THE EXPERIENCE USING OF FREEZE-DRIED MULBERRY LEAFS' AS COMPOSITION OF ARTIFICIAL DIET FOR FEEDING SILKWORM CATERPILLARS.

ABSTRACT

Aims: Due to the changing global climate and the increased spring frosts in recent years, leading to the death of silkworm caterpillars of the first ages from lack of feed and for the transition of sericulture to multiple feedings, there is an urgent need to find effective and cheap means to replace natural food - mulberry leaf. One of the promising ways to increase the forage base of sericulture is the preservation and enrichment of mulberry leaves. One of the new and progressive drying methods for products in sericulture is the method of freeze-drying them in a vacuum.
Place and Duration of Study: The experiments were conducted in the laboratory of the Scientific Research Institute of Sericulture in 2024, where the spring feeding of silkworm caterpillars of 4 breeds and 2 hybrids was carried out
Methodology: After the revival of the eggs, 500 caterpillars were selected and they began to be fed with the developed artificial food. The food was given once a day, the room temperature was 25-26 ° C, and the humidity was 65-70%. when the caterpillars reached the third age, some of the caterpillars in the amount of 150 each of the breed and hybrid were selected and continued to be fed on artificial diet. The remaining caterpillars were calculated and transferred to natural leaf feeding according to the generally accepted methodology.
Results: Depending on the number of layers, the drying of the mulberry leaf lasted from 15 to 25 hours.
All breeds and hybrids of lonesome have well received the developed artificial food. In the Ipakchi-5, Gulshan breeds, as well as the breeding hybrid L-501, the viability of caterpillars was 100%.
Conclusion: According to the totality of the results shown, the Musafo Tola and Gulshan breeds, as well as the T-1 and L-500 breeding hybrids, are promising for feeding them on artificial diet and breeding the corresponding lines and breeds from them.

***Keywords**: mulberry leaf, silkworm caterpillars, freeze drying, artificial diet.*

1. INTRODUCTION

Uzbekistan is a long-standing and significant global producer of cocoons, raw silk, silk goods and products. Currently, Uzbekistan ranks third in the world in harvesting silkworm cocoons and fourth in the world in exporting raw silk and nutcracker products. The intensive growth of cocoon production in the Republic in recent years requires the development of new technologies and high-performance equipment for processing cocoons in order to obtain raw silk that meets all the requirements of world market standards, as well as new directions in science on the use of sericulture products.
Despite the achievements of silkworm scientists in creating new highly productive silkworm breeds, as well as mechanization of the feeding process, this industry remains dependent on natural conditions, various mulberry pests, silkworm diseases, as well as the human factor

and is a seasonal production based on the breeding of caterpillars in farmers' households, far from industrial production methods. Due to the changing global climate and the increased spring frosts in recent years, leading to the death of caterpillars of the first ages from lack of feed, there is an urgent need to find effective and cheap means to replace natural food - mulberry leaf during spring frosts and for the transition of sericulture to multiple feeding.

The silkworm is a monophage. It feeds only on mulberry leaves due to the presence of the chemical compound Morin (flavonol) in them [1]. The nutritional value of mulberry leaves has a significant impact on the growth and development of caterpillars, as well as on the subsequent curling of cocoons. Nutrition plays a crucial role in enhancing the biological properties of the silkworm. The mulberry leaf provides almost all the nutrients necessary for the growth of the monophage silkworm. The protein of mulberry leaves, which is the highest quality for the successful production of cocoons, accounts for about 70% of the silk protein produced by the silkworm [2].

The most modern research methods in the field of sericulture include the addition or enrichment of mulberry leaves with various preparations, numerous compounds, including vitamins, minerals, amino acids, soy protein, hormones and plant extracts, when added to mulberry leaves as additives, they improve the characteristics of the cocoon, including the reproductive ability of the silkworm [3,4,5].

Therefore, one of the promising ways to increase the forage base of sericulture is the conservation and enrichment of mulberry leaves.

The main purpose of canning is to protect the product from physico-chemical, autolytic and microbiological decomposition. There are several directions in the technology of food preservation [6].

Studying them led us to the conclusion that the most promising is the preservation of the leaf in a state of potential (possible) active life after certain effects (suspended animation) [7,8,9].

One of the promising ways to strengthen the forage base of sericulture is the preservation and enrichment of natural diets.

In addition to the theoretical aspects, this path opens up a tempting prospect in sericulture of using the organic mass of mulberry leaves collected for autumn leaf fall.

