

**UNIVERSITY OF AGRICULTURAL SCIENCES  
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FACULTY OF ANIMAL BREEDING AND BIOTECHNOLOGIES**

**Cristina ZAH**

## **Ph.D THESIS SUMMARY**

**RESEARCH REGARDING THE EFFECT OF ENVIRONMENTAL  
FACTORS AND OF THE NUTRITION ON THE REARING OF  
SILKWORMS (BOMBYX MORI L.)**

**SCIENTIFIC COORDINATOR  
Prof. univ. dr. LIVIU ALEXANDRU MĂRGHITAŞ**

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## INTRODUCTION

Traditionally, in order to harvest quality silk from the *Bombyx Mori* L. species of silkworm, feeding with fresh mulberry leaves was necessary, substitution attempts with other vegetal matter yielding undesirable results. Research regarding the nutrition of the silkworm has progressed significantly since the introduction of artificial diets, which attempt to reproduce and even enhance the nutritional content of fresh mulberry leaves for an extended period past regular leafing intervals, in order to achieve an increase in the economic parameters.

## RESEARCH OBJECTIVES

The aim of the research was to observe the nutritional effect on the productivity of silkworms (length and quality of thread) for various races and hybrids reared in Transylvania.

The experiment was run in the springs of the years 2010 and 2011.

In the first year, a comparative study was made following the administering of types of oil and milk applied to the mulberry leaves, fed to one Korean race, and Japanese and Italian hybrids.

During the second year, the effects of treatment with Vitamin C, B1, B6, B12 and a multivitamin mix (all in various concentrations) on the productivity of silkworms (length and quality of thread) was observed, as administered to the leaves fed to larvae (starting with the 4<sup>th</sup> instar) of the Băneasa 75 race reared in Transylvania.

The incubation, hatching, the biological and technological indices were studied, as follows:

1. biological indices of the egg (serosa color, chorion color, hatching percentage)
2. biological indices of the larvae (color of the larvae in the last instar, adult larvae shape, larval signs, weight and length of the larvae, duration of the larval stage, mass of silk glands)

3. biological indices of the raw cocoon (mass, longitudinal and transversal axis size, shell thickness, silk percentage)
4. technological indices of the thread (length, finesse)

## RESEARCH ORGANIZATION

### Biological material

The biological material was represented by two races and three hybrids of *Bombyx mori* L. supplied by SC. SERICAROM (București, România).

Table 1

Provenience of the biological material (Matei, 2008)

Race /Hybrid	Country	Provider
Băneasa 75	Romania	Commercial society "Sericarom" – Research department
BaeGokJam	Korea	Department of Agriculture Biology, National Institute of Agricultural Science and Technology, Rural Development Administration Suwon
(71x70) x (125x121)	Italy	Sericulture Experiment Station, Padua
Shunrei x Shogetsu	Japan	Laboratory of Insect Genetics, National Institute of Agro biological Science, Kobuchisawa 6585, Kitakoma-gun, Yamanashi-hen
N 137 x C 146	Japan	Laboratory of Insect Genetics, National Institute of Agro biological Science, Kobuchisawa 6585, Kitakoma-gun, Yamanashi-hen

### Employed methods

In order to accomplish the proposed objectives, we analyzed the qualitative (shape, color, and dimensions of the larvae and cocoons) and quantitative (hatchability percentage, raw cocoon weight, shell weight, silk ratio, length and finesse of filament, reelability percentage) characteristics and we interpreted the results from a statistical standpoint.

The observations were made in two consecutive years.

The first year studied 4 silkworm races and hybrids (one pure Korean strain, 2 Japanese hybrids and 1 Italian hybrid) reared in the environmental conditions of Transylvania, treated with milk (3.5% fat content), linseed oil, hemp oil, sea buckthorn oil, and respectively olive oil (oils applied either directly on mulberry leaves or on filter paper squares subsequently placed between mulberry leaves) , starting from the 4<sup>th</sup> instar.

Experimental lots of 150 larvae in each were partitioned, 9 for treatment variants, and one control group. For the biological parameters of egg yields, we averaged 5 different samples. The mass and length of larvae were studied for 20 randomly selected samples from each of the experimental lots. The silk gland mass was determined via dissection of 10 larvae for each lot. The raw cocoon weight was determined for 100 samples each. The shell weight and silk ratio were measured for 10 samples each. The length of the longitudinal and transversal axis was determined for 10 cocoons each.

