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SILKWORMS (*BOMBYX MORI* L.) REARING USING ARTIFICIAL DIET DURING THE SUMMER

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Abstract

The purpose of the research is to investigate new methods of silkworms rearing for a longer period during the year. To accomplish our main goal, we used artificial diet for silkworms rearing during the summer. The tasks of our research were to apply an artificial diet from the first to the third instars, and in the fourth and instars we used mulberry leaves. There is a trend showing that the hatchability in September is higher than in July and August. The average values of the pupation rate and larval period duration also remained relatively high in September. Considering the fresh cocoon yield by one box of eggs, we found that the lowest values are in August and the highest - in September again. Based on the results obtained, it can be concluded that the rearing of the silkworms using artificial diet in the young instars is most appropriate in September. It is economically viable, leads to greater employment and to increasing of the income of sericulture farmers.

Key words: *artificial diet, Bombyx mori L., Morus alba L.*

INTRODUCTION

The change of the organizational structure of agriculture in 1990s in Bulgaria and its intensive development, as well as the climate changes and the decrease of the rainfall in a temperate climate zone in the last 10 years created serious difficulties related to the silkworms rearing (*Bombyx mori* L.).

Rainfall in the period May-September is one of the main factors influencing the growing of mulberries under non-irrigation conditions, as is the case in widespread practice in Bulgaria. Humidity plays an important role in the synthesis of organic compounds and the transfer of mineral elements and substances to different parts and organs of the tree, as well as in the course of growth processes and cooling of the leaves when heated by the sun. Synthesis of 1 kg of dry matter requires the consumption of 700-800 l of water. The shoots usually contain more than 60% water and the leaves contain 70-85%. This shows the extreme importance of sufficient water in the soil and in the air while maintaining the necessary turgor, succulence and tenderness of the leaves in order to use them to produce a quality leaf mass. On the other hand, the labour force during the period May-September is very often insufficient. This necessitates exploring of other opportunities for lucrative and cost effective methods of silkworms rearing during this period. One way to solve this problem is to use artificial diet, which on its turn could be able to minimize the need for labour.

Although the Mulberry (*Morus* sp.) leaf is still considered as the traditional food for silkworms, many attempts have been made to establish rearing on artificial diet. Nowadays, sericulture research developed number of supplement nutrients with mulberry leaves for silkworm rearing. There are number of foods used as an ingredient for artificial diet of silkworm. Artificial diet encourages the small landless farmers to take up sericulture and it also helps to reduce labour cost for mulberry cultivation (Bhattacharyya et al., 2016).

There are insects for which it is possible to grow large populations in laboratory conditions, such as *Drosophila*, which has been used for many years as a major target for genetic research. Large-scale farming of *Bombyx mori* L. and *Apis mellifera* have been described as being widely spread and practiced decades ago (Cohen, 2004).

According to Panizzi et al. (2012), 1,300 species of insects are farmed using artificial diet, 85% out of which are represented by Lepidoptera, Coleoptera and Diptera species.

Sahay et al. (2011) also developed artificial diet for rearing of *Anthereaea mylitta* D. under controlled conditions.

The use of artificial diet for *Ostrinia furnacalis* and *Harmonia axyridis* rearing has been reported in the literature (Teguh et al., 2018; Yuan-Xing Sun et al., 2019) used artificial diet for rearing, and the results obtained are similar to those of field-grown insects, allowing the production of a large number of insects in laboratory conditions and their use for biological control.

According to Petkov et al. (1980), one of the reasons for the unattractiveness of the silkworm industry is its extensive nature, which is associated with the consumption of a lot of labour for a short-term. In this respect, the cost of feed constitutes about 60% of the total cost, with 27-32% falling on the pruning and delivery of the mulberry leaves and 31-36% on the feeding process - mainly during the fourth and fifth instars.

Silkworm rearing on artificial diet has many advantages over existing practices, since it provides balanced nutrition and disease-free conditions, regardless of the seasons (Jula et al., 2011). This is especially important in the first, second and third instars.