The solution to these problems is possible by creating and using artificial food for silkworms.

The development and creation of artificial feed for silkworms will solve the urgent problem of standardization of the feed ration, insufficient food supply in spring frosts, eliminate the timely distribution of revived caterpillars after incubation to farms, i.e. make the production of cocoons less dependent on natural conditions, from diseases of mulberry and silkworm, as well as reduce the influence of the human factor.

The use of artificial feed will lead to centralized feeding in hatcheries and labor savings, especially important in the spring period, coinciding with field and household work, as well as due to the shortage of working resources of the rural population.

Therefore, the solution of these problems, i.e. the use of artificial diet by centralized feeding, is one of the most relevant areas of domestic sericulture.

The practical significance of the research results lies in the fact that the use of artificial feed will allow us to move to full-scale provision of farms in the republic with healthy and viable silkworm caterpillars. This will significantly increase the productivity and yield of cocoon raw materials and significantly reduce labor costs.

To put a living active organism into suspended animation, it is necessary to dry it. There are two main drying methods - this is the convective method (drying in hot air) and freeze-drying (frozen drying). One of the new and progressive drying methods for sericulture products is the method of freeze-drying them in a vacuum.

Convective drying is the most common method of dehydration of vegetable raw materials in order to extend its shelf life. The convective drying method in the traditional version provides for the transfer of heat to the dried raw materials using hot air. During the transfer of thermal energy, moisture is released from the raw material, which is carried away from the installation by a drying agent [10].

Freeze drying is the removal of moisture from frozen materials by sublimation of ice, i.e. its direct transition to a vaporous state, bypassing the liquid phase [11].
This research is aimed at choosing the method of drying the main raw material, that is, the mulberry leaf for using it as the main ingredient of artificial diet. .

2. MATERIAL AND METHODS

To test the possibility of feeding caterpillars with artificial diet based on freeze-dried mulberry leaf, spring in 2024y. feeding of silkworm caterpillars was carried out in the laboratory of the Scientific Research Institute of Sericulture. To implement the tasks set, samples of 4 silkworm breeds and 2 hybrids were selected: Ipakchi-5, Chingiz, T-1, L-500, Musoffo tola, Gulshan, Ipakchi1×Ipakchi2, in the amount of 500 pieces from the collection of silkworm breeds and laid for incubation according to the generally accepted method.

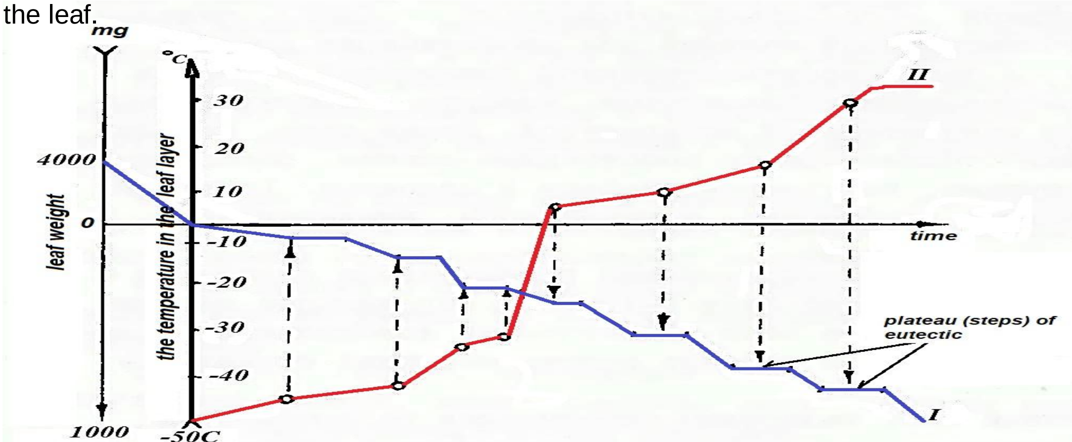
2.1. Using the freeze-drying method for mulberry leaf.

