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THE ROLE OF STIMULATIVE FEEDING OF NURSE BEES IN QUEEN REARING TECHNOLOGY

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Abstract

The aim of this study was to evaluate the role of stimulative feeding of nurse bees in the technological process of queen rearing. Under conditions of absent maintenance nectar flow, nurse colonies were fed 50% sugar syrup supplemented with the biostimulator Cloramicob. Supplemental feeding improved the physiological condition of the bees and stimulated royal jelly secretion for grafted larvae, which increased larval acceptance for rearing. It also promoted queen cell development, particularly length and diameter, and increased the body mass of both unmated and mated queens. The best overall response was generally obtained at the dose of 2.0 ml/L, while all stimulated groups outperformed the unstimulated control.

Key words: *biostimulants, queen cells, queen-rearing colonies, queens, sugar syrup.*

INTRODUCTION

The success of beekeeping largely depends on the organization and development level of the breeding base, queen-rearing technology, and colony management. Valuable breeding material can be rapidly multiplied through queen rearing. Queen quality is influenced by rearing methods, the condition of nurse colonies (cell builders), the number of grafted larvae, and the forage base, among other factors (Eremia, 2020).

Queen rearing by larval grafting involves transferring larvae from worker cells to artificial queen cups obtained by repeatedly dipping a template (2-3 times) in melted wax and then in cold water (Bura & Pătruică, 2004).

The production and breeding value of bee colonies is determined by hereditary traits and by the husbandry conditions provided for the queen. For breeder queens, rearing conditions are crucial: the main traits defining queen quality are formed during the larval stage. The development of the queen reproductive system depends on body size and overall body mass, which largely determine potential egg-laying capacity. Queen fecundity drives colony growth and development during the season and the expression of biological and productive traits (Borodachev & Savushkina, 2014).

Queen quality is also influenced by queen cell volume, which depends on colony strength and condition, nectar flow, bee race, etc. (Naumkin, 2000).

To obtain well-developed queens early in the season, special attention should be paid to nurse colonies and favourable conditions should be ensured, primarily warmth and sufficient feed (Belin, 1981).

In queen rearing, the influence of larval age was evaluated by comparing larvae aged 0-12 h, 24-36 h, and 48-60 h, as well as diets containing different proportions of pollen. It was found that the youngest larvae produced pupae with greater body mass, whereas the absence of pollen severely compromised rearing success, with acceptance falling to zero under complete pollen deprivation and being significantly higher at elevated protein levels (Kama & Shpigler, 2025).

Studies have shown that supplemental feeding significantly increased the acceptance rate of grafted larvae. Thus, 2-day-old larvae reached a mean acceptance rate of 82.3%, compared with 73.4% for 1-day-old larvae. At the same time, however, queens reared from younger larvae (1-day-old) exhibited a higher body weight (166.6 mg) than those developed from 2-day-old larvae (160.8 mg). These results suggest that, from a biological perspective, larval acceptance and the final quality of queens do not always coincide

and should therefore be interpreted together (Gençer et al., 2000).

Likewise, 1-day-old larvae produced queens with the best morphological traits, including a mean weight of 158.83 mg and a larger spermatheca volume, and supplemental feeding improved most traits except some more stable dimensions such as wing length (Mahbobi et al., 2012).

Among four pollen substitutes, the best-performing recipe for queen rearing consisted of brewer's yeast 30% + defatted soy flour 30% + skim milk 10% + cotton honey 20% + powdered sugar 10%. Orphaned colonies fed with this diet showed higher acceptance, more emerged queens, and better morphometric and reproductive traits than unfed controls (Taha et al., 2025). Pollen source strongly influences both queen-rearing success and queen morphophysiological quality (Abdel-Sattar et al., 2025).

Previous studies showed that feeding nurse colonies with stimulators during queen rearing under the absence of nectar flow increases graft acceptance and improves queen quality (Eremia & Zagareanu, 2012; Eremia et al., 2014; Eremia et al., 2015).

Queen-rearing success also depends on grafting technique and larval age. Testing different substrates placed in artificial cups showed that apple nectar gave the highest acceptance (81.06%), clearly above the control without substrate (47.04%), highlighting the importance of the larval microenvironment during the first 72 h after transfer (Contreras-Martinez et al., 2017). Similarly, queens reared from 1-day-old larvae were markedly superior to those from 2- or 3-day-old larvae (Zhong et al., 2024).

When queens originating from orphaned colonies fed with Apistimulin BM or Nanos-timul were introduced into swarms during the season, honey yields increased by 17% and 52% versus control, suggesting that improving queen quality during rearing has direct economic effects at colony level (Vedmed et al., 2016).