In the second year, observations were made on the Romanian race Băneasa 75, reared in the environmental conditions of Transylvania, treated with vitamins B1, B6, B12, C and multivitamin mix, starting with the 4<sup>th</sup> instar.

The experimental lots had 60 larvae each, with 13 treatment variants and one control group. The mass and length of larvae were studied for 20 randomly selected samples from each of the experimental lots. The silk gland mass was determined via dissection of 5 larvae for each lot. The raw cocoon weight was determined for 20 samples each. The shell weight and silk ratio were measured for 20 samples each. The length of the longitudinal and transversal axis was determined for 10 cocoons each.

## RESULTS AND DISCUSSIONS

Analyzing the biological indices of the egg for the studied races and hybrids, we observe that the majority have a grey colored serosa and a white or yellow chorion. The hatchability percentage was determined on 5 samples of 150 eggs for each race and hybrid, on the 3<sup>rd</sup> day after the start of the hatching period. The obtained results range from 91.9 % (BaeGokJam) and 98.9% (Shunrei x Shogetsu). These results are within the

values mentioned by Bacsa in the year 2006, for three testing centers (Azerbaijan, Ukraine and Bulgaria).

In figure 1 we can observe the average larval mass for the control group (lot code M) as being between 2.397g for BaeGokJam and 1.832g for N137 x C146.

For the BaeGokJam race, the minimum value of the average larval mass is 0.925g (TCT) and the maximum value is 2.397g (M).

For the Shunrei x Shogetsu hybrid, the minimum value of the average larval mass is 1.225g (TCTp) and the maximum value is 2.173g (M).

For the (71x70) x (125x121) hybrid the minimum average larval mass is 1.686g (TCT) and the maximum value is 2.102g (TL).

For the N137 x C146 hybrid the minimum value of the average larval mass is 1.063 g (TCNp) and the maximum value is 2.076g (TL).

The average length of larvae (figure 2) for the control group lies between a value of 4.75cm for the N137 x C146 hybrid and 5.31cm for the BaeGokJam race.

For the BaeGokJam race the minimum value of the average larval length is 3.55cm (TCT) and the maximum value is 5.31cm (M).

For the Shunrei x Shogetsu hybrid the minimum value of the average larval length is 3.95cm (TCTp) and the maximum value is 5.19cm (M).

For the (71x70) x (125x121) hybrid the minimum value of the average larval length is 4.65cm (TCN) and the maximum value is 5.26cm (TMp).

For the N137 x C146 hybrid the minimum value of the average larval length is 4.20cm (TCNp) and the maximum value is 5.16cm (TI).

