD test. Results showed significant effects of both diet and genotype ($p<0.001$). The freeze-dried mulberry diet ensured the highest survival (86-90%), cocoon weight (1.7-1.85 g), and silk ratio (18.5-20.3%), statistically comparable to natural feeding. Hybrids *Gulshan × Nafis* and *Navruz* exhibited superior adaptability and physiological stability, confirming strong genotype × environment interaction. The study demonstrates that locally developed freeze-dried diets enable sustainable, year-round, and industrially scalable sericulture in Uzbekistan, supporting both traditional textile and emerging biomedical applications of silk.

Keywords: Artificial diet, *Bombyx mori*, cocoon traits, freeze-dried mulberry leaves, Uzbek breeds and hybrids

INTRODUCTION

Sericulture has long been a vital component of Uzbekistan's agricultural heritage, providing rural employment and contributing substantially to the country's exports. The silkworm (*Bombyx mori* L.) and its product—natural silk—have sustained livelihoods for centuries, forming an integral part of cultural identity and economic development (Nagaraju 2002). Recently, silk has also emerged as a high-value biomaterial due to the unique properties of fibroin—its biocompatibility, tensile strength, and biodegradability—making it suitable for applications in biomedical engineering, including surgical sutures, tissue scaffolds, and drug delivery systems (Chen et al. 2023). This dual role of silk, both as a traditional textile material and as a modern biomaterial, underscores the strategic importance of revitalizing sericulture in Uzbekistan within a global innovation framework.

Traditional silkworm rearing depends entirely on the availability of fresh mulberry (*Morus alba* L.) leaves, which are harvested during a narrow seasonal window, typically from late April to June. This strong dependence on a short harvesting period restricts cocoon production to one or two cycles per year, limiting productivity and income stability for sericulturists. Seasonal fluctuations in temperature, precipitation, and pest incidence also affect leaf yield and nutritional composition, leading to unpredictable cocoon quality and reduced economic efficiency (Ito 1978). Consequently, the modernization of sericulture in Uzbekistan demands an innovative approach that minimizes climatic dependency and ensures continuous production cycles.

Artificial Diets (ADs) have been developed to address this limitation by replicating the nutritional profile of mulberry leaves using powdered leaf material combined with proteins, carbohydrates, vitamins, and minerals (Janarthanan et al. 1999; Cappellozza et al. 2005; Bhattacharyya et al. 2016; Paudel et al. 2020). These diets enable silkworm rearing under controlled indoor conditions, independent of leaf seasonality. However, large-scale use remains restricted due to the high cost of diet ingredients, the complexity of preparation, and genotype-dependent variability in larval growth and silk yield (Ramesha et al. 2012; Saviane et al. 2014; Zhao et al. 2024). The critical challenge is therefore to optimize diet formulation and identify silkworm strains that maintain high productivity and survival under artificial feeding conditions.

The drying process of mulberry leaves used in diet preparation plays a crucial role in determining nutritional quality. Convective drying, though inexpensive and widely used, causes significant losses of vitamins, amino acids, and chlorophyll due to heat exposure. In contrast, freeze-drying (lyophilization) preserves these nutrients by sublimation under vacuum, maintaining the biochemical integrity of the leaf powder (Katsube et al. 2009; Wang et al. 2023). Integrating freeze-dried leaf powder into artificial diets thus represents a promising direction for enhancing larval performance, disease resistance, and silk fiber properties.

Uzbekistan possesses a rich genetic resource base, maintaining over 120 *B. mori* genotypes within its national sericultural germplasm collection. Yet, systematic evaluation of these local breeds and hybrids on artificial diets remains limited. Earlier small-scale trials at the Sericulture Research Institute (Tashkent) demonstrated survival rates above 80% when using diets containing 70-75% freeze-dried leaf powder (Anvarovich et al. 2024). However, those preliminary tests covered only a few genotypes and lacked comparative assessment across multiple diet types. The genotype-by-diet interaction, which determines both adaptability and economic viability, has not yet been comprehensively characterized.

Understanding this interaction is essential for two main reasons. First, genotype responses to artificial diets vary widely, influenced by differences in metabolic efficiency, silk gland physiology, and gut microbiota composition (Dong et al. 2017, 2018; Chen et al. 2024). Second, identifying genotypes capable of maintaining high survival, rapid growth, and stable cocoon characteristics on artificial feed will enable selective breeding for diet adaptability. This genetic–nutritional optimization would form the basis for sustainable and industrial-scale sericulture in Uzbekistan.

Traditionally, sericulture in Uzbekistan has been confined to a single productive season, but the implementation of controlled-environment rearing combined with artificial diets offers the potential for four to five rearing cycles annually. Such intensification could effectively triple annual cocoon output, maximize the use of rearing facilities, and stabilize supply for both the textile and biomedical industries. In this context, the present study aims to evaluate the interaction between genotype and artificial diet type among Uzbek silkworm breeds and hybrids. By comparing larval survival, cocoon yield, and silk ratio under different feeding systems, this research seeks to identify the most diet-adaptable genetic lines capable of supporting year-round sericulture and high-quality silk production.

MATERIALS AND METHODS

The experiment was conducted in the spring of 2025 at the Research Institute of Sericulture (Tashkent, Uzbekistan). Ten genotypes of *B. mori* were used, including five pure breeds (*Ipakchi-5*, *UzNIISH-9*, *Ipakchi-1*, *Gulshan,* and *Nafis*) and five hybrids (*Zarafshon*, *Gulshan* × *Nafis*, *Oltin Vodiy*, *Kumush Tola*, and *Navruz*). Larvae were reared on four feeding variants: (i) an artificial diet based on freeze-dried mulberry leaves; (ii) an artificial diet based on convectively dried mulberry leaves; (iii) a standard Japanese artificial diet; and (iv) fresh mulberry leaves (control).