Overall, studies from different countries confirm that, when correctly applied and combined with efficient rearing methods, stimulative feeding improves graft acceptance and the morphological and reproductive quality of queens obtained (Gençer et al., 2000; Cengiz et al., 2019; Mahbobi et al., 2012; Taha et al., 2025). Thus, the analysed sources demonstrate that stimulative feeding is not only an energy-support measure, but a biotechnological tool capable of substantially shaping the response of cell-builder colonies and the quality of queens produced.

Based on the above, the aim of this study was to evaluate the role of stimulative feeding of nurse bees in the technological process of queen rearing.

MATERIALS AND METHODS

The research material consisted of bee colonies from a specialized and authorized queen-rearing apiary located in Dănceni village, Ialoveni district, maintaining a stock of 100 colonies and a nucleus yard with a capacity of 500 queen mating units. Based on the evaluation of morphoproductive traits (honey yield, early development, queen prolificacy, and overwintering resistance) and morphometric parameters (proboscis length; length and width of the 3rd tergite and 3rd sternite; wax glands; right forewing; tarsus; cubital index; and discoidal shift), the breeding stock of maternal and paternal colonies for queen reproduction was established. The experiment was conducted during May 2024.

Five groups of cell-builder colonies were formed by full queen removal; the queen and 2-3 frames with open brood were removed. The nest retained frames with honey, bee bread, and sealed brood. Open brood was removed to prevent the bees from building queen cells on larvae from those frames.

In the experimental colonies, the following morphoproductive traits were studied: the number of combs in the nest, colony strength (bee spaces), the amount of capped brood, and honey reserves.

Under the absence of maintenance nectar flow, each cell-builder colony received 1 L of 50% sugar syrup supplemented with the Cloramicob stimulator as follows: Group I - 1.0 ml/L; Group II - 2.0 ml/L; Group III - 3.0 ml/L; Group IV (Control I) - plain sugar syrup; Group V (Control II) - no stimulation.

In the first trial, conducted on 30 April 2024, a frame containing 36 grafted larvae was introduced between the frames with capped brood. From the first day after the introduction

of the grafted larval frame, nurse bees were fed 1 L of sugar syrup supplemented with the biostimulator, and feeding was continued daily until the queen cells were capped. Two days before queen emergence, the frame containing the capped queen cells was removed, and the diameter, length, and weight of the queen cells were measured, after which they were individually placed in cages.

The cages containing the queen cells were then introduced into incubator colonies and maintained there until queen emergence. The body weight of the unmated queens was determined using a torsion balance (measuring range: 500 mg), after which the queens were introduced into mating nuclei. After mating, the queens were marked and their body weight was reassessed.

In the second trial, the same procedures were followed. The number of grafted larvae introduced and accepted for rearing, the diameter and length of the queen cells, the weight of empty queen cells and queen cells containing queens, as well as the body weight of unmated and mated queens, were recorded.

Biometric processing of the data obtained was performed using the method of statistical variation, with determination of the standard value of Student's t-test to assess the significance of differences, according to the ANOVA method, using Microsoft Office.

RESULTS AND DISCUSSIONS

At the start of the experiment, nurse colonies had on average 9 frames in the nest, including 4 frames with sealed brood; colony strength was 8 bee spaces and honey reserves were 7.0 kg. Each colony received 36 grafted larvae. From the total number of larvae (36), acceptance was: Group I - 20 larvae (56%); Group II - 24 (67%); Group III - 20 (56%); Group IV (Control I) - 17 (47%); Group V (Control II) - 15 (42%) (Table 1).

Thus, colonies in Group II accepted 19% more larvae than Control IV and 25% more than Control V.

Table 1. Productive indicators of cell-builder colonies and number of grafted and accepted larvae for queen rearing, 10 May 2024 (I grafting)

Group	No. of frames, pcs	Colony strength, bee spaces	Honey stores, kg	No. of brood frames, pcs	No. of grafted larvae, pcs	No. of accepted larvae, pcs	No. of accepted larvae, %
I - Sugar syrup + Cloramicob, 1.0 ml/L	9	8	7.0	4	36	20	56
II - Sugar syrup + Cloramicob, 2.0 ml/L	9	8	7.0	4	36	24	67
III - Sugar syrup + Cloramicob, 3.0 ml/L	9	8	7.0	4	36	20	56
IV - Plain sugar syrup (Control I)	9	8	7.0	5	36	17	47
V - No stimulation (Control II)	9	8	7.0	4	36	15	42

Feeding nurse bees during larval rearing until queen cell capping increased queen cell length and the mass of capped queen cells, queen cells containing queens, and the body mass of unmated and mated queens.

The mean queen cell diameter in Group I was 12.81 mm, i.e., 0.54 mm larger than Group V; the difference was significant (*P1 >= 0.95). The largest queen cells were produced in Group II, with a length of 32.71 mm, which was 5.90 mm (22%) longer than Control IV and 4.7 mm (20%) longer than Control V (***P3 >= 0.999) (Table 2).

