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THE USE OF "CHLORAMICOB" BIOSTIMULATOR IN THE FEEDING OF NURSE BEES TO OBTAIN ROYAL JELLY

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Abstract

Royal jelly is a secretion of the hypopharyngeal and mandibular glands of young nurse bees, used to feed larvae in the first three days throughout the larval period. The research evaluated the impact of the biostimulator "Chloramicob" on royal jelly production. It was established that the optimal dose is 2.25 ml/L administered to nurse bees (1 L sugar syrup/day for three days). In the first stage, it increased the larval acceptance rate by 12.5-27.5%, the mass of the brood by 2.4-14.4% and the total royal jelly production by 25.45-40.04%. In the second stage, increases of 10.0-24.3% in the number of larvae raised, 1.49-1.79% in the diameter of the queen cells and 41.14-93.38% in total royal jelly production. In the third stage, there were increases of 1.12-1.32% in the diameter of the queen cells, 1.13-3.30% in their length and 4.82-27.82% in the total royal jelly production obtained compared to the control group. The conclusion highlights that the use of the biostimulator "Chloramicob" in the feeding of nurse bees leads to an increase in the number of larvae accepted by 7.27-16.36% and in the total royal jelly production by 21.11-48.06% compared to the control group.

Key words: *bee families, biostimulator, sugar syrup, morpho productive indices, royal jelly.*

INTRODUCTION

Royal jelly is a secretion of the hypopharyngeal and mandibular glands of young bees (nurses) at the age of 3-12 days, which is used to feed young larvae in the first three days, queen larvae throughout the larval period and queens (Ahmad et al., 2020; Ebubekir & Çağlayan, 2023; Eremia, 2020).

Freshly harvested royal jelly presents an opaque mass, consistently like cream, slightly viscous, white, yellowish-white or yellowish in color, sour taste with a burning shade and a specific pleasant smell, slightly aromatic (Eremia et al., 2000).

Royal jelly contains 58.0-67.0% water and 33.0-42.0% dry matter, of which: 13.8-18.0% protein, 7.5-12.5% carbohydrates, 3.0-6.0% lipids, 0.8-1.5% ash, 8.7-4% undetermined substances (Lazăr & Vornicu, 2007).

To produce royal jelly, the nurse colony must have at least 3 kg of bees of all ages and a lot of broods, 10-12 kg of honey and considerable reserves of fodder (Krasochko & Eremia, 2022). The amount of royal jelly harvested depends on the number of larvae proposed for rearing, as well as the number accepted by the nurse colony (Ebubekir & Çağlayan, 2023).

Feeding the brood colonies with the nutritional composition containing sugar syrup and peppermint essential oil makes it possible to increase the percentage of larvae reception, increases the productivity of bee colonies in the production of royal jelly, improves the physiological state of worker bees and stimulates the flight activity when collecting both with and without pollen balls (Krivtsov & Lebedev, 1993).

The process of obtaining royal jelly includes forming brood colonies, making combs and transferring larvae; after 72 hours, the royal jelly is extracted (https://revista-ferma.ro/7-conditii-pentru-obtinerea-laptisorului-de-matca/).

During periods of lack of collection, it is mandatory that the production of royal jelly be stimulated by artificial feeding with syrup (https://agromedia.md/agricultura-moderna/zootehnie/cresterea-albinelor/cum-se-produce-laptisorul-de-matca-procedeul-pas-cu-pas) or with food rich in proteins

(https://apilink.ro/utile/produse-apicole/laptisor-de-matca/59-producerea-laptisorului-de-matca).
Based on the above, the purpose of the work is to study the influence of stimulating nurse bees feeding with a mixture of sugar syrup and a biostimulator on the number of larvae accepted for rearing and the production of royal jelly.

