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STUDY OF THE TECHNOLOGICAL CHARACTERS OF SILKWORMS (*Bombyx mori* L.) ON ARTIFICIAL DIET

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Abstract

The purpose of the research is to prolong the period of silkworm rearing during the summer and autumn season. The rearing was carried out in the months of July, August and September at the Experimental Station of Sericulture at the Agricultural University in Plovdiv, Bulgaria. Silkworms in the first, second and third instars were fed by applying artificial diet, and in the last two larval instars – on mulberry leaves from Morus alba L. The results obtained from the studied technological characters show that there is a decrease in the basic values of the cocoon weight and filament length. For other characters, the decrease is negligible. The main conclusion is that the rearing of silkworms during the summer months by using combination of feeding on artificial diet in the first three instars and on mulberry leaves in the fourth and fifth instars is successful and leads to the possibility for more employment of people and increasing of the income of the silkworm rearers.

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

INTRODUCTION

The specificity of the climatic conditions in Bulgaria allows the rearing of silkworms with mulberry leaves to be carried out during the half of the year – from the beginning of May to the middle of October (about five months). Accordingly, there is an existing scheme of silkworm eggs production, in which spring and late-spring rearing are carried out with eggs produced in the previous year, summer rearing – with treated with hydrochloric acid eggs from spring generation, and autumn rearing – with hydrochloric acid-treated eggs laid by silkworms of spring or summer generation.

At the same time, in subtropical countries, the rearing begins as early as March, while in most tropical countries, it occurs throughout the year. In the countries of the Southern Hemisphere, the seasons of silkworms rearing are reciprocal to those in Bulgaria.

In India and Thailand, some of the best coconut crops are harvested during the so-called "winter" season from December to February. In the last decade in countries such as Japan, Italy, USA, Canada, UK and others, year-round silkworms rearing with so-called "artificial food" has been developed.

The idea of developing artificial diet as a replacement of the fresh mulberry leaves originated decades ago out of a simple query "Why do silkworms feed only on mulberry leaves?". This question caused a number of studies on the composition of mulberry leaves and in particular on the compounds which attracts the silkworms. Globally, continuous efforts are being made to improve the performance of artificial diet through permutations and combinations. However, all existing silkworm breeds and hybrids might not thrive on artificial diet on default; instead they should be made adapted to the diet through breeding process (Nair et al., 2013).

Each of the ingredients involved in the preparation of artificial diet can have a positive or negative effect on the main characters. According to some studies, ascorbic acid in food affects the yield of cocoon. Rearing of silkworms in their first and last instars on ascorbic acid-free diet has been shown to have a beneficial effect to cocoon production without affecting the survival rate or delaying the larval cycle (Cappellozza et al., 2005).

In Japan, where the sericulture industry is one of the most developed in the world, in about 50% of the silkworms farming an artificial diet is used during the young instars (Shimbo et al., 1994). Although the use of artificial diet is costly, since 6% of the costs are for agar-agar and mulberry leaves (Shimbo et al., 1994).

Some recipes have also been developed in order to feed silkworms on artificial diet using different percentages of mulberry leaves (5%, 15%, 25%) and applying the diet at different larval stages (Sbrenna et al., 2000).

According to (Cappellozza et al., 2011), hormone-based preparations can be added to the artificial diet, such as Methoprene (Manta) having a stimulating effect on the silkworms, with an increase in protein accumulation in the body that can be used later for biomedical purposes. Different diets could also have a negative effect on protein accumulation in the body, immune system, digestion, nutrient uptake, metabolism and silk synthesis (Zhou et al., 2008).

Sukirno et al. (2005) evaluate artificial diets that can be used to successfully culture the atlas silk moth, *Attacus atlas* L. The results of their studies show that in some of the characters there are no significant differences, while there are some in others. Based on the results that were obtained, they recommend adding extra protein to the artificial diet.