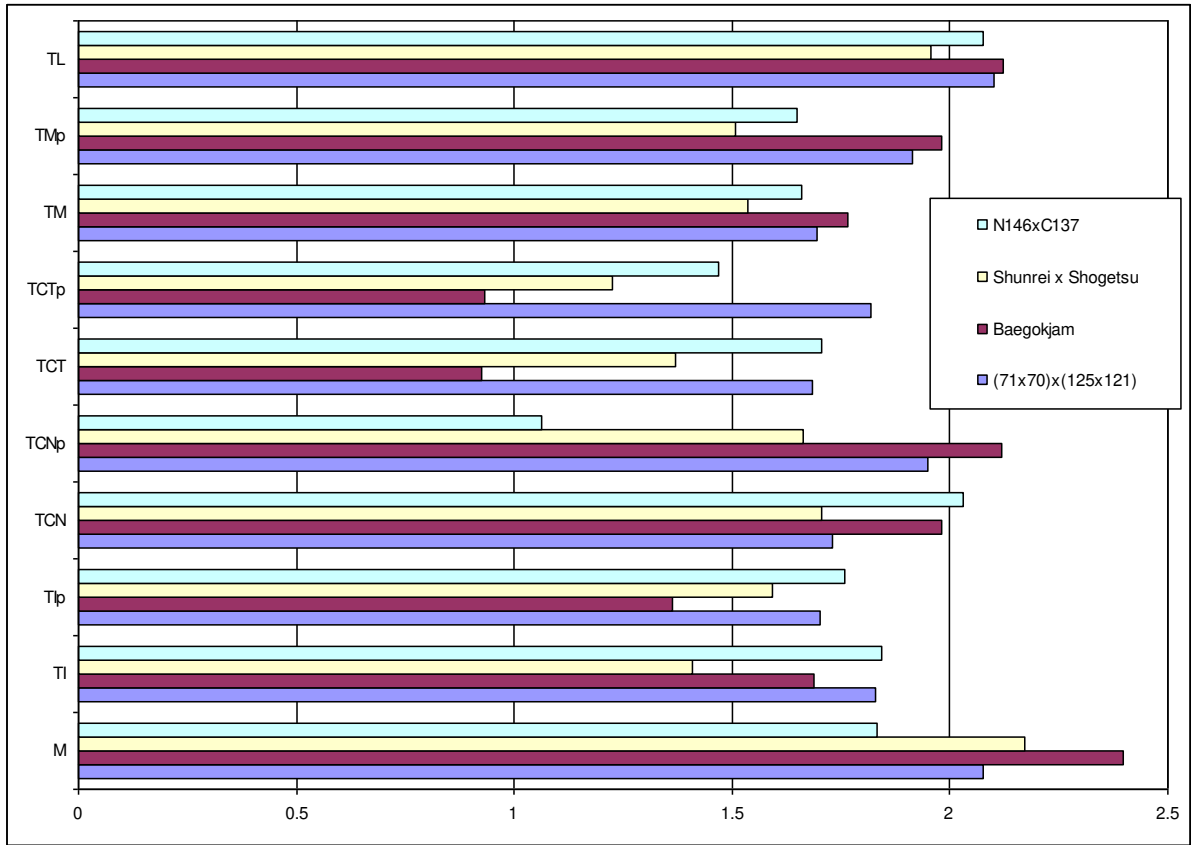


Figure 1. Average values of larval mass in day 2 of instar 5

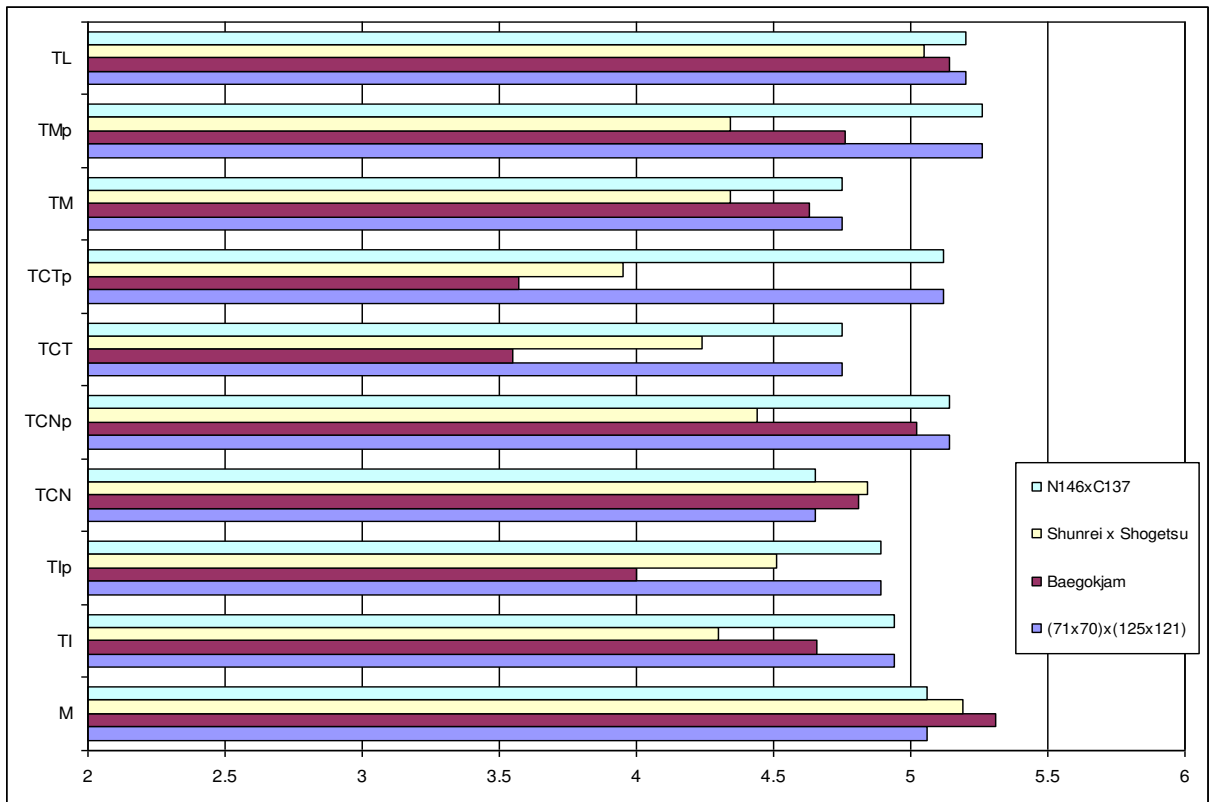


Figure 2. Average values of larval length in day 2 of instar 5

Table 2

Average values of the fresh cocoon mass, shell weight, silk percentage, length and finesse of the thread filament for the lots treated with linseed oil