According to Nair et al. (2013), mulberry leaves, especially in tropical conditions, does not always contain the necessary balanced nutrients that meet the needs of silkworms throughout the year.

It is widely accepted that the high cost of the artificial diet is a major problem for the spread of silkworm rearing in cooperative houses, especially in the young instars. The cost of the artificial diet amounts to about 35% and 50% of the total cost of silkworms rearing in cooperative houses from the first to the second instars and from the first to the third instars, respectively. Therefore, it is important to reduce the costs of artificial diet in order to extend the period of rearing so that farmers to be able to develop more robust breeding management systems (Shinbo et al, 1994).

Tzenov et al. (2010) creates a balanced artificial diet for rearing over the whole larval period. Silkworms reared on artificial diet have a shorter larvae period during the fifth instar and during the rearing period as a whole. Considering the other characters, there are no significant differences to the silkworms reared on mulberry leaves. Artificial diet is appropriate to be applied during all seasons of the year, which, on its turn will lead to higher incomes for the silkworm rearers.

We consider all those facts as reasonable enough to set as our main objective to explore new methods of silkworms rearing for a longer periods during the year and thus, for higher employment of the farm workers.

MATERIALS AND METHODS

Strains. Strains named Svila 1 and Svila 2 were tested. Three replicates with 200 larvae each were employed. As a reference we used silkworms reared on mulberry leaves only. Svila is uni-bivoltine 4 molting pure line, created in Bulgaria in 2005. The egg serosa color is greenish gray, chorion color is yellow and the eggs are sticky. The last instar silkworm larvae are bluish-white in color and plain. The body shape is thicker and shorter. The cocoons are white in color, with oval shape.

Environmental conditions. The incubation of silkworm eggs was carried out in accordance to the standard method for the summer season.

The standard conditions for silkworm rearing with mulberry leaf are shown in Table 1.

Table 1. Environmental conditions (larvae fed on fresh mulberry leaves)

Instar	Temperature, °C	Relative humidity, %
I	26-27	85-90
II	26-27	85-90
III	25-26	80-85
IV	23-25	70-75
V	20-25	65-70
Cocooning	24-26	70-75

The environmental conditions required for the rearing on artificial diets are slightly different than those established for the rearing on fresh mulberry leaves. The conditions for silkworm rearing with artificial diet are as shown in Table 2 and it can be seen that the temperatures are slightly higher during the all instars and cocooning.

Immediately after removal from the refrigeration chamber, the silkworm eggs were placed at a temperature of 24-25°C. From the third day the temperature was raised to 26-27°C. During the first and the second instars, the temperature was set to be such as 29 to 30°C, being higher by 1-2°C than the one that has been set for mulberry-reared larvae. The same difference can be observed in regards to the temperature during the third instar. It is assumed that at a high temperature larvae might gain more weight than at a low temperature. The rearing temperature is recommended to be higher in the fourth instar also (compared to mulberry-fed rearing). It should be anyway kept low, such as at 24°C, in the fifth instar, similar to the case of mulberry rearing where the optimum temperature interval is established to be 20- 25°C.

Table 2. Environmental conditions (larvae fed on artificial diet)

Instar	Temperature, °C	Relative humidity, %
I	29–30	90
II	29–30	90
III	27–28	80
IV	26	70–75
V	24	70
Cocooning	25–27	55–60

The relative humidity was kept as required (50-85%). Higher humidity in the rearing room is recommended at least in order to prevent the drying of diet.

The incubation is carried out in room which the natural light can enter; artificial lighting is rather not used. 12-hours light and 12-hours dark rhythm is recommended as photoperiodism can also affect the silkworm rearing at some extend.

On the 10th day after the incubation has started, some silkworm eggs turn white and the first larvae appear. On the 11th day the hatching begins and, in order to prevent the larvae from crawling out, a few mulberry leaves or artificial food are placed around them. On the 12th day a "mass" hatching is observed and then the larvae can be fed with artificial diet for first time. Only the larvae, hatched on the day of "mass" hatching are brushed for rearing.