Preparation of freeze-dried mulberry leaves

Mulberry leaves collected in spring 2025 from the institute's plantation were freeze-dried using a "Scientz-50F/A" lyophilizer under the following conditions: preliminary freezing at -40°C with the maximum cooling rate; primary drying at a residual pressure of 0.3 mm Hg with a gradual increase in condenser temperature up to +35°C; total process duration 18-23 h depending on layer thickness. Optimal results were achieved with deep freezing (-40 to -42°C) followed by rapid stabilization, which ensured the preservation of cellular integrity and nutritional components. Drying above –30°C deteriorated palatability and increased larval mortality. The resulting freeze-dried leaves contained 7-8% moisture, were brittle, and readily absorbed ambient humidity. When stored in sealed polyethylene bags at room temperature, they retained nutritional value for an extended period (Lamberti et al. 2019).

Preparation of convectively dried mulberry leaves

For the second feed variant, fresh mulberry leaves were dried in a hot-air convective oven ("Eyela WFO-1020W") at 60±2°C for 8-10 h until a final moisture content of 7-9%. Although widely used for feed additive preparation, this method leads to partial vitamin loss and protein instability (Trajković et al. 2025).

Preparation of artificial diets

Freeze-dried and convectively dried leaves were ground into a fine powder and mixed with cellulose and agar powder. Vitamins, trace minerals, and fructose were dissolved separately in distilled water (Chen et al. 2024). The liquid phase was gradually poured into the pre-measured dry mixture packaged in heat-resistant polyethylene bags and stirred until a homogeneous paste was formed. The mixture was thermally treated in a microwave oven (200 g portion) at 900 W for 5 min (3 min heating + 2 min holding + 2 min re-heating), reaching an internal temperature of 88-90°C, which ensured complete gelatinization. After cooling, the final pH of the diet was 6.4±0.1, a mildly acidic environment optimal for *B. mori* larvae.

Two separate diets were thus obtained —one based on freeze-dried and another on convectively dried mulberry leaves. The final moisture content of the diets was 70-72%. The prepared mixtures were stored at 3±1°C for no more than 7 days. Agar used in the artificial diets had a bloom strength of ≈900 g cm^{-2}. Each batch underwent quality control for pH, moisture, and microbial load (CFU<10^3 g^{-1}); no spoilage was detected.

A feed composition was formulated for two rearing variants —one based on freeze-dried mulberry leaves and another based on convectively dried leaves (Table 1).

Preparation of standard Japanese artificial diet

The commercial Japanese standard diet (Shinbo and Yanagawa 1994; Horie 1995) was prepared according to the manufacturer's instructions by rehydrating 200 g of diet powder in 600 mL of distilled water and sterilizing at 90°C for 5 min. No composition modifications were made.

Table 1. Percentage composition of the artificial diet

Component	% of dry mass
Mulberry leaf powder (freeze-dried or convectively dried)	71.0
Cellulose	18.0
Sucrose	5.0
Inositol	0.06
Choline	0.04
Ascorbic acid	1.0
Citric acid	1.8
Agar-agar	2.7
Propionic acid	0.2
KH_2PO_4 (potassium dihydrogen phosphate)	0.2
Distilled water	600 mL

Experimental feeding design and rearing conditions

Egg incubation followed the standard procedure of Nagaraju et al. (2010), while larval rearing was carried out according to the modern protocol (Cappellozza et al. 2005; Bhattacharyya et al. 2016). Experimental groups reared on artificial diets were maintained at 26.5-27°C and 70-75% relative humidity (Yuri and Sahara 2017; Chen et al. 2024). Control groups were maintained under conventional rearing conditions at 25-26°C and 65-70% relative humidity (Hussain et al. 2011). Environmental parameters were continuously monitored and later included as covariates in the ANOVA model to account for microclimatic effects. Each shelf measured 0.45 m^2 and housed 150 larvae. Ventilation rate was 0.2 m s^{-1} with a 14 h light: 10 h dark photoperiod.

Larvae fed on artificial diets were given food once daily at 8:00 a.m. until cocoon formation. Rearing was conducted under controlled semi-sterile conditions on three-tier shelves simulating production environments. During the first three instars, approximately 4 g of artificial diet were supplied per 100 larvae per day; in later instars, up to 200 g. Feed residues were removed daily to prevent microbial contamination (Wu et al. 2024).

Control groups reared on natural mulberry leaves were fed six times daily during the first three instars (every 4 h) with approximately 10 g of fresh leaf per 100 larvae per day. During later instars, feeding was reduced to four times daily (every 6 h) with up to 300 g of leaf per 100 larvae per day (Ahmed et al. 2025). Rearing was also conducted under controlled semi-sterile conditions on three-tier shelves.

Differences in microclimate between feeding regimes reflected the established technological standards of artificial versus natural rearing systems and were therefore regarded not as experimental error but as inherent to each respective rearing method. Similarly, differences in feeding frequency reflected standard sericultural practice and were not treated as confounding factors.

Evaluated parameters

Throughout the experiment, both biological and technological parameters were evaluated, including larval survival (%), duration of development (days), cocoon weight (g), shell weight (g), and shell ratio (%). Each "Diet × Genotype" combination included three replicates (shelves) with 150 larvae each (n = 450 per combination). Cocoon parameters were determined from 50 randomly selected cocoons per replicate (n = 150 per genotype × diet). The shell ratio was calculated as: Shell ratio (%) = (Shell weight / Cocoon weight) × 100.