Race/hybrid	Code	Raw cocoon weight (g)	Shell weight (g)	Silk ratio (%)	Filament length (m)	Filament finesse (den)
BaeGokJam	TI	1.246	0.266	21.34%	678	3.03
BaeGokJam	TIp	1.374	0.284	20.65%	707	3.10
Shunrei x Shogetsu	TI	1.545	0.315	20.40%	819	2.99
Shunrei x Shogetsu	TIp	1.733	0.385	22.19%	892	3.35
(71x70)x(125x121)	TI	1.127	0.210	18.64%	565	2.88
(71x70)x(125x121)	TIp	1.319	0.251	19.01%	648	3.00
N137 x C146	TI	1.433	0.278	19.37%	740	2.88
N137 x C146	TIp	2.009	0.363	18.05%	932	2.99

Table 3

Average values of the fresh cocoon mass, shell weight, silk percentage, length and finesse of the thread filament for the lots treated with hemp oil

Race/hybrid	Code	Raw cocoon weight (g)	Shell weight (g)	Silk ratio (%)	Filament length (m)	Filament finesse (den)
BaeGokJam	TCN	1.413	0.277	19.62%	718	2.98
BaeGokJam	TCNp	1.576	0.344	21.82%	859	3.09
Shunrei x Shogetsu	TCN	1.359	0.286	21.04%	735	3.02
Shunrei x Shogetsu	TCNp	1.664	0.385	23.17%	997	3.00
(71x70)x(125x121)	TCN	1.344	0.231	17.22%	608	2.95
(71x70)x(125x121)	TCNp	1.466	0.256	17.49%	680	2.92
N137 x C146	TCN	1.250	0.255	20.39%	678	2.89
N137 x C146	TCNp	1.572	0.282	17.93%	734	2.95

Table 4

Average values of the fresh cocoon mass, shell weight, silk percentage, length and finesse of the thread filament for the lots treated with sea buckthorn oil

Race/hybrid	Code	Raw cocoon weight (g)	Shell weight (g)	Silk ratio (%)	Filament length (m)	Filament finesse (den)
BaeGokJam	TCT	1.063	0.187	17.60%	513	2.81
BaeGokJam	TCTp	1.108	0.293	26.44%	756	2.99
Shunrei x Shogetsu	TCT	1.391	0.287	20.64%	669	3.34
Shunrei x Shogetsu	TCTp	1.524	0.363	23.79%	877	3.21
(71x70)x(125x121)	TCT	1.103	0.220	19.92%	598	2.85
(71x70)x(125x121)	TCTp	1.463	0.258	17.64%	664	3.01
N137 x C146	TCT	1.244	0.223	17.97%	609	2.82
N137 x C146	TCTp	1.456	0.288	19.75%	766	2.88

Table 5

Average values of the fresh cocoon mass, shell weight, silk percentage, length and finesse of the thread filament for the lots treated with olive oil

Race/hybrid	Code	Raw cocoon weight (g)	Shell weight (g)	Silk ratio (%)	Filament length (m)	Filament finesse (den)
BaeGokJam	TM	1.747	0.359	20.55%	891	3.11
BaeGokJam	TMp	1.641	0.363	22.10%	897	3.12
Shunrei x Shogetsu	TM	1.303	0.320	24.52%	771	3.22
Shunrei x Shogetsu	TMp	1.727	0.370	21.42%	870	3.30
(71x70)x(125x121)	TM	1.735	0.294	16.97%	762	2.99
(71x70)x(125x121)	TMp	1.075	0.271	25.21%	726	2.90
N137 x C146	TM	1.203	0.255	21.22%	684	2.86
N137 x C146	TMp	1.510	0.269	17.81%	709	2.91



Table 6

Average values of the fresh cocoon mass, shell weight, silk percentage, length and finesse of the thread filament for the lots treated with milk (3.5% fat content)

Race/hybrid	Code	Raw cocoon weight (g)	Shell weight (g)	Silk ratio (%)	Filament length (m)	Filament finesse (den)
BaeGokJam	TL	1.563	0.351	22.44%	869	3.12
Shunrei x Shogetsu	TL	1.622	0.399	24.62%	936	3.32
(71x70)x(125x121)	TL	1.709	0.279	16.34%	709	3.05
N137 x C146	TL	1.961	0.371	18.91%	933	3.05

From the data presented in table 2 (linseed oil treatments) we observe that the highest values for the silk thread length were obtained for the N137 x C146 hybrid (TIp) (932 m) followed by de Shunrei x Shogetsu hybrid (TIp) (892m). Regarding the silk ratio we observe the Shunrei x Shogetsu hybrid (TIp) has the highest value (22.19%).