Experimental plan. The experiments were conducted during the period 2017-2018 at the Agricultural University of Plovdiv. For the achievement of our main objective, we tested the use of artificial diet in silkworms rearing during the summer. The tasks of our research were to feed the silkworms on artificial diet during the first, second and third instars, and the silkworms in their fourth and fifth instars we fed on mulberry leaves. The main technological characters were studied: fresh cocoon weight; cocoon shell weight; filament length and shell ratio.

RESULTS AND DISCUSSIONS

Table 3 shows the mean values of the hatchability of the employed strains Svila 1 and Svila 2 during the months of July, August and September. The average values for Svila 1 range from 1,109 to 1,683, indicating that the values of hatchability in 2017 are within acceptable limits, with the average error ranging from 0.671 to 1.011.

Table 3. Hatchability (%)

Strains	Months	2017			2018		
		$\bar{x}$	*Sx*	*Vc*	$\bar{x}$	*Sx*	*Vc*
Svila 1	July	85.56	0.67	1.11	84.35	0.621	1.04
	August	82.24	0.76	1.31	82.95	1.00	1.70
	September	84.91	1.01	1.68	85.23	0.35	0.58
Svila 2	July	83.44	0.90	1.53	81.25	0.73	1.27
	August	82.88	2.50	4.26	82.32	0.27	0.46
	September	87.53	0.40	0.65	85.30	0.43	0.71

We have the lowest results of the character in August 2017 for Svila 1 with a value of 82.24% and with a value 81.25% in July 2018. For Svila 2, the highest hatching rate of 87.53% was observed in September 2017, being with 2.23% more than in the same month of the following year. In Svila 1, the difference in hatching in September in both years is minimal and has a value below 1%, and in both strains we noticed a slight decrease in values in

August, and then in the following month they increase. The lowest value of the hatchability character was recorded in Svila 2 in July 2018 with a value of 81.25%, and the highest one - 87.53%, was registered in September 2017 for by Svila 2 again (the difference between them being 6.28%).
There is a trend showing that the hatchability in July is lower than in September, which is most likely due to the negative impact of the high temperatures in July and August.
The average values of the pupation rate of the both studied strains remained relatively high in September (Table 4). There is a greater variation of the values of this character for the Svila 1 in July 2017 and for the Svila 2 in September 2018. The difference between the average values of the pupation rates in September (in terms of the surveys conducted in two consecutive years), is minimal with a value of 0.13% for the Svila 1 and 0.73% for Svila 2.
One of the factors that influence the pupation rate is the diseases during the larval stage. In regards to this character, there are slight differences between the larvae fed on artificial diet and those fed on fresh mulberry leaves. This difference is due to the fact that when feeding on artificial diet we can better control the humidity and the development of microorganisms that can cause some diseases. For larvae fed on mulberry leaves, the mortality is slightly higher and hence a slight decrease in the pupation rate might be observed.

Table 4. Pupation rate (%)

Strains	Months	2017			2018		
		$\bar{x}$	*Sx*	*Vc*	$\bar{x}$	*Sx*	*Vc*
Svila 1	July	87.33	0.820	1.33	86.67	0.41	0.68
	July (reference)	87.70	0.25	0.41	86.93	0.10	0.17
	August	86.23	0.55	0.90	85.29	0.24	0.40
	August (reference)	86.17	0.18	0.29	86.03	0.10	0.18
	September	89.40	0.37	0.59	89.53	0.39	0.62
	September (reference)	88.80	0.14	0.22	88.73	0.177	0.28
Svila 2	July	88.57	0.41	0.66	88.27	0.45	0.73
	July (reference)	87.90	0.19	0.30	86.60	0.39	0.64
	August	85.30	0.43	0.71	86.80	0.14	0.23
	August (reference)	85.37	0.25	0.41	86.96	0.15	0.23
	September	89.07	0.15	0.23	89.80	1.04	1.64
	September (reference)	88.90	0.07	0.11	89.16	0.47	0.74