For experiments, mulberry leaves were used, collected before leaf fall in autumn 2023 at the experimental plantation of the mulberry varieties collection of the Institute of Sericulture and freeze-dried.
Based on the analysis of recyclable materials for freeze drying of biological objects, the mode of freeze drying of mulberry leaves was chosen: pre-freezing to t = -45°C, then freeze drying at a condenser temperature of t = +35°C with a residual vacuum pressure of P = 0.3 mmHg [12].
At low temperature sublimation, 7-8% of the absolute moisture content of the leaf is preserved, although it looks very dry and brittle. This leaf is very lyophilic and softens quickly because it has the ability to absorb vapors from the atmosphere.
When the leaf was subjected to supercooling (nitrification) and then sublimation, the cells remained potentially alive.
Such a sheet can be stored for a long time at room temperature in sealed plastic bags. At the same time, the nutritional properties of such a leaf are only slightly reduced.
The freeze-dried mulberry leaf is ground to a floury state on a high-speed shredder. The required amount of cellulose powder is injected into the mulberry powder and everything is also stirred. The necessary ingredients included in the artificial feed are dissolved in the required volume of distilled water on a magnetic stirrer. The prepared mixture of mulberry leaf and cellulose is poured into a heat-resistant plastic bag and then a solution with ingredients is introduced. All this is mixed to a paste-like homogeneous mass. Then the prepared mixture in the bag is placed in the microwave for 3 minutes, the mixture is removed and after two minutes of exposure it is re-placed in the microwave for 2 minutes. The total heating time is 5 minutes. After cooling, the feed is ready for use.

3. RESULTS AND DISCUSSION

3.1. Drying of mulberry leaf by sublimation method.

The drying speed ultimately determines the size of the drying unit, i.e. the number of mulberry leaf layers placed on the shelves, which means its cost, i.e., to a large extent, the cost of the drying process of the material.Depending on the layer, drying lasts from 15 to 25 hours.
During the freeze-drying process, many eutectic plateaus (5-14) are found in the mulberry leaf (depending on the varieties). The last of them occurs at a temperature of about -40÷42 ° C. This suggests that the freezing of salt groups in the sheet does not occur at the same time.
The averaged drying curves and temperature curves of the mulberry leaf with the resulting eutectic plates shown in Fig. 1. give the most complete description of the freezing process of the leaf.



I is the curve of change in the weight of the leaf; II is the curve of temperature inside the leaf layer;

Fig. 1 Averaged drying curves and temperature curves of mulberry leaf with emerging eutectic plates

If drying takes place at temperatures higher than -30 ° C, then such a caterpillar leaf eats worse and does not develop, gradually dying in the second age.

The lower the pre-freezing temperature of the leaf and the faster this process proceeds to the zone of the last plateau (-40-42 ° C), the higher the biological effect of feeding caterpillars with such a leaf. This fact also indicates that up to -42 ° C in the mulberry leaf, not all solutions solidify yet.

These changes in the drying curve are related to changes in the drying rate. Depending on the drying conditions and the intensity of heat supply, moisture can evaporate at one rate or another, as a result of which the moisture content of the product will also decrease at different rates. The results of the freeze drying time are shown in Figure 2.

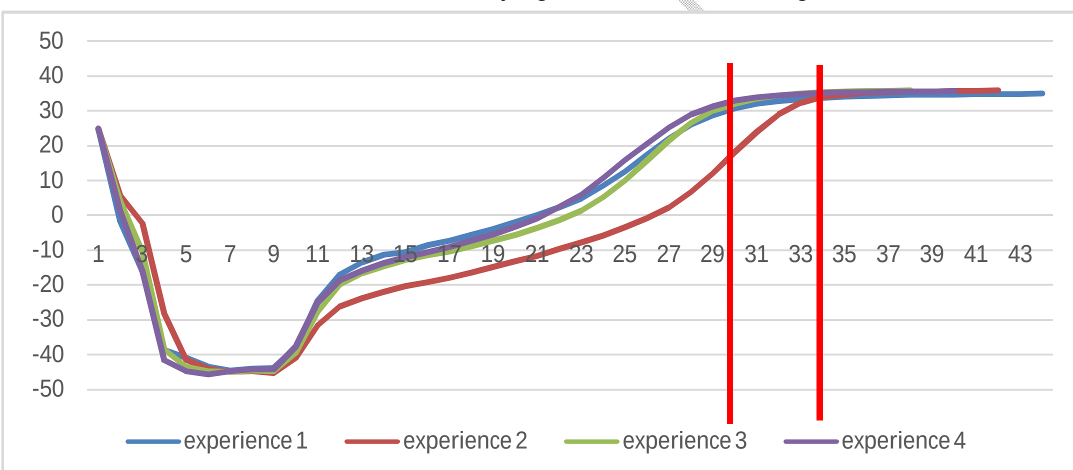


Figure 2. Duration of freeze drying of mulberry leaf

The graph shows that the leaf reaches the set temperature of -45 degrees after 2-3 hours. then the vacuum pump is turned on and after 2 hours the heating is turned on, then the sheet is evenly dried for 9-12 hours. The total drying time varied and ranged from 15-17 hours. The variation in duration, indicated on the graph by red lines, occurs due to the uneven location of the sheet on the shelves of the sublimation chamber.