Table 2. Diameter, length and mass of empty queen cells (I grafting), 22 May 2024

Group	Queen cell diameter, mm	Queen cell length, mm	Mass of empty queen cells, mg
I - Sugar syrup + Cloramicob, 1.0 ml/L	12.81±0.14*	27.89±0.47	120.37±4.53
II - Sugar syrup + Cloramicob, 2.0 ml/L	12.36±0.13	32.71±0.56***	143.19±2.73**
III - Sugar syrup + Cloramicob, 3.0 ml/L	12.73±0.41	30.17±0.61**	169.35±7.21**
IV - Plain sugar syrup (Control I)	12.79±0.24	26.81±0.38	134.27±2.83
V - No stimulation (Control II)	12.27±0.20	27.93±0.68	137.0±0.05

Queen cell diameter, Group I / Group V - *P1 >= 0.95; Queen cell length, Group II / Group V - ***P3 >= 0.999; Queen cell length, Group II / Group IV - ***P3 >= 0.999; Queen cell length, Group III / Group IV - **P3 >= 0.999; Queen cell length, Group III / Group V - **P2 >= 0.99; Mass of empty queen cells, Group II / Group IV - **P2 >= 0.99; Mass of empty queen cells, Group II / Group V - *P1 >= 0.95; Mass of empty queen cells, Group III / Group IV - **P2 >= 0.99; Mass of empty queen cells, Group III / Group V - **P2 >= 0.99.

The heaviest empty queen cells after queen emergence were obtained in Group II (143.19 mg) and Group III (169.35 mg), which were 8.92 mg and 32.35 mg heavier than Control IV and Control V (**P2 >= 0.99).

The mass of queen cells containing queens averaged 1.066 g in Group II and 1.301 g in Group III, exceeding both controls.

Queen quality is primarily assessed by body mass. The mass of unmated queens in experimental groups ranged from 175.82 to 179.93 mg, whereas in Control I and Control II it ranged from 168.33 to 174.80 mg (Table 3).

The highest body mass of mated queens was recorded in Group I: 267.14 mg, which was 37.39 mg (16%) higher than Control IV (***P3 > = 0.999) and 44.33 mg (22%) higher than Control V (***P3 > = 0.999). Thus, feeding nurse bees from the introduction of grafted larvae until queen cell capping for 5 days with 1 L of sugar syrup supplemented with 1.0 ml/L Cloramicob ensured queens with increased body mass, which was 7% higher than previously reported results (Eremia & Zagareanu, 2012; Eremia et al., 2015).

Table 3. Mass of queen cells and body mass of unmated and mated queens (I grafting)

Group	Mass of queen cells containing queens, g	Mass of unmated queens, mg	Mass of mated queens, mg
I - Sugar syrup + Cloramicob, 1.0 ml/L	0.987±0.09	175.82±3.57	267.14±1.96***
II - Sugar syrup + Cloramicob, 2.0 ml/L	1.066±0.02*	179.93±5.07	264.00±7.57**
III - Sugar syrup + Cloramicob, 3.0 ml/L	1.301±0.04**	179.80±5.46	261.00±4.93**
IV - Plain sugar syrup (Control I)	1.128±0.06	174.80±4.65	229.75±2.95
V - No stimulation (Control II)	0.932±0.05	168.33±3.71	219.67±0.88

Mass of queen cells containing queens, Group II / Group V - *P3 >= 0.95; Mass of queen cells containing queens, Group III / Group IV - *P3 >= 0.95; Mass of queen cells containing queens, Group III / Group V - **P3 >= 0.95; Mass of mated queens, Group I / Group IV - ***P3 >= 0.999; Mass of mated queens, Group I / Group V - ***P3 >= 0.999; Mass of mated queens, Group II / Group IV - **P3 >= 0.999; Mass of mated queens, Group II / Group V - **P3 >= 0.999; Mass of mated queens, Group III / Group IV - **P3 >= 0.999; Mass of mated queens, Group III / Group V - **P3 >= 0.999.

In the second trial, a second frame containing grafted larvae was introduced into the nurse colonies for rearing.

From the 36 larvae, the highest acceptance was recorded in Group II - 23 larvae (64%). With an increased dose, acceptance decreased to 53% (Table 4).

Table 4. Number of grafted and accepted larvae, 13 May 2024 (II grafting)

Group	No. of grafted larvae, pcs	No. of accepted larvae, pcs	No. of accepted larvae, %
I - Sugar syrup + Cloramicob, 1.0 ml/L	36	21	58
II - Sugar syrup + Cloramicob, 2.0 ml/L	36	23	64
III - Sugar syrup + Cloramicob, 3.0 ml/L	36	19	53
IV - Plain sugar syrup (Control I)	36	20	56
V - No stimulation (Control II)	36	19	53

The number of accepted larvae in Group II was 8% higher than Control IV and 11% higher than Control V.