Statistical analysis

All data were analyzed using two-way ANOVA with fixed factors (Diet, Genotype) and their interaction (Diet × Genotype), with shelf treated as a random effect. Environmental parameters (temperature, humidity) were included as covariates in a mixed-model framework. Replicates were treated as random effects. Percentage data were arcsine-transformed prior to analysis; homogeneity of variances was tested using Levene's test. Post-hoc comparisons were performed using Tukey's HSD at $\alpha = 0.05$. All results are reported as mean ± SE based on three biological replicates (n = 3), and coefficient of variation (CV%) = $(SD/\bar{X}) \times 100$. Statistical processing was performed using IBM SPSS v.26.

RESULTS AND DISCUSSION

The survival of *B. mori* larvae reared on different types of artificial diets showed considerable variation, determined by both the genetic characteristics of the tested breeds and hybrids and the specific composition of the feed mixtures. The level of adaptation to artificial feeding varied among genotypes, indicating the presence of genotype–environment interactions (G×E effect) typical for the transition from traditional mulberry leaf feeding to artificial diets. It should be emphasized that the studied breeds and hybrids were exposed for the first time to artificial diets without prior selective breeding for adaptation to this type of nutrition, which may have contributed to the increased sensitivity of certain genotypes to changes in the nutritional environment and to the observed physiological instability of larvae during early instar stages. Such differences could potentially be minimized in the future through targeted selection or gradual adaptation of breeding lines to artificial feeding conditions. The results of the biological parameters of *B. mori* breeds and hybrids reared on different diets are presented in Tables 2 and 3, while Figure 1 illustrates the comparative survival rates under the various feeding treatments.

The data clearly demonstrate that larval survival and cocooning performance are strongly affected by both diet composition and genotype. Across all breeds and hybrids, the freeze-dried mulberry leaf diet provided the most favorable biological outcomes, followed by the Japanese artificial diet, whereas the convectively dried diet consistently showed the lowest performance. These results emphasize the crucial role of the leaf preservation method in determining the nutritional and physiological quality of artificial feeds.

Among pure breeds, Ipakchi-5 and Gulshan exhibited the highest and most stable survival rates across all artificial diets —85.3% and 84.3% on the freeze-dried diet and 84.6% and 84.1% on the Japanese diet, respectively. These values were only 10-12% lower than those observed under natural feeding (95.1-91.1%), indicating strong adaptive potential to artificial nutrition. Ipakchi-1 maintained intermediate survival levels (≈79.3-81.1%) under both freeze-dried and Japanese diets, while *UzNIISH-9* and *Nafis* were less tolerant, with survival decreasing to 69-74% on the convective diet. These findings confirm that lyophilization preserves essential nutrients such as proteins, vitamins, and chlorophylls, thereby supporting larval metabolism and silk synthesis. In contrast, convective drying causes partial protein denaturation and vitamin degradation, prolonging larval development (29-30 days versus 26-27 days for natural feeding) and reducing overall vitality.

Table 2. Biological characteristics of silkworm breeds reared on artificial diets compared with those reared on natural mulberry leaves

Breed	**Number of spun cocoons (pcs)**						**Survival rate (%)**						**Duration of rearing (days)**	
	AD			**Leaf**			**AD**			**Leaf**			**AD**	**Leaf**
	$\bar{X}$±SD	**SE**	**Cv (%)**	**$\bar{X}$±SD**	**SE**	**Cv (%)**	**$\bar{X}$±SD**	**SE**	**Cv (%)**	**$\bar{X}$±SD**	**SE**	**Cv (%)**		
Based on freeze-dried mulberry leaves														
Ipakchi-5	128.0±0.50	0.29	3.4	142.7±1.20	0.73	1.9	85.3±0.33	0.19	3.3	95.1±0.80	0.49	1.8	28	27
UzNIISH-9	108.0±1.50	0.87	5.1	134.0±1.04	1.04	2.8	72.0±1.00	0.58	5.1	89.3±1.10	0.65	2.8	27	26
Ipakchi-1	121.7±2.08	1.20	3.9	142.3±1.04	0.60	1.9	81.1±0.84	0.49	3.8	94.9±0.69	0.40	1.8	28	27
Nafis	111.3±1.04	0.60	4.8	139.0±0.50	0.29	2.3	74.2±0.70	0.40	4.9	92.7±0.33	0.19	2.3	28	27
Gulshan	126.7±1.25	0.72	3.5	136.7±1.25	0.72	2.6	84.3±0.83	0.48	3.5	91.1±0.83	0.48	2.5	27	26
Based on convectively dried mulberry leaves														
Ipakchi-5	112.7±1.52	0.88	4.7	143.7±1.26	0.73	2.0	75.1±1.02	0.59	4.6	95.8±0.84	0.49	2.0	30	27
UzNIISH-9	104.0±2.30	1.33	5.4	134.0±1.80	1.04	2.8	69.3±1.54	0.89	5.4	89.3±1.12	0.65	2.7	29	25
Ipakchi-1	108.3±1.52	0.88	5.1	143.0±0.50	0.29	1.9	72.2±1.02	0.59	5.0	95.3±0.33	0.19	1.9	29	26
Nafis	98.0±2.25	1.30	6.0	140.0±0.50	0.29	2.3	65.3±1.50	0.87	6.1	93.3±0.33	0.19	2.3	30	27
Gulshan	109.7±2.00	1.15	5.0	138.7±1.26	0.73	2.6	73.1±1.17	0.67	5.1	92.4±0.84	0.49	2.5	29	26
Based on Japanese artificial diet														
Ipakchi-5	127.0±2.29	1.33	3.5	143.3±0.76	0.44	1.9	84.6±1.53	0.89	3.5	95.6±0.51	0.30	2.0	28	27
UzNIISH-9	106.7±1.26	0.73	5.2	137.7±0.76	0.44	2.8	71.1±0.84	0.49	5.1	91.8±0.51	0.30	2.6	27	25
Ipakchi-1	119.0±2.30	1.33	4.2	142.0±0.87	0.50	1.9	79.3±1.53	0.88	4.3	94.7±0.58	0.33	1.9	28	26
Nafis	109.3±2.25	1.30	5.0	138.3±0.76	0.44	2.3	72.9±1.50	0.87	5.0	92.2±0.51	0.30	2.4	28	27
Gulshan	126.3±1.77	1.02	3.6	139.3±0.44	0.55	2.6	84.1±1.17	0.67	3.7	92.9±0.51	0.30	2.5	27	26