In table 3 (hemp oil treatments) we observe the highest values of the silk filament length were obtained for the Shunrei x Shogetsu hybrid (TCNp) (997 m) followed by the BaeGokJam race (TCNp) (859m). Regarding the silk ratio we find that the Shunrei x Shogetsu hybrid (TCNp) has the highest value (23.17%).

In table 4 (sea buckthorn treatments) we observe the highest values of the silk filament length were obtained for the Shunrei x Shogetsu hybrid (TCTp) (877 m) followed by the N137 x C146 hybrid (TCTp) (766m). Regarding the silk ratio we find that the BaeGokJam race (TCTp) has the highest value (26,44%).

In table 5 (olive oil treatments) we observe the highest values of the silk filament length were obtained for the BaeGokJam race (TMp) (897m) followed by the same race (TM) (891m). Regarding the silk ratio we find that the (71x70)x(125x121) hybrid (TMp) has the highest value (25,21%).

In table 6 (milk with 3.5% fat contents treatment) we observe the highest values of the silk filament length were obtained for the Shunrei x Shogetsu hybrid (936 m) followed by the N137 x C146 hybrid (933m). Regarding the silk ratio we find that the Shunrei x Shogetsu hybrid (TIp) has the highest value (24,62%).

Following the statistical significance analysis of the differences in measurements of the silk thread length between each treated lot and the control group of each studied hybrid and race, we observe for the BaeGokJam race positive differences for TI, TIp, TCN (significant) și TCT (distinctly significant). The rest of the treatments did not cause statistically significant differences.

For the Shunrei x Shogetsu hybrid, positive differences were observed for TCN, TM (significant), TCT (distinctly significant). The rest of the treatments did not cause statistically significant differences.

For the the (71x70)x(125x136) hybrid the treatments did not cause statistically significant differences.

For the N137xC146 hybrid, positive differences could be observed for TCN, TM, Tmp (significant) and distinctly significant positive differences were observed for the TCT treatment variant. The rest of the treatments did not cause statistically significant differences.

Following the statistical significance analysis of the differences in measurements of the silk thread finesse between each treated lot and the control group of each studied hybrid and race, we observe for the BaeGokJam race, positive differences could be observed for TCT (significant). The rest of the treatments did not cause statistically significant differences.

For the Shunrei x Shogetsu hybrid, we observe negative differences for TIp and TCT (significant). The rest of the treatments did not cause statistically significant differences.

For the (71x70)x(125x136) hybrid, positive differences could be observed for TI and TCT (significant). The rest of the treatments did not cause statistically significant differences.

For the N137xC146 hybrid, positive differences could be observed for TCT (significant), with negative differences for TIp (significant) and TL (distinctly significant). The rest of the treatments did not cause statistically significant differences.

Table 7

Average values of the fresh cocoon mass, shell weight, silk percentage, length and finesse of the thread filament for the lots treated with vitamins

Race/hybrid	Code	Raw cocoon weight (g)	Shell weight (g)	Silk ratio (%)	Filament length (m)	Filament finesse (den)
Băneasa 75	B75	1.936	0.450	23.24%	1160	2.94
Băneasa 75	VC1	1.796	0.429	23.89%	1101	2.95
Băneasa 75	VC2	1.876	0.421	22.44%	1084	2.94
Băneasa 75	VC3	1.769	0.427	24.14%	1103	2.93
Băneasa 75	VB1-250	1.797	0.441	24.54%	1134	2.95
Băneasa 75	VB1-500	1.958	0.454	23.19%	1167	2.95
Băneasa 75	VB1-1000	1.803	0.441	24.46%	1137	2.94
Băneasa 75	VB6-250	1.818	0.414	22.77%	1077	2.91
Băneasa 75	VB6-500	1.895	0.462	24.38%	1182	2.96
Băneasa 75	VB6-1000	1.889	0.419	22.18%	1075	2.95
Băneasa 75	VB12-2,5	1.826	0.413	22.62%	1058	2.96
Băneasa 75	VB12-5	1.864	0.411	22.05%	1056	2.95
Băneasa 75	VB12-10	2.094	0.427	20.39%	1105	2.93
Băneasa 75	MV 4,5	2.021	0.472	23.35%	1176	3.04

As can be observed in table 7, for the Băneasa 75 race we observe that the highest value for silk ratio (24.54%) is encountered for the vitamin B<sub>1</sub> 250ppm treatment variant, whereas the silk thread length has the highest value for the vitamin B<sub>6</sub> 500ppm treatment variant (1182m).