The results on the larval period duration show a variation of the character from 0.897 to 1.97 for Svila 1 and from 0.71 to 1.22 for Svila 2 (Table 5).
The lowest values of the variation coefficient for both Svila 1 and Svila 2 strains are observed in September. The deviations are insignificant and no firm conclusions can be drawn. There is no trend or significant difference compared to the reference values.
For Svila 1 strain fed on artificial diet, we observed a difference in the duration of the larval period of 92 h.
The lowest duration was in September 2018 with values of 776.67 h. For the reference of the same strain, a difference of 50 h between the lowest and the highest value is observed.
For Svila 2 strain, the difference in larval period duration is relatively small, with a value of 36.67 h for the larvae fed on artificial diet, and 63.33 h for the reference.
In silkworms rearing, the only character that targets lower values is the larval period duration. With longer larval period duration, an extended workflow for feeding and rearing can be expected, and hence a higher labor costs.

Table 5. Larval period duration (h)

Strains	Months	2017			2018		
		$\bar{x}$	Sx	Vc	$\bar{x}$	Sx	Vc
Svila 1	July	851.67	5.40	0.89	863.33	10.80	1.77
	July (reference)	850	7.07	1.17	870	7.07	1.14
	August	868.67	8.52	1.38	830	7.07	1.20
	August (reference)	856	8.60	1.42	826.66	4.08	0.69
	September	803.33	10.80	1.90	776.67	10.80	1.97
	September (reference)	830	7.07	1.20	800	14.14	2.8
Svila 2	July	870	7.07	1.14	820	7.07	1.22
	July (reference)	870	7.07	1.14	826.66	14.71	2.48
	August	876.67	10.80	1.74	863.33	4.08	0.74
	August (reference)	876.66	4.08	0.65	836.66	14.71	2.48
	September	813.33	4.08	0.71	783.33	4.08	0.74
	September (reference)	813.33	8.16	1.41	826.66	26.77	4.58

With reference to the fresh cocoon yield by one box of eggs, it was found that the highest values for both tested strains are in September (Table 6).

Larvae being fed on artificial diet show no significant differences compared to the reference - 0.37 kg for Svila 1 in 2017 and 0.46 kg in 2018. For Svila 2 the difference is 0.33 kg in 2017 to 0.67 kg in 2018.

The lowest values are in August, with a variance from 0.680 to 2.350.

These differences are probably due to the optimal conditions created during the young instars.

This finding proves the fact that the conditions during the first instars lead to higher yields in the latter instars.

Table 6. Fresh cocoon yield by one box of eggs (kg)

Strains	Months	2017			2018		
		$\bar{x}$	Sx	Vc	$\bar{x}$	Sx	Vc
Svila 1	July	23.74	0.61	3.68	23.13	0.39	2.38
	July (reference)	24.26	0.86	5.03	25.16	0.21	1.21
	August	22.47	0.10	0.68	23.03	0.29	1.81
	August (reference)	23.23	0.34	2.12	23.76	0.177	1.06
	September	25.93	0.04	0.22	25.47	0.39	2.16
	September (reference)	25.56	0.34	1.93	25.93	0.10	0.58
Svila 2	July	24.43	0.33	1.93	25.3	0.31	1.72
	July (reference)	24.43	0.28	1.65	24.56	0.35	2.00
	August	23.43	0.38	2.35	23.13	0.23	1.39
	August (reference)	23.3	0.43	2.61	24.03	0.22	1.27
	September	26.57	0.41	2.20	26.13	0.47	2.55
	September (reference)	25.9	0.07	0.38	25.8	0.18	1.02

The fresh cocoon yield by one box of eggs character is the indicator that shows the actual results of the rearing. Higher values of the character are aimed at each test and selection. While with the larval period duration lowering the values is the aim, here we consider the higher values as the best results.

CONCLUSIONS

Silkworm rearing during the summer season (from July to August) by applying artificial diet during the first three instars and mulberry leaves during the later instars does not lead to significant differences compared to the rearing of silkworms on mulberry leaves only.

Therefore, the results obtained allow us to recommend silkworm rearing during the summer season in Bulgaria on artificial diet during their first three instars and then with mulberry leaves up to the cocooning, which on its own turn will provide optimal nutrition and sanitary conditions, as well as reducing labor costs and raising the income of the silkworms rearers.

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