Table 3. Biological characteristics of silkworm hybrids reared on artificial diets compared with those reared on natural mulberry leaves

Hybrid	**Number of spun cocoons (pcs)**						**Survival rate (%)**						**Duration of rearing (days)**	
	AD			**Leaf**			**AD**			**Leaf**			**AD**	**Leaf**
	$\bar{X}$±SD	**SE**	**Cv (%)**	**$\bar{X}$±SD**	**SE**	**Cv (%)**	**$\bar{X}$±SD**	**SE**	**Cv (%)**	**$\bar{X}$±SD**	**SE**	**Cv(%)**		
Based on freeze-dried mulberry leaves														
Zarafshon	128.0±1.00	0.58	3.4	141.0±0.58	0.58	2.0	85.3±0.67	0.39	3.5	94.0±0.67	0.39	2.0	28	27
Gulshan × Nafis	133.0±0.50	0.29	2.9	139.0±0.50	0.29	2.1	88.7±0.34	0.19	3.0	92.7±0.33	0.19	2.2	27	26
Oltin Vodiy	125.0±1.00	0.58	4.0	142.3±0.76	0.44	2.1	83.3±0.67	0.39	4.0	94.9±0.51	0.30	2.0	28	26
Kumush Tola	126.3±0.76	0.44	3.5	143.7±1.52	0.88	1.7	84.2±0.51	0.30	3.0	95.8±0.84	0.49	1.7	28	27
Navruz	134.3±0.76	0.44	2.8	144.0±1.00	0.58	2.0	89.6±0.51	0.30	2.7	96.0±0.67	0.39	2.1	27	26
Based on convectively dried mulberry leaves														
Zarafshon	114.3±2.08	1.20	4.7	141.7±0.76	0.73	2.0	75.1±1.02	0.59	4.7	95.8±0.84	0.49	2.1	30	27
Gulshan × Nafis	117.3±2.50	1.45	4.2	141.0±0.50	0.29	2.1	78.2±1.67	0.96	4.1	94.0±0.34	0.19	2.1	29	25
Oltin Vodiy	108.0±2.00	1.15	5.1	141.0±1.00	0.58	2.1	72.0±1.33	0.77	5.2	94.0±0.67	0.39	2.2	29	26
Kumush Tola	105.0±2.65	1.53	5.5	143.7±0.77	0.44	1.7	70.0±1.77	1.02	5.1	95.8±0.51	0.30	1.6	30	27
Navruz	120.7±2.52	1.46	4.1	142.3±1.04	0.60	2.0	80.4±1.68	0.97	4.1	94.9±0.69	0.40	1.9	29	26
Based on Japanese artificial diet														
Zarafshon	120.0±1.52	0.88	3.4	141.7±0.76	0.44	2.0	85.3±1.02	0.59	3.5	94.4±0.51	0.30	1.9	28	27
Gulshan × Nafis	130.7±2.25	1.30	3.1	141.0±0.50	0.29	2.1	87.1±1.50	0.87	3.1	94.0±0.34	0.19	2.0	27	25
Oltin Vodiy	122.0±2.00	1.15	3.9	141.0±1.00	0.58	2.1	81.3±1.33	0.77	4.0	94.0±0.67	0.39	2.1	28	27
Kumush Tola	124.7±2.08	1.20	3.7	143.7±0.77	0.44	1.7	83.1±1.33	0.77	3.8	95.8±0.51	0.30	1.8	28	27
Navruz	132.0±2.00	1.15	3.0	142.3±1.04	0.60	2.0	88.0±1.33	0.77	3.0	94.6±0.67	0.39	1.9	27	26

Hybrid lines demonstrated generally higher adaptability and physiological stability compared to pure breeds. Navruz and *Gulshan* × *Nafis* showed the highest survival—89.6% and 88.7% on the freeze-dried diet and 88.0% and 87.1% on the Japanese diet—values that were nearly equivalent to the natural leaf control (96.0% and 92.7%, respectively). *Zarafshon*, *Kumush Tola*, and *Oltin Vodiy* also maintained high and consistent survival (83-85%) on the freeze-dried diet but displayed more pronounced declines on the convective diet (70-75%). These results clearly demonstrate the manifestation of heterosis (hybrid vigor), which enables hybrids to utilize nutrients more efficiently under artificial feeding conditions. Moreover, the hybrids exhibited lower coefficients of variation (CV<5%), indicating greater phenotypic stability and consistency across all diet types.

Two-way ANOVA (Diet × Genotype) revealed significant main effects of both factors ($F_{3,56}=24.3$, $p<0.001$) and a significant interaction between them ($p<0.05$). The analysis results are summarized in Table 4.

Two-way ANOVA for larval survival revealed significant main effects of diet and genotype, as well as a weak but significant Diet × Genotype interaction ($p=0.045$). When the analysis was restricted to artificial diets only, post-hoc comparisons showed that survival was highest on the freeze-dried diet, intermediate on the standard Japanese diet, and lowest on the convective diet. The natural mulberry leaf group is therefore used in this study as a technological reference rather than as a fully comparable diet treatment, and no formal statistical ranking between artificial diets and natural feeding is inferred. Tukey's HSD post-hoc comparison showed significant differences between freeze-dried and convective diets ($p<0.001$) and between convective and leaf control ($p<0.01$), whereas no statistical difference was found between freeze-dried and Japanese diets ($p>0.05$). The coefficients of variation remained below 5%, confirming the high reproducibility and reliability of the obtained results.