By analyzing the significance of the differences between the average values of the silk filament length we only notice one statistically significant positive difference, for the vitamin B<sub>1</sub> 250ppm treatment variant (significant). The rest of the treatments did not cause statistically significant differences.

## CONCLUSIONS

1. The larval stage duration ranged from 25 days for the (71x70)x(125x121) hybrid and the BaeGokJam race, up to 26 days for the Shunrei x Shogetsu and N137 x C146 hybrids.
2. For the treatment variants with linseed oil, the overall best result is obtained for the Shunrei x Shogetsu hybrid filter paper variant (rank 2 in the overall ranking of the treatments with oils or milk), rank 2 being taken by the N137xC146 hybrid filter paper variant (rank 11 in the overall ranking), and rank 3 is also occupied by the Shunrei x Shogetsu hybrid for the treatment variant with oil sprinkled on leaves (rank 13 in the overall ranking). The average of all obtained scores (for both the sprinkled oil and filter paper variants) places the treatment with linseed oil as rank 3.
3. For the treatment variants with hemp oil, the overall best result is obtained for the Shunrei x Shogetsu hybrid filter paper variant (rank 3 in the overall ranking of the treatments with oils or milk), rank 2 being taken by the BaeGokJam race filter paper variant (rank 10 in the overall ranking), and rank 3 is also taken by the Shunrei x Shogetsu hybrid for the treatment variant with oil sprinkled on leaves (rank 17 in the overall ranking). The average of all obtained scores (for both the sprinkled oil and filter paper variants) places the treatment with hemp oil as rank 4.
4. For the treatment variants with sea buckthorn oil, the overall best result is obtained for the Shunrei x Shogetsu hybrid filter paper variant (rank 6 in the overall ranking of the treatments with oils or milk), rank 2 being taken by the BaeGokJam race filter paper variant (rank 14 in the overall ranking), and rank 3 is also taken by the Shunrei x Shogetsu hybrid for the treatment variant with oil sprinkled on leaves (rank 15 in the overall ranking). The average of all obtained scores (for both the sprinkled oil and filter paper variants) places the treatment with sea buckthorn oil in the last place (rank 5).
5. For the treatment variants with olive oil, the overall best result is obtained for the Shunrei x Shogetsu hybrid filter paper variant (rank 4 in the overall ranking

of the treatments with oils or milk), rank 2 being taken by the BaeGokJam race filter paper variant (rank 5 in the overall ranking), and rank 3 is also taken by the BaeGokJam race for the treatment variant with oil sprinkled on leaves (rank 7 in the overall ranking). The average of all obtained scores (for both the sprinkled oil and filter paper variants) places the treatment with olive oil as rank 2.

6. For the treatment variants with milk (3.5% fat), the overall best result is obtained for the Shunrei x Shogetsu hybrid (also taking rank 1 in the overall ranking of all the treatments with oils or milk), rank 2 being taken by the N137xC146 hybrid (rank 8 in the overall ranking), and rank 3 is also occupied by the BaeGokJam race (rank 9 in the overall ranking). The average of all obtained scores places the treatment with milk (3.5% fat contents) in the first place (rank 1).
7. For the Băneasa 75 race, rank 1 is occupied by the lot fed with mulberry leaves sprayed with a multivitamin mix in a water solution at 4.5% concentration. Making a general ranking including all races, hybrids and treatment variants from both study years, we can conclude that this multivitamin treatment had the best overall results, scoring better on every of the studied parameters. Rank 2 is taken by the lot treated with vitamin B<sub>6</sub> 500ppm. However, for a lower dosage of the same vitamin (lot VB6-250), the results rank in the last place for this second year alone.
8. Sea buckthorn oil has the highest overall potential to improve the silk mass, with linseed oil on second place, olive oil in third place, while hemp oil and milk do not show a statistically significant difference for this parameter. However, taking into account all biological parameters, the milk treatment takes first place, the only one that has overall average results above those of the control group.

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