According to Saviane et al. (2014), the availability of quality mulberry leaves in the temperate climate zone is restricted to the spring-summer season, which is a limiting factor in the selection of silkworm strains. Therefore, they carry out coupled traditional rearing on mulberry leaves with rearing on an artificial diet, in order to obtain increased larval efficiency in converting food and high silk production.

Some authors (Tzenov et al., 2000) studied the pupation rate and fresh cocoon yield in different Bulgarian silkworm breeds and hybrids under optimal and adverse rearing conditions during the last two larval instars.

It was estimated that there existed clearly expressed genetically determined differences between the breeds and hybrids for pupation rate under adverse silkworm rearing conditions. However, no any correlation was detected between the pupation rate under the optimal and adverse rearing conditions in one the same strain.

MATERIALS AND METHODS

The experiments were conducted during the period 2017-2018 at the Agricultural University of Plovdiv. 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.

Two uni-bivoltine 4 molting pure lines, created in Bulgaria in 2005, namely Svila 1 and Svila 2, were tested.

From each strain, there were employed three replicates with 200 larvae each. Same strains but reared on mulberry leaves only were used as references to collate the main technological characters that have been studied.

The standard method for the summer season was applied for carrying out the incubation of silkworm eggs. The conditions for silkworm rearing on artificial diet compared to those applied for rearing with mulberry leaves only are shown in Table 1:

Table 1. Environmental conditions for silkworms rearing on artificial diet and on mulberry leaves

	Instar	Temperature, °C	Relative humidity, %
Larvae fed on fresh mulberry leaves	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
Larvae fed on artificial diet	I	29–30	90
	II	29–30	90
	III	27–28	80
	IV	26	70–75
	V	24	70
	Cocooning	25–27	55–60

It can be seen that the temperatures established for the rearing on artificial diet differs from environmental conditions required for rearing on fresh mulberry leaves, being slightly higher during the all instars and cocooning.

In regards to the different instars, the rearing temperature when artificial diet is used, is recommended to be kept higher (compared to rearing on mulberry leaves only): such as 29 to 30°C in the first and the second instars, 27 to 28°C in the third instar, and 26°C in the fourth instar. It is assumed that at a high temperature larva might gain more weight than at a low temperature. In the fifth instar however, there is also a difference (24°C for artificial diet-fed rearing) but it is still comparable with the temperature established for rearing on mulberry leaves only (20- 25°C).

In regards to the relative humidity, it can be seen from Table 1 that the required interval is about 50 - 85%. Lowering this parameter is not recommended as it can cause at least drying of the diet. Only daylight is applied during the incubation (no artificial light) and thus 12 hours light and 12-hours dark rhythm is established.

The "mass" hatching is observed on the 12th day after the incubation has started 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.

The following main technological characters were studied: fresh cocoon weight; cocoon shell weight; filament length and shell ratio.

For determining the fresh cocoon weight and shell weight, the following approaches were used: (1) all good quality cocoons/shells from the replicate were weighted and after that divided by their number; (2) a random sample consisting of 30 female and 30 male good quality cocoons/shells was taken and after weighting the weight is divided by the number. For shell percentage determination, the ratio between the weight of cocoon shell and the weight of fresh cocoon was used. The filament length was determined on a random sample of 30 good quality cocoons after single cocoon reeling test.

RESULTS AND DISCUSSIONS

The fresh cocoon weight has a direct effect on the yield of the cocoon and also on the filament length.

The results in Table 2 show that we have the highest values for the references of both strains in September 2017 and 2018.

The variation of the characters in silkworms fed on artificial diet ranges from 0.55 to 1.52. The lowest average values of the character were observed in July of 2017 in both the Svila 1 and Svila 2.

The highest values were recorded in September in both years 2017 and 2018 for the both strains that have been fed on artificial diet, probably due to the favourable climatic conditions.