Technological parameters of the cocoons obtained from the studied breeds and hybrids reared on different diets are presented in Tables 5 and 6 and Figure 2. Figure 2 shows generalized technological indicators of silkworm breeds and hybrids for various types of feed.

The results revealed a clear dependence of cocoon characteristics on diet type ($p<0.001$). For all genotypes, the type of artificial diet had a significant effect on cocoon weight and raw silk content, although the pattern of variation differed between pure breeds and hybrids.

Among the pure breeds (Table 5), the highest cocoon weights were recorded in those reared on the freeze-dried mulberry-based diets, ranging from 1.71 to 1.79 g, statistically comparable to natural feeding (1.73-2.09 g). Under convective drying, cocoon weights declined to 1.40-1.71 g, accompanied by an increase in the coefficient of variation (CV ≈18-22%), indicating instability in cocoon size. The Japanese diet produced intermediate results (1.51-1.78 g), confirming that lyophilization preserved the nutritional quality of the feed and allowed cocoon weights nearly equivalent to those obtained from natural feeding, whereas convective drying resulted in a 10-15% reduction in cocoon mass.

Table 4. Larval survival (%)

Source	SS	df	MS	F	p	Partial η^2	ω^2
Diet	1120.4	3	373.5	24.3	<0.001	0.56	0.52
Genotype	870.1	9	96.7	6.3	<0.001	0.50	0.44
Diet × Genotype	420.5	27	15.6	1.8	0.045	0.27	0.18
Error	860.2	56	15.4				
Total	3271.2	95					

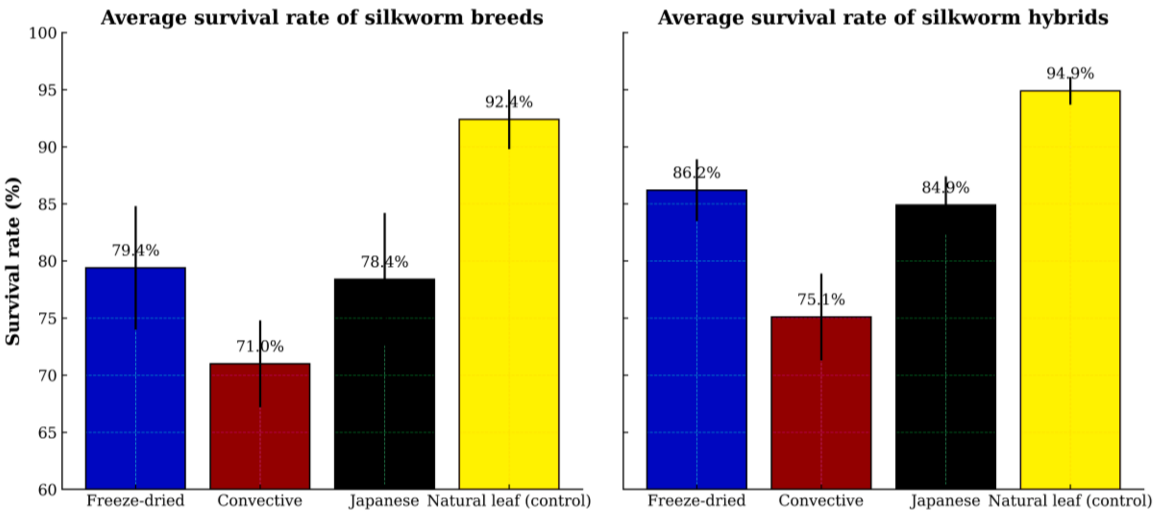

Figure 1. Comparison of mean survival rates of silkworm breeds and hybrids on different feed types

Table 5. Technological characteristics of silkworm breeds reared on artificial diets compared with those reared on natural mulberry leaves

Breed	**The average weight of the cocoons (g)**						**Content of raw silk (%)**					
	AD			**Leaf**			**AD**			**Leaf**		
	X̄±SD	**SE**	**Cv (%)**	**X̄±SD**	**SE**	**Cv (%)**	**X̄±SD**	**SE**	**Cv (%)**	**X̄±SD**	**SE**	**Cv(%)**
Based on freeze-dried mulberry leaves												
Ipakchi-5	1.71±0.33	0.05	17.3	1.73±0.27	0.05	17.2	18.2±2.3	0.34	12.5	22.4±3.1	0.44	13.6
UzNIISH-9	1.54±0.34	0.10	17.5	1.69±0.28	0.07	17.4	17.9±3.3	0.58	13.6	19.7±3.5	0.58	14.7
Ipakchi-1	1.79±0.33	0.07	16.9	2.01±0.30	0.04	16.9	17.7±3.1	0.48	19.3	22.3±2.6	0.48	13.5
Nafis	1.76±0.34	0.08	17.4	1.75±0.29	0.08	17.3	18.0±3.5	0.75	20.1	21.8±2.9	0.42	14.5
Gulshan	1.78±0.33	0.05	16.2	2.09±0.27	0.06	16.5	18.2±2.1	0.42	12.2	22.9±3.0	0.43	12.8
Based on convectively dried mulberry leaves												
Ipakchi-5	1.58±0.44	0.08	19.3	1.82±0.28	0.06	17.0	16.0±2.9	0.59	13.6	23.4±3.1	0.49	13.8
UzNIISH-9	1.40±0.43	0.09	22.1	1.35±0.28	0.07	17.2	15.1±3.6	0.89	22.2	19.9±3.4	0.65	12.6
Ipakchi-1	1.67±0.39	0.07	20.4	2.01±0.29	0.05	16.8	16.5±3.9	0.49	21.4	23.5±2.6	0.59	13.5
Nafis	1.55±0.40	0.10	19.3	1.75±0.30	0.09	17.1	17.1±4.4	0.87	22.3	22.8±3.0	0.43	14.3
Gulshan	1.71±0.38	0.09	18.2	2.42±0.27	0.07	16.3	17.3±2.4	0.45	17.5	24.4±2.9	0.42	12.9
Based on Japanese artificial diet												
Ipakchi-5	1.68±0.33	0.06	17.8	1.97±0.29	0.04	15.3	18.1±2.5	0.37	12.8	23.7±3.4	0.43	13.5
UzNIISH-9	1.51±0.36	0.07	18.2	2.10±0.31	0.05	16.5	17.8±3.0	0.59	13.8	24.2±3.1	0.46	13.1
Ipakchi-1	1.78±0.35	0.08	17.7	1.99±0.28	0.06	17.3	17.5±3.4	0.51	20.4	23.5±2.9	0.41	14.3
Nafis	1.76±0.34	0.09	17.6	1.87±0.30	0.07	17.2	17.6±2.9	0.77	22.0	22.1±2.7	0.40	14.5
Gulshan	1.77±0.33	0.06	16.6	2.23±0.28	0.05	16.5	18.2±2.4	0.67	13.9	24.4±3.1	0.39	12.5