From the graph, you can calculate the drying rate per unit of time using the formula:

$V = \frac{m_{кg} - m_{кc}}{t}$,g per minute or hour,

Depending on the sheet layer, the drying rate is ≈ 3,5 - 8 mg per hour, and during the falling drying rate is ≈ 2,5 – 6,5 g per hour.

After sublimation, the sheet is sealed in plastic bags and stored under normal temperature and humidity conditions.

3.2. Experimental silkworm feeding with artificial diet

After the revival of the eggs, 500 caterpillars were selected and they began to be fed with the developed artificial diet. The food was given once a day, the room temperature was 25-26 ° C, and the humidity was 65-70%. when the caterpillars reached the third age, some of the caterpillars in the amount of 150 each of the breed and hybrid were selected and continued to be fed on artificial diet. The remaining caterpillars were calculated and transferred to natural leaf feeding according to the generally accepted methodology. The caterpillars continued to be fed once a day on artificial diet. The results of experimental feeding of caterpillars on artificial diet and with the transition to a leaf are shown in Tables 1-2

Table 1 shows the biological indicators of breeds and hybrids based on the results of feeding, and histogram 3 clearly shows data on the viability of caterpillars in comparison on a leaf and artificial diet.

Table 1. Biological indicators of breeds and hybrids

№	Breeds, hybrids	The beginning of caterpillar feeding		The beginning of cocoon curling		Number of caterpillar pcs		The number of cocoons received		Viability, %		Duration of feeding, days	
		A.D.	**leaf**	**A.D.**	**leaf**	**A.D**	**leaf**	**A.D**	**leaf**	**A.D**	**leaf**	**A.D**	**leaf**
1	**Ipakchi-5**	06.05	17.05	02.06	03.06	150	350	150	257	100	63	27	28
2	**Chingiz**	10.05	20.05	03.06	03.06	150	350	146	127	92	75	25	25
3	**T-1**	06.05	17.05	01.06	03.06	150	350	48	311	94	93	26	28
4	**L-501**	13.05	22.05	08.06	08.06	150	1035	50	723	100	70	26	26
5	**Mussafo tola**	10.05	19.05	07.06	07.06	150	336	50	306	100	91	28	29
7	**Ip1xIp2**	11.05	19.05	05.06	06.06	150	510	41	312	82	61	25	26
8	**Gulshan**	07.05	17.05	03.06	03.06	150	59	50	46	100	78	27	27
10	**Ip1xIp2 (contr.)**		11.05		08.06		50		41		82		28

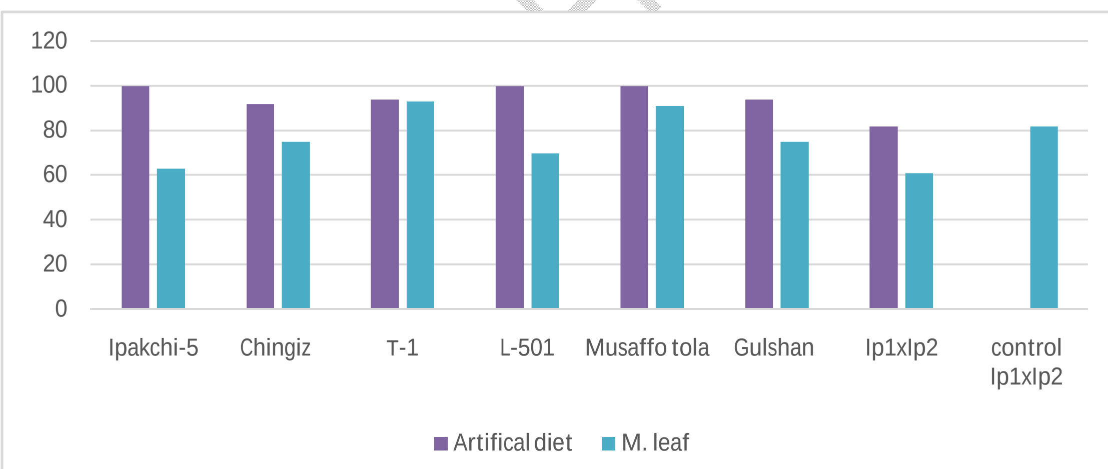


Figure 3. Histogram of caterpillar viability in comparison on a leaf and artificial feed.

