

## Susceptibility of some silkworm, *Bombyx mori* L. hybrids towards artificial diet containing reduced by 50% amount of mulberry leaf powder

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### Abstract

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The present study aimed at testing the performance of 3 new Bulgarian four-way F1 silkworm hybrids, fed on standard artificial diet, containing 30% mulberry leaf powder and on an artificial diet, containing only 15% mulberry leaf powder. It was established that mulberry leaf powder content in the artificial diets had a direct effect on the larval survivability rate and average weight of one larva. All hybrids showed higher values of both characters studied at feeding on diet, containing 30% of mulberry leaf powder compared to those fed with diet having lower amount of mulberry powder. The results obtained clearly brought out that the hybrids Vratsa63 × Line22 × NIG2 × Merefafa6 and NIG2 × Merefafa6 × Vratsa63 × Line22 displayed a higher susceptibility to feeding on artificial diet containing 50% less mulberry leaf powder, compared with the hybrid I1 × VB1 × N2 × HB2.

**Keywords:** *Bombyx mori* L.; survivability rate; mulberry powder; artificial diet

### Introduction

The silkworm *Bombyx mori* L. feeds with mulberry leaves as it transforms leaf nutrients into a silk thread. With relation to the seasonal cultivation of mulberry tree and the impossibility for silkworms to be bred all year round, more and more research studies have been carried out worldwide in order to use artificial diets as an alternative of mulberry leaf. The use of artificial diets allows larval rearing all the year round without any seasonal limitations.

Vitamin content, including thiamine, biotin, alfa-tocopherol and riboflavin in the hemolymph, in silkworms fed on artificial diet is considerably lower than vitamin content in silkworms fed with mulberry leaves (Dong et al., 2017).

Wang et al. (2023) found that in silkworms fed on artificial diet, expression levels of genes related to amino acid metabolism syntheses were reduced, while levels of antimi-

crobial peptide genes were higher. These observed changes likely contributed to the slower growth and lower viability observed in the first instars of the larvae fed on artificial diet.

Nutrients in mulberry leaves (*Morus* sp.) serve as a golden standard in the preparation of artificial diets. Mulberry leaves contain sugars, lipids, proteins, minerals, vitamins and flavonoids (Horie, 1985; Wen, 2020).

Qin et al. (2020) have studied the mechanism causing slow growth and low efficiency of protein synthesis in silkworms fed on artificial diet compared to silkworms fed with mulberry leaf. Content of amino-acids, carbohydrates and lipids in excrements with silkworms on an artificial diet is obviously reduced, while some organic acids, such as urea and citric acid, are significantly increased. Larval growth depends to a great extent on proteins. In cases when artificial diets contain nutrients with low levels of protein, such as gluten and zein, the discharge of uric acid is accelerated (Horie, 1985).

The artificial diets could significantly decrease labour expenses. Labour productivity in sericulture has increased three times, sericulture scale per household has increased 2.5 times, and labour time for 1 kg of cocoon has decreased from 2.13 to 0.6 h via the introduction of artificial diets (Huang et al., 2013).

According to Bhattacharyya (2016), the higher amount of mulberry leaf in artificial diets increases the nutrition response of larvae. Guncheva (2016) has studied the receptivity of the silkworms towards artificial diet containing low percent of mulberry leaf – 15%. The author has established that this indicator /receptivity/ varies within wide ranges depending on larvae age. According to Dong et al. (2017), Hamano et al. (1986), Horie & Watanabe (1986), silkworm low pupation rate could be due to their low adaptability to artificial diets.

The present study aims at testing the performance of 3 new Bulgarian four-way F1 silkworm hybrids, fed on standard artificial diet, containing 30% mulberry leaf powder and on an artificial diet, containing only 15% mulberry leaf powder.

## Materials and Methods

The present research study was conducted at the Scientific Center of Sericulture – Vratsa in the period 2019–2023. The artificial diet used as a control contained 30% of mulberry powder, 34% of defatted soybean powder and the rest 36% – corn starch, wheat flour, citric acid, ascorbic acid, agar-agar, microelements and vitamins, antibiotic and conservatives. During the experiment the artificial diet contained the same ingredients, but 50% of mulberry powder content was replaced with wheat flour. The powder was mixed with tap water in a ratio – 1 part of food: 2.6 parts of water, and after that – stirred up with a mixer. The mixture obtained was poured in plastic containers – from 1.5 to 2 cm thickness of the layer. After that it was treated in a microwave with 850 KW of duty and 6 min. of exposure.

After heat treatment the prepared food was left for 4 h at room temperature, and then it was stored in a fridge at a temperature of 4°C until its use for silkworm larvae feeding. Before feeding the food was taken out of the fridge, cut in thin fillets – 0.5 cm wide. The study was carried out with the new four – way hybrid silkworms Vratsa63 × Line22 × NIG2 × Merefafa6, NIG2 × Merefafa6 × Vratsa63 × Line22, and I1xVB1 × N2xHB2. The parents of the hybrid Vratsa63 × Line22 × NIG2 × Merefafa6 and the reciprocal have been chosen also for a higher artificial diet susceptibility. The parents of the hybrid I1xVB1 × N2xHB2 have been selected for higher tolerance to adverse rearing conditions. The four-way hybrids were fed with artificial diet containing 30% mulberry powder and artificial diet containing 50% less mulberry powder.