MATERIALS AND METHODS

For the experiment, four groups of bee colonies we had formed, according to the principles of the methods of analogs according to the number of combs, the strength of the colony, the number of combs with brood capped and the honey reserve in the hive.
In the absence of maintenance honey collection, the bee colonies were administered one liter of a mixture of sugar syrup in a concentration of 50% and the biostimulator "Chloramicob" group I - with 1.25 ml/L, group II - with 2.25 ml/L, group III - with 3.25 ml/L, group IV (control) pure sugar syrup.
In the first half, a frame with 40 transferred larvae was introduced into the nurse (breeding) families, in the second half – with 70 larvae (2 frames: one with 30 and the second with 40 larvae) and in the third – with 110 larvae (3 frames: two with 40 larvae and the third with 30 transferred larvae).
The nurse bees were given one liter of a mixture of sugar syrup in a concentration of 50% and with biostimulator, three days in a row after the introduction of the frame with transferred larvae, after 3 days the frames with the bees were removed from the hive to collect royal jelly and the second and third half were repeated.
During the royal jelly collection experiments, the following indices were studied: the percentage of acceptance of transferred larvae for growth, the diameter, length and mass of the brood, the amount of royal jelly obtained from a brood and from a nurse family per session. The results obtained were processed by the method of statistical variations using the computer program Microsoft Office.

RESULTS AND DISCUSSIONS

The experimental bee colonies had 12-13 combs, the strength of the colonies was 10 alleys (spaces between the combs occupied by bees), the honey reserve was 9.0-9.5 kg and 6.0 combs with brood. In the first half, the nurse colonies were given a frame with 40 transferred larvae for rearing.
The results of the research showed that feeding bee colonies during the period of lack of honey collection with a mixture of sugar syrup with a concentration of 50% and biostimulator stimulates an increase in the percentage of larvae acceptance for rearing by 12.5-27.5% compared to the control group (Table 1).

Table 1. Productive indices of breeding families, number of larvae transferred and accepted for breeding, 03-06.06.2024 (I transfer)

Group	No. of frames, pcs.	Colony strength, No. of alleys	Honey reserves in the nest, kg	Amount of capped brood, hundred cells	No. of transferred larvae, pcs.	No. of accepted larvae	
						Pcs.	%
I-Sugar syrup + Chloramicob, 1.25 ml/l	12	10	9.0	6	40	32	80.0
II-Sugar syrup + Chloramicob, 2.25 ml/l	13	10	9.5	6	40	30	75
III-Sugar syrup + Chloramicob, 3.25 ml/l	12	10	9.0	6	40	36	90
IV-Pure sugar syrup (control)	12	10	9.0	6	40	25	62.5

The diameter of the queen cells on the third day in the experimental groups was on average 10.32-10.63 mm and the length – 23.91-25.90 mm. The mass of the queen cells with larvae and royal jelly in group II was 0.18 g (14.4%) higher than in the control group (*$B_3 \geq 0.999$), and in group I - by 0.03 g (2.4%). The largest amount of royal jelly in one queen cell was obtained in group II on average - 0.587 g, which was 84 mg (16.70%) more than in the control group IV, the difference being authentic **$B_3 \geq 0.999$ (Table 2).

The coefficient variation of the studied indices ranged between 5.24 (queen cell diameter) and 13.05 (amount of royal jelly obtained from a queen cell). The amount of royal jelly obtained from one beehive was 1.8-2.3 times higher than the results obtained by other local researchers (Belioglo, 2001).

Considering the number of larvae accepted and the average amount of royal jelly from a queen cell, it was found that the total amount of royal jelly obtained in the first half varied between 15.776 g and 17.610 g, or 3.201-5.035 g (25.45-40.04%) more than in the control group.

It was revealed that the largest amount of royal jelly (17.610 g) was obtained in group II, which was administered a mixture of sugar syrup and 2.25 ml/l of biostimulator. The results obtained confirm the deductions of some Romanian researchers, such as (Mărghitaș, 2008) that from 5 bee colonies, an average of about 50 g of royal jelly can be obtained in one session. Feeding nurse bees during the larval growth period for three days with one liter of a mixture of sugar syrup and 2.25 ml/l of biostimulator increases the production of royal jelly by 40.04% more than in the control group.