Queen cell diameter in experimental groups varied slightly, from 12.23 mm to 12.93 mm. By queen cell length, Group II was superior: 34.06 mm, which was 6.3 mm (23%) longer than Control IV and 6.13 mm (22%) longer than Control V (***P3 >= 0.999) (Table 5).

Compared with the data reported by Canverdi et al. (2023), queen cell length in Caucasian bees was 23.19 mm at low altitude and 22.75 mm at high altitude. In our case, particularly in Group II, queen cell length was greater, exceeding 32 mm after the first grafting and 34 mm after the second grafting. Queen cell dimensions and queen performance depend on the entire technological chain, which includes the type of cell cup, the rearing method, environmental conditions, process intensity, and supplemental feeding (Abou-shaara, 2023; Ahmat et al., 2024; Derafsh et al., 2025; Lashari et al., 2022; Mattiello et al., 2022; Poudel et al., 2024).

Table 5. Diameter, length and mass of queen cells containing queens (II grafting)

Group	Queen cell diameter, mm	Queen cell length, mm	Mass of queen cells containing queens, g
I - Sugar syrup + Cloramicob, 1.0 ml/L	12.96±0.23	28.09±0.51	1.25±0.47
II - Sugar syrup + Cloramicob, 2.0 ml/L	12.23±0.17	34.06±0.54***	1.47±0.05
III - Sugar syrup + Cloramicob, 3.0 ml/L	12.55±0.26	23.97±0.27	1.17±0.04
IV - Plain sugar syrup (Control I)	12.84±0.14	27.76±0.29	1.35±0.04
V - No stimulation (Control II)	12.27±0.19	27.93±0.67	1.37±0.05

Queen cell length, Group II / Group IV - ***P3 >= 0.999; Queen cell length, Group II / Group V - ***P3 >= 0.999.

After emergence, at the time of introducing queens into nuclei for maturation and mating, body mass in experimental groups was 182.19-186.50 mg, while in Control I and Control II it was 173.67-179.50 mg (Table 6).

Feeding nurse bees from the introduction of grafted larvae until queen cell capping with 1 L of 50% sugar syrup supplemented with Cloramicob at doses of 1.0-3.0 ml/L increased the body mass of mated queens that started laying; differences versus both controls were significant (*P1 >= 0.95).

The best results were obtained in Group II, where mated queens had an average body mass of 250.0 mg, which was 22.50 mg (10%) higher than Control IV and 24.5 mg (11%) higher than Control V.

The obtained data confirm the view of several authors that supplemental feeding of nurse colonies results in improved indicators of queen quality (Kama & Shpigler, 2025; Sakla & El-Shafeiy, 2022).

Table 6. Body mass of unmated and mated queens (II grafting)

Group	Mass of unmated queens, mg	Mass of mated queens, mg
I - Sugar syrup + Cloramicob, 1.0 ml/L	182.61±3.28	243.60±2.97*
II - Sugar syrup + Cloramicob, 2.0 ml/L	186.50±5.25	250.0±5.02*
III - Sugar syrup + Cloramicob, 3.0 ml/L	182.19±2.50	246.40±5.55*
IV - Plain sugar syrup (Control I)	179.50±4.42	227.50±3.50
V - No stimulation (Control II)	173.67±1.98	225.5±5.50

Mass of mated queens, Group I / Control IV - *P1 >= 0.95; Mass of mated queens, Group I / Control V - *P1 >= 0.95; Mass of mated queens, Group II / Control IV - *P1 >= 0.95; Mass of mated queens, Group II / Control V - *P1 >= 0.95; Mass of mated queens, Group III / Control IV - *P1 >= 0.95; Mass of mated queens, Group III / Control V - *P1 >= 0.95.

Therefore, stimulative feeding of nurse bees during queen rearing ensured, in the first grafting, an increase in larval acceptance by 19-25%, queen cell length by 20-22%, and mated queen body mass by 16-22%; in the second grafting, larval acceptance increased by 8-11%, queen cell length by 22-23%, and mated queen body mass by 10-11%.

CONCLUSIONS

Based on the results obtained, a queen-rearing method was developed and patented, which involves feeding nurse bees from the first day after the introduction of the frame containing grafted larvae with 1 L of sugar syrup supplemented with a biostimulator, and subsequently on a daily basis until the queen cells are capped.

It was established that, in the absence of a maintenance nectar flow, feeding nurse bees involved in the technological process of queen rearing with 50% sugar syrup supplemented with the biostimulator Cloramicob improves the physiological condition of the bees and stimulates royal jelly secretion for the rearing of grafted larvae.

This, in turn, increases the number of grafted larvae accepted for rearing and promotes the development of queen cells (length and diameter), as well as increasing the body weight of both unmated and mated queens.

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