Table 6. Technological characteristics of silkworm hybrids reared on artificial diets compared with those reared on natural mulberry leaves

Hybrid	**The average weight of the cocoons (g)**						**Content of raw silk (%)**					
	AD			**Leaf**			**AD**			**Leaf**		
	X̄±SD	**SE**	**Cv (%)**	**X̄±SD**	**SE**	**Cv (%)**	**X̄±SD**	**SE**	**Cv (%)**	**X̄±SD**	**SE**	**Cv(%)**
Based on freeze-dried mulberry leaves												
Zarafshon	1.89±0.30	0.04	16.3	1.89±0.26	0.04	15.4	19.8±2.3	0.32	12.2	23.9±2.5	0.34	11.9
Gulshan × Nafis	1.95±0.32	0.08	16.5	2.12±0.24	0.06	16.3	22.4±2.2	0.52	12.6	24.5±2.8	0.44	12.4
Oltin Vodiy	1.80±0.33	0.06	15.9	2.01±0.28	0.05	16.1	20.0±2.4	0.46	13.3	23.3±2.3	0.45	13.5
Kumush Tola	1.75±0.34	0.07	16.4	1.88±0.29	0.08	16.2	19.6±2.3	0.45	15.1	21.8±2.8	0.40	12.5
Navruz	1.87±0.34	0.05	15.2	2.22±0.24	0.06	16.1	20.3±2.4	0.40	12.0	24.5±2.6	0.38	11.8
based on convectively dried mulberry leaves												
Zarafshon	1.61±0.33	0.08	18.3	1.83±0.22	0.06	16.0	16.5±3.5	0.55	13.2	23.4± .0	0.38	12.4
Gulshan × Nafis	1.62±0.40	0.10	21.9	1.96±0.21	0.06	16.1	17.7±3.2	0.72	18.2	22.7±1.9	0.40	12.6
Oltin Vodiy	1.59±0.36	0.07	20.2	2.03±0.27	0.07	15.8	17.5±3.4	0.47	19.4	23.4±2,6	0.49	13.4
Kumush Tola	1.60±0.38	0.10	18.1	1.85±0.22	0.09	17.3	18.0±2.9	0.97	22.1	22.2±3,1	0.39	12.3
Navruz	1.68±0.40	0.07	17.8	2.12±0.24	0.07	16.0	20.3±2.6	0.55	18.5	24.5±2.8	0.40	11.9
Based on Japanese artificial diet												
Zarafshon	1.88±0.40	0.05	16.8	1.87±0.24	0.04	15.3	18.1±2.2	0.37	12.8	23.7±2.4	0.33	12.0
Gulshan × Nafis	1.84±0.38	0.07	17.2	2.10±0.32	0.05	16.2	20.1±2.3	0.56	13.0	24.2±3.0	0.45	12.1
Oltin Vodiy	1.80±0.34	0.07	17.0	1.99±0.28	0.06	16.3	17.4±3.0	0.48	19.0	23.5±2.9	0.46	13.3
Kumush Tola	1.70±0.36	0.09	17.4	1.87±0.30	0.08	17.0	17.0±2.7	0.57	17.1	22.1±2.7	0.40	12.6
Navruz	1.89±0.38	0.06	16.2	2.23±0,22	0.05	15.9	20.1±2.5	0.50	14.1	24.4±2.7	0.39	12.0

All breeds exhibited a similar trend: under freeze-dried diets, silk content ranged between 17.7-18.2%, while under convective drying, it dropped to 15.1-17.3%. The control group fed on natural leaves achieved 22.3-24.4%, approximately 4-6 percentage points higher, but the difference between freeze-dried and natural diets was not statistically significant (p>0.05). The Japanese diet produced silk contents of 17.5-18.2%, close to those obtained with the freeze-dried diet. Thus, the freeze-dried mulberry-based diet maintained physiological activity and fibroin synthesis efficiency comparable to natural feeding, while convective drying caused partial protein denaturation and reduced silk quality.

In hybrid groups (Table 6), the highest cocoon weights were recorded under freeze-dried feeding, ranging from 1.75-1.95 g, nearly matching natural feeding controls (1.88-2.22 g). Under convective drying, cocoon weight decreased to 1.59-1.68 g, particularly in *Kumush Tola* and *Oltin Vodiy*, where the coefficient of variation exceeded 20%. The Japanese diet produced consistent results (1.70-1.89 g) with lower variation (≈16-17%), indicating high uniformity of cocoon quality. The highest silk content was recorded for *Gulshan* × *Nafis* and *Navruz* (22.4-24.5%) under freeze-dried feeding, comparable to the natural leaf control (24.2-24.5%). Under convective drying, silk content decreased to 16.5-20.3%, especially in *Oltin Vodiy* and *Kumush Tola*, while the Japanese diet yielded intermediate results (17.0-20.1%). These findings clearly indicate that hybrids demonstrate higher adaptability to artificial diets than pure breeds, with heterosis ensuring stable cocoon mass and silk content even under altered nutritional conditions.