In the second half, two frames (one with 40 larvae, the second with 30 larvae) were introduced into the breeding families, a total of 70 transferred larvae. Nurse bees were fed from the introduction of the frames with transferred larvae with one liter of a mixture of sugar syrup with biostimulator for three days until the frame with the beehives was removed from the hive to collect the royal jelly.

It was found that the breeding families in the experimental groups in the second half where 70 transferred larvae were given accepted 28-38 larvae or 10.0-24.3% more than the control group IV (Table 3).

Table 2. Indices of the brood and the amount of royal jelly obtained, 03-06.06.2024 (I transfer - 40 larvae)

Group	Indices	Queen cell's diameter, mm	Queen cell's lenght, mm	The queen cell's mass with larvae and royal jelly, g	The amount of royal jelly obtained from a queen cell, g	The total amount of royal jelly, g
I - Sugar syrup + Chloramicob, 1.25 ml/l	$\bar{X}\pm S_{\bar{x}}$	10.32±0.103	23.91±0.415	1.28±0.018	0.493±0.008	15.776
	V, %	5.66	9.83	7.99	9.79	-
II - Sugar syrup + Chloramicob, 2.25 ml/l	$\bar{X}\pm S_{\bar{x}}$	10.41±0.105	25.90±0.425	1.43±0.031*	0.587±0.012**	17.610
	V, %	5.24	8.98	11.80	11.25	-
III - Sugar syrup + Chloramicob, 3.25 ml/l	$\bar{X}\pm S_{\bar{x}}$	10.63±0.096	23.94±0.369	1.21±0.021	0.454±0.010	16.344
	V, %	5.43	9.24	10.62	13.05	-
IV - Pure sugar syrup (control)	$\bar{X}\pm S_{\bar{x}}$	10.48±0.125	25.04±0.268	1.25±0.021	0.503±0.010	12.575
	V, %	5.95	5.35	8.45	9.78	-

*Mass of the queen cell with larva and royal jelly: group II / group IV – $*B_3 \geq 0.999$
**Quantity of royal jelly obtained from a queen cell: group II / group IV – $**B_3 \geq 0.999$.

Table 3. Number of larvae transferred and accepted for rearing, 06-09.06.2024 (II transfer)

Group	No. of transferred larvae, pcs.	No. of accepted larvae	
		pcs.	%
I - Sugar syrup + Chloramicob, 1.25 ml/l	70	28	40.0
II - Sugar syrup + Cloramicob, 2,25 ml/l	70	38	54.3
III - Sugar syrup + Chloramicob, 3.25 ml/l	70	34	48.6
IV-Pure sugar syrup (control)	70	21	30.0

It was revealed that the diameter of the queen cells in the experimental groups was on average 10.23-10.26 mm or 0.15-0.18 mm (1.49-1.79%) higher than in the control group IV, the length of the queen cells respectively – 21.41-22.05 mm (2.47-2.94%) (Table 4).

From one queen cell in the experimental groups, an average of 0.413-0.420 g of royal jelly was obtained or 0.020-0.027 g (5.10-6.87%) more than in the control group. The largest amount of royal jelly was obtained from

group II - 15.960 g or 93.38% higher than the control group.

The coefficient of variation of the studied indices ranged between 5.79 (diameter of the queen cell) and 13.41 (amount of royal jelly obtained from one queen cell).

So, in the second half, when 2 frames with 70 larvae (30+40) were raised, and the feeding of nurse bees stimulated the acceptance of larvae by 10.0-24.3%, the growth of the queen cells (diameter - by 1.49-1.79%, length - 2.47-2.80%, mass - 2.86-84.76%) and the amount of royal jelly obtained from one queen cell by 5.09-6.87%, and the total amount - by 41.14-93.38%.

In the third half, three frames were introduced into the experimental colonies (the first and second with 40 larvae each, the third - with 30 larvae), a total of 110 transferred larvae.

The research results demonstrated that in the third round, when a larger number of larvae (110 pcs.) was administered, 41-51 pcs. were accepted in experimental groups, or 2-12 pcs. (1.9-10.9%) more than in the control group (Table 5).