Table 2 shows the technological parameters of cocoons obtained from caterpillars fed on IR and with the transition to leaf feeding. Histograms 4 and 5, respectively, clearly show data on cocoon weight and **Content of raw silk.**

Table 2. Technological indicators of cocoons.

№	Породы/ гибриды	The average weight of the cocoon is g .				Shell weight, g				Cocoon diameter mm				Content of raw silk, %			
		A.D.		**leaf**		**A.D.**		**leaf**		**A.D.**		**leaf**		**A.D.**		**leaf**	
		$X \pm S^2x$	**Cv**	**$X \pm S^2x$**	**Cv**	**$X \pm S^2x$**	**Cv**	**$X \pm S^2x$**	**Cv**	**$X \pm S^2x$**	**Cv**	**$X \pm S^2x$**	**Cv**	**$X \pm S^2x$**	**Cv**	**$X \pm S^2x$**	**Cv**
1	**Ipakchi-5**	1,43±0,26	18	1,82±0,23	12	0,25±0.04	16	0,41±0,05	12	17,5±1,01	6	18,9±0,23	5	18,0±2,92	16	23.4±3.0	13
2	**Chingiz**	1,50±0.26	18	1,35±0,09	7	0,26±0.04	17	0,26±0,09	14	15,4±1,10	6	16,7±1,23	7	17,7±3,2	18	19,7±1.9	9

3	**T-1**	1,79±0.32	18	2,01±0,30	15	0,30±0.05	17	0,46±0,05	11	19,3±1,35	7	20,4±1,21	6	17,5±3,4	19	23,3±2,6	11
4	**L-501**	1,76±0.33	19	1,75±0,22	13	0,35±0.04	12	0,39±0,05	12	18,7±1,15	6	20,0±0,76	4	18,0±2,3	12	22,8±3,1	13
5	**Mussafo tola**	2,20±0.34	15	2,42±0,34	15	0,44±0.04	10	0,50±0,06	12	20,6±1,26	6	20,0±1,14	6	20,3±2,4	12	24,5±3,0	12
7	**Ip1xIp2**	1,72±0.40	22	1,88±0,40	22	0,30±0.08	21	0,40±0,05	13	18,3±1,42	8	19,2±1,40	7	17,1±2,9	17	21,8±2,8	13
8	**Gulshan**	1,78±0.34	19	1,69±0,34	19	0,31±0,06	19	0,39±0,07	17	18,5±1,39	8	18,8±1,46	8	18,0±2,2	12	23,9±3,5	14
10	**Ip1xIp2 (contr.)**			1,67±0,36	22			0,36±0,07	19			18,1±2,2	12			22,3±2,9	13

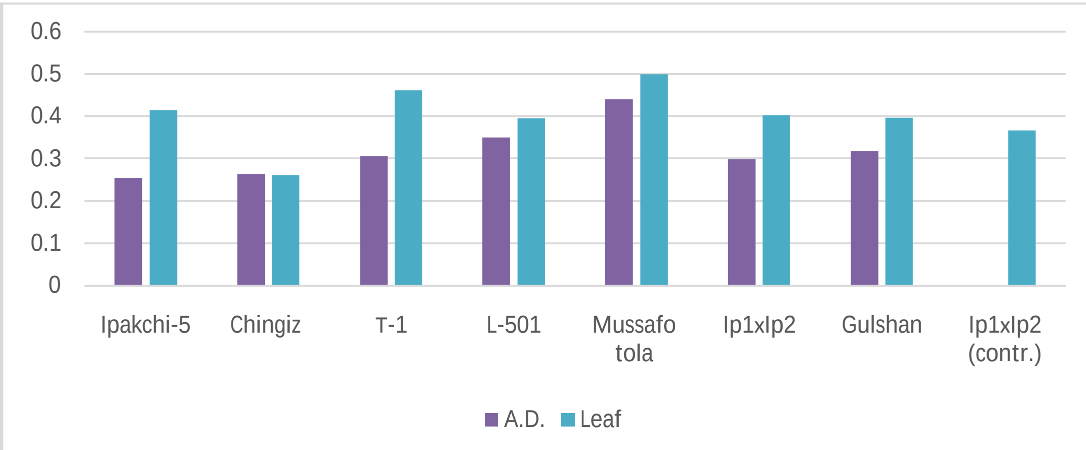


Figure 4. Comparative indicators of the cocoons obtained by weight.