A two-way ANOVA confirmed that both diet and genotype significantly influenced cocoon weight and content of raw silk (Tables 7 and 8). Cocoon weight differed strongly by diet and genotype; freeze-dried ≈ leaf > Japanese > convective. Interaction was not statistically significant ($p>0.05$). Two-way ANOVA demonstrated highly significant main effects of both diet ($p<0.001$) and genotype ($p<0.001$) for all traits except the content of raw silk. Interaction effects were weak but significant for survival ($p<0.05$). The strongest contribution to variance was due to diet (η^2 = 0.47-0.60), confirming that the preservation method of mulberry leaves was the primary determinant of silkworm performance.

Discussion

The present study provides comprehensive evidence that both the nutritional composition of the artificial diet and the genetic background of *B. mori* play decisive roles in determining larval survival, cocoon yield, and silk quality. The pronounced interaction between diet type and genotype indicates that feed efficiency and physiological adaptability are not universal across silkworm strains, but rather genotype-specific, which is consistent with earlier findings by Ramesha et al. (2012), Saviane et al. (2014) and Lamberti et al. (2019).

Table 7. Cocoon weight (g)

Source	SS	df	MS	F	p	Partial η^2	ω^2
Diet	0.312	3	0.104	19.8	<0.001	0.52	0.47
Genotype	0.228	9	0.025	4.7	<0.001	0.43	0.36
Diet × Genotype	0.112	27	0.0041	1.5	0.095	0.22	0.13
Error	0.293	56	0.0052				
Total	0.945	95					

Table 8. Content of raw silk (%)

Source	SS	df	MS	F	p	Partial η^2	ω^2
Diet	38.1	3	12.7	11.9	<0.001	0.39	0.33
Genotype	25.6	9	2.8	2.6	0.014	0.30	0.20
Diet × Genotype	13.7	27	0.51	0.8	0.70	0.12	0.00
Error	60.0	56	1.07				
Total	137.4	95					

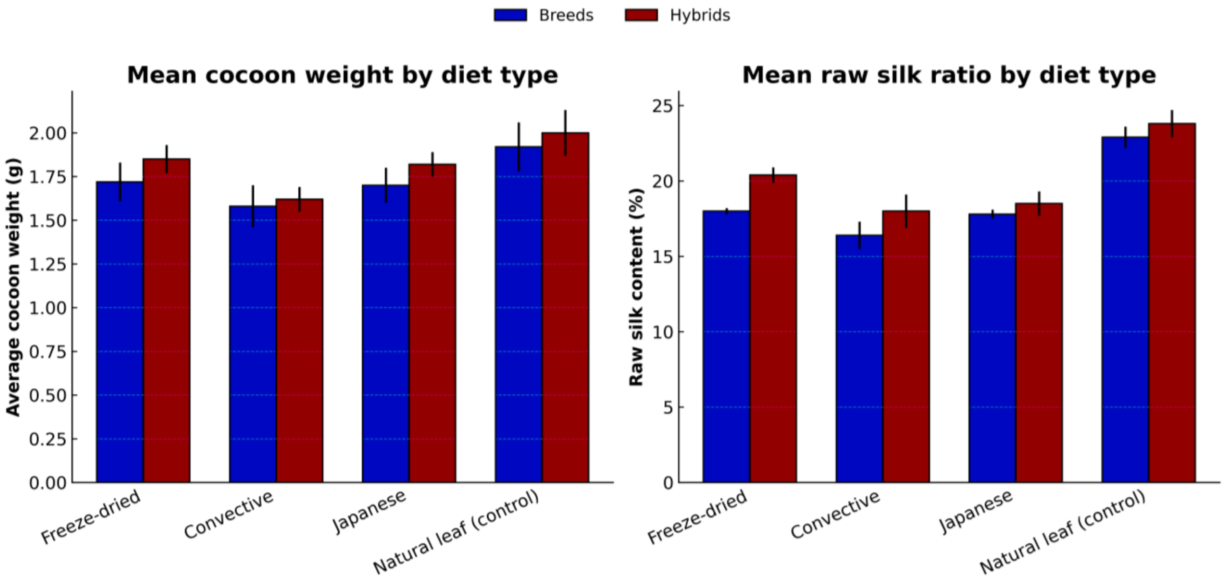

Figure 2. Technological indicators of silkworm breeds and hybrids on different feed types

The superior performance of larvae reared on the freeze-dried mulberry leaf diet compared to the convective and standard Japanese diets can be attributed to the preservation of nutrients during low-temperature dehydration. Freeze-drying maintains the structural integrity of proteins, amino acids, and vitamins, preventing oxidative degradation and nutrient loss (Ma et al. 2018; Wang et al. 2023). Similar results were reported by (Dong et al. 2017, 2018), who observed that larvae fed with lyophilized mulberry leaf powder exhibited enhanced fibroin gene expression and improved metabolic stability under nutritional stress. Our results align with these observations, confirming that the lyophilization process supports more balanced larval metabolism and cocoon formation.

Convective drying, although simpler and less energy-demanding, caused a 10-20% reduction in cocoon weight and silk ratio, indicating partial denaturation of proteins and loss of heat-sensitive micronutrients such as ascorbic acid and chlorophylls. Comparable findings were reported by Paudel et al. (2020), who demonstrated that high-temperature processing negatively affected larval immunity and reduced silk gland development. Moreover, Wu et al. (2024) reported that stable humidity and moderate feeding moisture (70-72%) are crucial for microbial safety and effective digestion in artificial diet rearing systems. The present results confirm that maintaining this moisture balance in freeze-dried diets ensures superior larval performance under semi-sterile rearing conditions.