Table 4. Indices of the brood and the amount of royal jelly obtained, 06-09.06.2024
(II transfer - 70 larvae)

Group	Indices	Queen cell's diameter, mm	Queen cell's lenght, mm	The queen cell's weight with larvae and royal jelly, g	The amount of royal jelly obtained from a queen cell, g	The total amount of royal jelly, g
I - Sugar syrup + Chloramicob, 1.25 ml/l	$\bar{X}\pm S\bar{x}$	10.26±0.133	21.41±0.326	1.08±0.024	0.416±0.008	11.648
	V, %	6.86	8.06	11.51	10.16	-
II - Sugar syrup + Chloramicob, 2.25 ml/l	$\bar{X}\pm S\bar{x}$	10.25±0.088	22.05±0.231	1.94±0.017*	0.420±0.009	15.960
	V, %	5.79	6.46	9.73	13.41	-
III - Sugar syrup + Chloramicob, 3.25 ml/l	$\bar{X}\pm S\bar{x}$	10.23±0.129	21.95±0.257	1.09±0.016	0.413±0.009	14.042
	V, %	7.35	6.81	8.49	12.64	-
IV - Pure sugar syrup (control)	$\bar{X}\pm S\bar{x}$	10.08±0.129	21.42±0.386	1.05±0.030	0.393±0.008	8.253
	V, %	5.88	8.25	13.1	9.49	-

*Mass of the queen cell with larva and royal jelly: group II/group IV – *B3 ≥ 0.999

Table 5. Number of larvae transferred and accepted for rearing, 09-12.06.2024
(III transfer)

Group	No. of transferred larvae, pcs.	No. of accepted larvae	
		pcs.	%
I-Sugar syrup + Chloramicob, 1.25 ml/l	110	41	37.3
II-Sugar syrup + Chloramicob, 2,25 ml/l	110	49	44.5
II-Sugar syrup + Chloramicob, 2.25 ml/l	110	51	46.3
IV-Pure sugar syrup (control)	110	39	35.4

The diameter of the queen cells obtained from the experimental groups varied, on average, between 9.97 and 9.99 mm, or 0.11 and 0.13 larger than the control group, the length - 20.14-20.98 mm and the mass of queen cells with larva and royal jelly - 0.87-0.95 g. The coefficient of variation ranged between 6.45 (queen cell length) and 96.58 (amount of royal jelly obtained from a queen cell).

The largest amount of royal jelly from a queen cell was obtained from group II, on average 0.352 g, and the total amount - 17,248 g or 3.754 g more than the control group (Table 6).

Therefore, we can mention that increasing the number of frames (3), the number of larvae (110 pcs.) and stimulating the nurse bees with sugar syrup in a concentration of 50% and with a biostimulator increased the growth of the diameter of the queen cells by 1.12-1.32%, the length - by 1.13-3.30%, the mass of the queen cells with larvae and royal jelly - by 2.20-4.40%, the amount of royal jelly obtained from a queen cell – by 1.73% and the total amount of royal jelly obtained per group by 4.82-25.28% compared to the control group.

In total, over three rounds, the breeding colonies that were introduced with frames (1-3) with 40, 70 and 110 transferred larvae and the feeding of nurse bees with a mixture of sugar syrup and biostimulator in doses of 1.25-3.25 ml/l increased the number of transferred larvae accepted for rearing by 7.27-16.36% and the total amount of royal jelly obtained by 21.11-48.06% more than the control group (Table 7). It was found that the largest amount of royal jelly (50.818 g) over three rounds was collected from bee colonies in group II, which were introduced to grow 1-3 frames with 40-70-110 transferred larvae and nurse bees fed with a mixture of sugar syrup and a 2.25 ml/l biostimulator, one liter each when introducing the frames with larvae and daily for three days until the frames were removed from the hive, then the second and third rounds were repeated similarly.