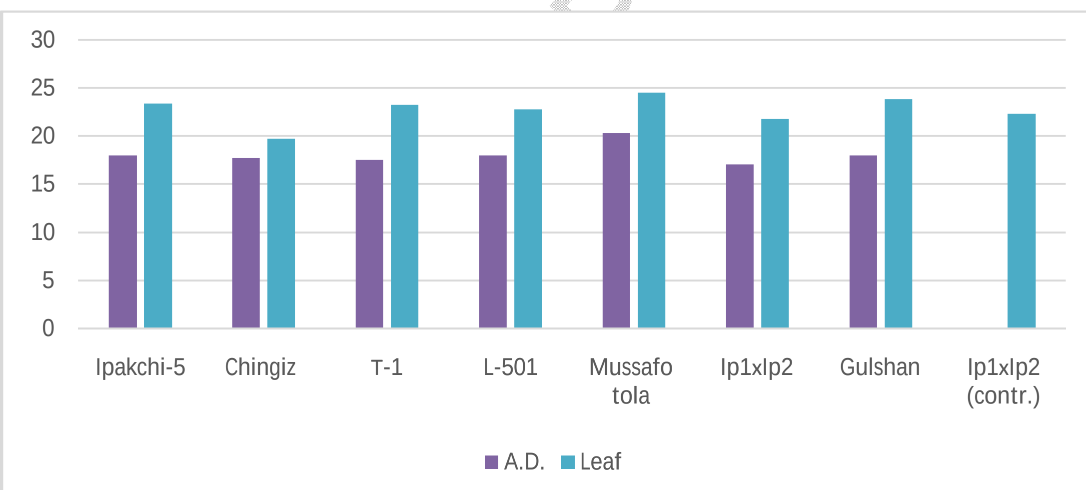


Figure 5. Comparative indicators of the cocoons obtained by **Content of raw silk**.

The analysis of tables and histograms showed the following results;

All breeds and hybrids of Ionesome have well received the developed artificial food. In the breeds of the Ipakchi-5, Gulshan and the breeding hybrid L-501, the viability of the caterpillars was 100%. And when switching from artificial diet at the third age to a leaf, caterpillars of T-1 hybrids showed the best viability - 93% and Musoffo Tola breeds - 91%. The highest average weight of cocoons during artificial diet feeding was shown by the Musoffo Tola breed and the T-1 hybrid, respectively, 2.2 g and 1.79 g. Also, the Musoffo Tola and Gulshan breeds showed a high shell mass, respectively 440mg and 370mg, and the hybrid L-501 – 350mg. During the transition from artificial diet to leaf, the highest average cocoon weight was shown by the Musaffo tola breed - 2.42 g. and the T-1 hybrid-

2.01 g. These same breed and hybrid showed the best shell weight of 500 mg and 460 mg, respectively.
According to the totality of the results shown, the Musafo Tola and Gulshan breeds, as well as the T-1 and L-500 breeding hybrids, are promising for feeding them on artificial feed and breeding the corresponding lines and breeds from them. Despite the fact that these indicators were lower than the control indicators, except for the viability indicator, this most likely indicates that the first generation (F1) was not adapted to artificial diet. Therefore, it is necessary to carry out breeding work.
Studies have shown that several productive silkworm breeds, which initially reacted poorly to artificial diet, gradually adapted to it through breeding from generation to generation. [13,14]. Five multi- and six bivoltine strains were created, which readily accepted an artificial diet and demonstrated favorable economic characteristics comparable to those of their counterparts grown on mulberry leaves. They also conducted tests on hybrids, and in connection with the confirmed resistance of the silkworm to breeding with artificial nutrition, they proposed to make diets with different contents of dried mulberry leaves [15].

4. CONCLUSION

1. The freeze drying mode was selected, the drying time in this mode was 14-18 hours.
2. Based on previous experiments of artificial feed feeding of caterpillars, as well as a comprehensive analysis of previously developed artificial feeds, a recipe for artificial feed was created, in which the content of mulberry leaf powder was 72%.
3. All breeds and hybrids equally well accepted the developed artificial food. In the breeds of the Pack-5, Gulshan and the breeding hybrid L-501, the viability of the caterpillars was 100%. And when switching from artificial feed at the third age to a leaf, cocoons of T-1 hybrids showed the best viability - 93% and Musoffo Tola breeds - 91%.
4. According to the totality of the results shown, the Musafo Tola and Gulshan breeds, as well as breeding hybrids T-1 and L-500 are promising for feeding them on artificial feed and the removal of the corresponding lines and breeds from them.

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