3 repetitions by 400 normal eggs were counted from each hybrid. After the silkworms were hatched, they were fed on artificial diet until their 4<sup>th</sup> moult. The number of fed silkworms was calculated via samples regarding hatching ability within 3 repetitions by 200 normal eggs. Silkworm cultivation with artificial diet was carried out in plastic containers put in a thermostat disinfected. During the silkworm rearing the following temperature and relative humidity were maintained:

- during first and second instars of silkworm rearing: 29–30°C, 85% relative humidity;
- during third instar: 27°C, 80% relative humidity;
- during fourth instar: 26°C, 75% relative humidity;

After the fourth moult the silkworm larvae were counted and weighed. The values of the following characters were examined: survival ratio of the larvae; average weight of 1 larva. Data were processed statistically, by the conventional methods.

## Results and Discussion

Table 1 shows the data related to the comparative examination of the new tetra-hybrids reared on standard artificial

**Table 1. Survival rate of the larvae and average weight of one larva fed on artificial diet containing 15% and 30% of mulberry powder**

Hybrid/variant	Survival ratio of the larvae, % $\bar{x}$	Average weight of 1 larva, mg $\bar{x}$
Vratsa63 × Line22 × NIG2 × Merefafa6 15%	63.46	403.5
Vratsa63 × Line22 × NIG2 × Merefafa6 30%	93.18	472
$t_d$	5.22***	2.06
NIG2 × Merefafa6 × Vratsa63 × Line22 15%	63.90	448
NIG2 × Merefafa6 × Vratsa63 × Line22 30%	86.31	426.5
$t_d$	6.83***	2.12
I1xVB1 × N2xHB2 15%	51.09	285
I1xVB1 × N2xHB2 30%	82.95	480
$t_d$	6.23***	4.93***

diet containing 30% of mulberry powder and diet containing 15% of mulberry powder. Taking into account all the hybrids, silkworm survival ratio is higher for those fed with 30% mulberry powder food. Regarding the average weight of 1 larva, the tetra-hybrids Vratsa63 × Line22 × NIG2 × Merefa6 and NIG2 × Merefa6 × Vratsa63 × Line22 showed higher or the same value when reared on diet containing 15% or 30% of mulberry powder. Nevertheless, the average weight of silkworms for the hybrid I1×VB1 × N2×HB2 fed with 15% mulberry powder food was lower compared to those fed with 30% mulberry powder food. On the other hand, the average weight of 1 larva for the hybrids Vratsa63 × Line22 × NIG2 × Merefa6 and NIG2 × Merefa6 × Vratsa63 × Line22 fed with 15% mulberry powder diet was higher compared to the tetra-hybrid I1×VB1 × N2×HB2.

It was established that the higher content of mulberry leaf in artificial diets influenced not only the larval survivability, but also the average weight of 1 larva, as it improved growth values. The tetra-hybrid I1×VB1 × N2×HB2 fed with 15% mulberry powder diet showed lower values for both characters. The larval survivability rate character was registered with a value of 51.09%, and the average weight of one larva was 285 mg, which proved the statement of some authors that the reduction of mulberry powder content in artificial diets leads to lower values of some characters. The three examined tetra-hybrids fed with 15% mulberry powder food registered values of survivability rate varying within 51% and 64% compared to larvae fed with 30% mulberry powder food, where all hybrids had over 80% survivability. According to the studies of Dong et al. (2017), Hamano et al. (1986), Horie & Watanabe (1986), the lower survival ability rate could be due to weak adaptability of silkworms to artificial diets.

With relation to larval weight, all groups were established with values over 400 mg except for I1×VB1 × N2×HB2, where the weight of 1 larva was extremely low – 285 mg. It could be due to the hybrid's lower susceptibility towards artificial diet, which also influenced directly silkworm survival rate.

The hybrid NIG2 × Merefa6 × Vratsa63 × Line22 fed with 15% mulberry powder food showed lower by 21.5 mg average larval weight compared with the same hybrid fed with 30% mulberry powder. At the same time, NIG2 × Merefa6 × Vratsa63 × Line22 showed higher weight of 1 larva when cultivated with 15% mulberry powder diet compared to Vratsa63 × Line22 × NIG2 × Merefa6. Regarding larval rearing with diets containing 30% mulberry leaf powder, the lowest survivability values were reported for the hybrid NIG2 × Merefa6 × Vratsa63 × Line22.

Furthermore, direct dependence was not established between silkworm survival rate and larvae average weight.

## Conclusions

It was established that mulberry leaf powder content in the artificial diets had a direct effect on the larval survivability rate and average weight of one larva. All hybrids showed higher values of both characters studied at feeding on diet, containing 30% of mulberry leaf powder compared to those fed with diet having lower amount of mulberry powder. The results obtained clearly brought out that the hybrids Vratsa63 × Line22 × NIG2 × Merefa6 and NIG2 × Merefa6 × Vratsa63 × Line22 displayed a higher susceptibility to feeding on artificial diet containing 50% less mulberry leaf powder, compared with the hybrid I1×VB1 × N2×HB2.

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