Table 6. Morphometric indices of the brood and the amount of royal jelly obtained, 09-12.06.2024 (III transfer - 110 larvae)

Group	Indices	Queen cell's diameter, mm	Queen cell's lenght, mm	The queen cell's mass with larvae and royal jelly, g	The amount of royal jelly obtained from a queen cell, g	The total amount of royal jelly, g
I - Sugar syrup + Chloramicob, 1.25 ml/l	$\bar{X}\pm S\bar{x}$	9.97±0.101	20.14±0.305	0.93±0.016	0.345±0.007	14.145
	V, %	6.47	9.80	11.24	12.59	-
II - Sugar syrup + Chloramicob, 2.25 ml/l	$\bar{X}\pm S\bar{x}$	9.99±0.098	20.98±0.195	0.95±0.017	0.352±0.008	17.248
	V, %	6.80	6.45	12.13	11.06	-
III - Sugar syrup + Chloramicob, 3.25 ml/l	$\bar{X}\pm S\bar{x}$	9.98±0.134	20.54±0.337	0.87±0.038	0.308±0.011	15.708
	V, %	9.55	11.25	17.75	24.95	-
IV - Pure sugar syrup (control)	$\bar{X}\pm S\bar{x}$	9.86±0.103	20.31±0.280	0.91±0.020	0.346±0.053	13.494
	V, %	6.54	8.87	13.79	96.58	-

Table 7. Number of transferred larvae and amount of royal jelly obtained during three rounds (03-06; 06-09 and 09-12.06.2024)

Group	No. of larvae transferred at one round/ accepted			The total number of transferred larvae / accepted/%	No. of larvae transferred at one round / the amount of obtained royal jelly g/%			The total amount of obtained royal jelly, g	Difference from the control group	
	40	70	110		40	70	110		G	%
I - Sugar syrup + Chloramicob, 1.25 ml/l	32	28	41	220/101/ 45.91	15.776/ 125.46	11.648/ 141.14	14.145/ 104.82	41.569	7.247	121.1 1
II - Sugar syrup + Chloramicob, 2.25 ml/l	30	38	49	220/117/ 53.18	17.610/ 140.04	15.960/ **193.38**	17.248/ 127.82	50.818	16.49 6	148.0 6
III - Sugar syrup + Chloramicob, 3.25 ml/l	36	34	51	220/121/55. 0	16.344/ 129.97	14.042/ 170.14	15.708/ 116.41	46.094	11.77 2	134.3 0
IV - Pure sugar syrup (control)	25	21	39	220/85/ 38.64	12.575/ 100.0	8.253/ 100.0	13.494/ 100.0	34.322	-	100.0 0

CONCLUSIONS

1. It was evaluated that the optimal dose of the biostimulator "Chloramicob" used in the nutrition of nurse bees is 2.25 ml/L sugar syrup.
2. Feeding nurse bees with a mixture of 50% sugar syrup and 2.25 ml/L of biostimulator, one liter each when introducing frames with 40 transferred larvae and daily for three days until removing the frames from the hive, stimulates an increase in the percentage of larvae acceptance for growth by 12.5-27.5%, the mass of queen cells with larvae and royal jelly by 2.4-14.4% and the total amount of royal jelly

obtained by 25.46-40.04% higher than the control group.

3. In the second half, when introducing 2 frames with 70 (30+40) larvae transferred after feeding the nurse bees, it was revealed that in the experimental groups the number of larvae increased by 10.0-24.3%, the diameter of the queen cells by 1.49-1.79%, the amount of royal jelly obtained from a queen cell by 5.10-6.87%, the total amount of royal jelly obtained in the second half by 41.14-93.38% higher than in the control group.

4. In the third round, the introduction of three frames with 110 (30+40+40) transferred larvae increased the growth of the diameter of the queen cells by 1.12-1.32%, the length - by 1.13-3.30%, the mass of queen cells with larvae and royal jelly - by 2.20-4.40%, the amount of royal jelly obtained from one queen cell - by 1.73% and the total amount of royal jelly obtained per group by 4.82-27.82% compared to the control group.

5. It was found that the use of the biostimulator in the nutrition of nurse bees during three feedings ensures an increase in the number of transferred larvae accepted for growth by 7.27-16.36% and the total amount of royal jelly obtained by 21.11-48.06% higher than the control group.

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