Table 2. Fresh cocoon weight (mg)

Strains	Months	2017			2018		
		$\bar{x}$	*Sx*	*Vc*	$\bar{x}$	*Sx*	*Vc*
Svila 1	July	1743.33	28.57	2.32	1810	7.07	0.55
	July (reference)	1806.66	14.72	1.15	1870	7.07	0.53
	August	1763.33	10.81	0.86	1830	7.07	0.54
	August (reference)	1843.33	24.83	1.90	1836.66	22.73	1.75
	September	1810	7.07	0.55	1830	7.07	0.54
	September (reference)	1840	7.07	0.54	1870	7.07	0.53
Svila 2	July	1743.33	28.57	2.32	1790	7.07	0.55
	July (reference)	1800	7.07	0.55	1820	18.70	1.45
	August	1740	18.70	1.52	1843.33	14.72	1.12
	August (reference)	1803.33	10.81	0.84	1833.33	4.08	0.30
	September	1833.33	14.72	1.13	1850	7.07	0.54
	September (reference)	1870	7.07	0.53	1890	7.07	0.52

When observing the results of for Svila 1 strain in July, it can be seen that the values in both years had been decreased for larvae fed on artificial diet compared to the values of larvae fed entirely with mulberry leaf. The decrease compared to the reference is in the range of 63.33 mg in 2017 and 60 mg in 2018. The same trend is observed in the Svila 2 strain where we report a difference of 56.67 mg in 2017 compared to the reference and 30 mg in 2018 to reference.

Comparing the results from July and August of larvae fed on artificial food, we can see a decrease in the values of the cocoon weight at all. An increase in the values of the same character of the reference is observed in September. As a possible cause we consider the more favorable climatic conditions, since the summer months in our country exceed 30℃, which impedes the silkworms rearing and it leads to cocoons with lower weight. In September, the maximum temperature in reaches 25℃, which is the optimum temperature for silkworms rearing.

In regard to the cocoon shell weight (Table 3), it can be seen that the variation of the values is from 1.30 to 5.63%. We have a large variation within 5.63 % for Svila 1 reference values in September.

The highest average values of the character were observed in September and the lowest in August 2017 for the Svila 2 with a value of 410 mg. The values are slightly higher in July and August of 2018 again for Svila 2 - within 420 mg. In 2018, the highest reported mean values are observed for Svila 1 and the lowest - for Svila 2.

In regards to the results from July 2018, we see an increase in values compared to 2017, being higher with 28.34 mg. The results of the reference during same months retains relatively constant for both surveyed years. The lowest average values for the whole rearing period for larvae fed on artificial diet were observed in August (low values were noticed for both strains). In September there is already an increase in values, but at the same time we do not consider as significant the difference between individuals fed on artificial diet and those fed on mulberry leaves.

Table 3. Cocoon shell weight (mg)

Strains	Months	2017			2018		
		$\bar{x}$	*Sx*	*Vc*	$\bar{x}$	*Sx*	*Vc*
Svila 1	July	431.66	7.36	2.41	460	14.14	4.35
	July (reference)	460	7.07	2.17	466.66	14.71	4.46
	August	440	7.07	2.27	430	7.07	2.32
	August (reference)	453.33	4.08	1.27	460	7.07	2.17
	September	466.66	10.80	3.27	460	7.07	2.17
	September (reference)	470	18.70	5.63	460	7.07	2.17
Svila 2	July	443.33	10.80	3.44	420	14.14	4.76
	July (reference)	460	7.07	2.17	426.66	4.82	1.35
	August	410	7.07	2.44	420	7.07	2.38
	August (reference)	456.66	10.80	3.34	440	7.07	2.27
	September	476.66	10.80	3.20	446.66	4.08	1.30
	September (reference)	476.66	10.80	3.20	460	7.07	2.17

Table 4 shows the results of one of the most important technological characters - the filament length. The average filament length varies from 823.33 in Svila 1 in 2017 to 1016.66 m in the Svila 2 (reference values) in September 2018. The variation of this character is in the range of 1.05 to 2.90. The table clearly shows an increase in the reference values of the character in September 2018 for the Svila 2 and a decrease in the values in August for both tested strains in both years.