Genotypic variation remains a crucial determinant of adaptation to artificial feeding systems. Hybrids such as *Gulshan* × *Nafis* and *Navruz* exhibited consistently high survival rates and uniform cocoon characteristics across all feeding regimes, indicating a pronounced heterosis effect and broader adaptability. Similar trends were described by Lamberti et al. (2019), as also indicated by proteomic data comparing pupae reared on artificial versus natural diets (ProteomeXchange Consortium 2019), who demonstrated that hybrids generally exhibit improved nutrient assimilation efficiency and higher silk protein synthesis when reared on standardized artificial diets. In contrast, pure breeds such as *Ipakchi-5* and *UzNIISH-9* displayed greater variability and narrower physiological tolerance. These differences underscore the need for targeted selective breeding programs aimed at improving feed tolerance and metabolic plasticity. Developing diet-adapted genetic lines would substantially increase the reproducibility of artificial rearing systems and reduce genotype-dependent performance fluctuations.

Another critical observation concerns the interaction between the environment and the feeding system. Despite controlled laboratory conditions, minor differences in temperature and humidity between natural and artificial diet rearing may have influenced larval metabolism. However, such conditions reflect the intrinsic technological requirements of each rearing system and were accounted for as covariates in the statistical model. The inclusion of environmental parameters in ANOVA analysis ensured that observed effects were genuinely associated with diet type rather than uncontrolled microclimatic variation.

From a production standpoint, the implications of these results for industrial sericulture in Uzbekistan are highly significant. Traditional rearing, limited by seasonal leaf availability, allows only one or two annual cycles. The use of freeze-dried diets under controlled rearing environments could enable four to five cycles per year, effectively tripling cocoon yield. This approach is consistent with recent international trends toward continuous sericulture (Gheorghe et al. 2023; Chen et al. 2023). For Uzbekistan, where industrial silk production is emerging as a national priority, locally developed artificial diets based on domestic mulberry resources provide a sustainable alternative to imported feed formulations—reducing production costs and environmental dependency.

Equally important is the demonstration that properly balanced artificial diets can sustain physiological development comparable to natural feeding. The absence of significant differences in survival and cocoon weight between freeze-dried and natural leaf groups confirms that the nutritional and microbial profiles of the artificial diet effectively mimic fresh leaves. Similar outcomes were reported by Chen et al. (2024) and Ahmed et al. (2025), who stressed the role of microbiota–diet balance in maintaining larval immunity and silk quality. Our findings extend this concept, showing that microbial stability and controlled moisture are critical for achieving equivalence between artificial and natural rearing systems.

Finally, the integration of nutritional optimization with genetic selection emerges as the cornerstone for sustainable sericulture modernization. Future research should focus on refining amino acid and carbohydrate ratios, incorporating probiotic and antioxidant supplements to stabilize gut microbiota and prolong feed shelf life, and implementing systematic breeding programs for feed tolerance. Moreover, expanding trials across multiple seasons, locations, and production scales will be essential to validate external reproducibility and cost-effectiveness.

Several methodological limitations of the present study should be acknowledged. First, the comparison between artificial diets and the natural mulberry leaf control is partially confounded by differences in microclimate (temperature and humidity) and feeding frequency, which reflect practical rearing standards but do not allow a strict attribution of all observed differences solely to diet composition. For this reason, our strongest inferences are restricted to contrasts among artificial diets, which were tested under identical environmental and handling conditions. Second, relatively high coefficients of variation (up to 16-22% for some technological traits) indicate substantial biological variability under semi-production conditions and reduce the precision of fine-scale differences. Although the main effect of diet remained statistically robust despite this variability, future experiments should include more homogeneous genetic material, additional replicates, and fully factorial designs with unified environmental regimes to further isolate diet effects and improve statistical power.

By combining these nutritional, genetic, and technological innovations, Uzbekistan can develop a resilient model of year-round sericulture, ensuring stable cocoon yields and high-quality silk production under fully controlled, environmentally safe, and economically efficient conditions.

In conclusion, this study demonstrated that both the composition of artificial diets and the genetic background of *B. mori* significantly affect larval survival, development, and silk productivity, with a clear genotype × diet interaction determining the degree of adaptation to artificial feeding. The locally developed freeze-dried mulberry leaf–based diet provided biological and technological indicators—survival, cocoon weight, and silk ratio—statistically comparable to those obtained from natural leaf feeding, proving its efficiency and nutritional stability, with survival rates of 86-90%, cocoon weights of 1.70-1.85 g, and silk content of 18.5-20.3 %. The high adaptability and productivity of the hybrids *Gulshan* × *Nafis* and *Navruz* indicate valuable hereditary traits of their original parental breeds, which can be used in the development of new hybrids and in the selection of strains adapted to artificial diets. The findings confirm that freeze-drying preserves essential nutrients better than convective drying and ensures microbial safety and physiological stability under controlled rearing. In practical terms, the use of freeze-dried artificial diets enables up to four to five rearing cycles annually in Uzbekistan, significantly increasing cocoon output and promoting sustainable, year-round sericulture. The experiment was limited to a single rearing season under controlled laboratory conditions. Factors such as long-term microbial stability, feed storage duration, and large-scale economic feasibility were not assessed. Additionally, genetic responses were tested in only ten genotypes, which may not fully represent the diversity of Uzbekistan's sericultural germplasm. Future efforts should integrate nutritional optimization, probiotic supplementation, and selective breeding to create resilient silkworm lines and establish an eco-efficient sericulture system aligned with industrial silk production.

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