Table 4. Filament length (m)

Strains	Months	2017			2018		
		$\bar{x}$	*Sx*	*Vc*	$\bar{x}$	*Sx*	*Vc*
Svila 1	July	903.33	10.80	1.69	906.66	10.80	1.68
	July (reference)	930	18.70	2.84	940	7.07	1.06
	August	826.66	17.80	3.04	890	7.07	1.12
	August (reference)	910	7.07	1.10	923.33	10.80	1.65
	September	823.33	10.80	1.85	920	7.07	1.08
	September (reference)	886.66	8.16	1.30	976.66	10.81	1.56
Svila 2	July	883.33	10.80	1.73	900	7.07	1.11
	July (reference)	920	7.07	1.08	950	7.07	1.05
	August	910	18.70	2.90	980	7.07	1.02
	August (reference)	926.66	10.81	1.65	1010	7.07	10.80
	September	930	7.07	1.07	980	7.07	1.02
	September (reference)	976.66	10.80	15.6	1016.66	10.80	1.5

In regards to this technical character, as well as with the other technological characters that have been studied, the lowest values are observed during the summer months.

In September 2018 we registered the highest value for larvae fed on artificial diet, having in September 2018 filament length of 920 m (the same being 823.33 m in 2017). The difference comprises 96.67 m more in 2018, but despite the increase that has been observed, these values remain lower compared to the reference results for the same month and year. The difference between the reference and the test variant is 56.66 m in favor of the former.

The same trend is observed in Svila 2 where we have higher values in September for both years of rearing. In September 2018, the highest value of filament length is reported for Svila 2 - 1016.66 m for larvae fed on artificial diet. The difference between the lowest (833.3 m in July 2017) and highest (980 m in August and September 2018) values is within 146.67 m, which is slightly different than the observed values for the reference, where the difference is 96.66 m.

When considering the shell percentage (Table 5), we observed a gradual increase of the average values from July to August in 2017.

The variation in the same year ranges from 0.93 in August for Svila 1 (reference) to 3.75 for Svila 2 in August for the larvae fed on artificial diet. As with the other characters that have been analysed, the highest values were observed in September and the lowest values were registered in August.

Table 5. Shell percentage (%)

Strains	Months	2017			2018		
		$\bar{x}$	*Sx*	*Vc*	$\bar{x}$	*Sx*	*Vc*
Svila 1	July	24.77	0.56	3.21	25.41	0.68	3.79
	July (reference)	25.46	0.48	2.70	24.95	0.77	4.36
	August	24.96	0.51	2.9	23.49	0.29	1.77
	August (reference)	24.59	0.16	0.93	25.04	0.45	2.57
	September	25.78	0.64	3.50	25.13	0.29	1.62
	September (reference)	25.54	1.10	6.13	24.60	0.43	2.49
Svila 2	July	25.43	0.56	3.11	23.46	0.74	4.50
	July (reference)	25.55	0.29	1.61	23.44	0.46	2.77
	August	23.57	0.62	3.75	22.78	3.82	2.37
	August (reference)	25.32	0.46	2.58	23.36	0.41	2.54
	September	25.99	0.4	2.34	24.14	0.24	1.40
	September (reference)	25.49	0.64	3.56	24.33	0.28	1.64

CONCLUSIONS

Based on the results that have been obtained and the analysis that has been made therefore, the following conclusion can be drawn: rearing of silkworms on artificial diet in their young instars leads to a slight decrease in the values of the considered technological characters and provides a successful and economically viable cocoon crop.

Hence, as a summary, I recommend rearing of silkworms during their first three instars to be carried out on artificial diet in the summer months, in order to increase the competitive power of cocoon farmers and to beneficiate the silk entrepreneurs/ industries.

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