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Influence of the qualitative and quantitative composition of the queen-rearing colony and the number of grafted larvae on the weight of queen bees

Mykola Voinalovych*, Valerii Brovarskiy, Ihor Golovetskyi

National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine

Abstract. The relevance of the study is conditioned by the practical need to determine the optimal number of grafted larvae in the queen-rearing colony when breeding queen bees. The purpose of the study was to investigate the influence of the qualitative and quantitative composition of bees and brood in the queen-rearing colony on the reception of queen larvae and the weight of reared queen bees. The study was conducted using bees of breed *Apis mellifera sossimai*. To complete the tasks set, control and experimental groups of three bee colonies each were formed. The difference between the control and experimental groups was the different age ratios of bees and the number of open and sealed broods. In each group of colonies, 30, 40, 50, 60 larvae were alternately grafted at a time with an interval of 5 days, the percentage of their reception, the output of queens and their weight were determined by counting the number of larvae that the bees fed and from which the queens came out, and the weight of queens was determined on laboratory scales during the first four hours after leaving the queen cell. A total of 862 queen bees were bred and studied. During the research, it was found that in the colonies of the experimental group, if they were grafted from 30 to 40 larvae, the percentage of their reception was 88.1-86.2%, respectively. Analysing the weight indicators of queens obtained with a given number of grafted larvae, it was found that it was 195.0-197.3 mg, there was no significant difference with the corresponding indicators of the control group. However, with the subsequent loading of queen-rearing colonies in the experimental group with 50 larvae, the percentage of their reception prevailed by 18.2%, and the weight of queens exceeded similar indicators of the control group by 10%. Notably, the average weight of queens in the control group was 176.3 mg, which is 8.7 mg less than the requirements for the weight of infertile queens established by the breed standard. With a further increase in simultaneously grafted larvae in the queen-rearing colony to 60 units, the rate of their admission in the experimental group decreased by 5% and amounted to 80.5%. At the same time, in the control group, larval reception and queen weight decreased to unacceptable levels and amounted to 67.5% and 172.4 mg, respectively. Therefore, by increasing the number of nurse bees and reducing the number of open brood in the queen-rearing colony, better conditions are created for growing high-quality queen bees, which, in turn, allows simultaneously grafting 48% more larvae compared to the conventional method. The results of the study are of practical value for improving the methods of establishing queen-rearing colonies during the artificial breeding of queen bees

Keywords: *Apis mellifera sossimai*, bee brood, larval reception, queen quality, royal jelly, queen larvae

Introduction

Honeybees (*Apis mellifera* L.) are social insects that live in colonies characterised by a cooperative system of brood care, generational overlap, and reproductive distribution of functions. In such an organised biosocial structure, the

queen is a fertile female, whose main duty is to lay eggs [1]. In addition, the queen maintains the cohesion of the colony by continuously producing a “bouquet” of pheromones that actively spread in the nest. They prevent worker bees

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*Corresponding author

from replacing the queen and developing their ovaries [2]. The development, productivity, and survival of a bee colony largely depend on the health and physical fitness of its queen and the drones with which it mates [3]. The bee colony is negatively affected if the queen shows any defects or gets sick and stops laying eggs [1; 4]. In addition to pesticides, parasites, and pathogens, queen loss is considered the most important factor leading to the loss of bee colonies worldwide, especially when it occurs outside the active queen breeding season [5]. There are many indicators that can be related to the quality of the queen, which is a result of its genetic origin, development conditions, mating quality, and its habitat, including beekeeper care [6; 7]. The most intuitive are the physical indicators of the queen, such as body weight, which significantly correlates with its morphological measurements and the productivity of the bee colony [8-10]. Weight has also been found to positively correlate with successful queen reception in new colonies [11]. Body weight also positively correlated with the reproductive organs of the queen, such as the ovaries and the number of egg tubes, the diameter of the sperm, and the number of sperm stored [12]. Some researchers [13] have argued that queen body weight may represent an integral measure of size and physiological state. Therefore, it can be considered one of the most informative indicators of the quality of the queen.

The formation of the queen bee's body occurs in the early stages of ontogenesis. It is known that the quality of feeding the brood largely depends on the quality of future queens [14; 15]. Nurse bees under the age of 18 days actively take part in this process. This group of bees has royal jelly, which is necessary for the development of the queen. At the same time, it was found that bees secrete two fractions of royal jelly, so bees of 12 days of age secrete a white fraction of jelly enriched with proteins, and slightly older (17 ± 2) – a transparent one rich in carbohydrates [16]. In the process of feeding queen larvae, the quality of feed is constantly changing, which is determined by the ratio of these fractions [17]. Consequently, the shortage of young bees in the nest of queen-rearing colonies can negatively affect the development of the queen bee brood, and, accordingly, the quality of the resulting queens. There are no recommendations for compliance with certain requirements of the state of the queen-rearing colony and the specifics of its formation in different periods of the queen breeding season.

Given these circumstances, *the purpose of the study* was to investigate the influence of bee age groups and the ratio of brood (open and sealed) in the nests of queen-rearing colonies on the reception of queen larvae and the weight of reared queen bees

Literature Review

The quality of artificially bred queen bees undergoes significant changes during the season. Researchers explain this by the different states of queen-rearing colonies, the amount of feed, weather conditions, etc. [18]. In addition, some researchers have argued that the quality of queen bees depends on the genotype, nutritional factors, growing methods and period, the age of the grafted larvae, and the number of larvae that are grafted into each queen-rearing colony [19]. The number of grafted larvae and their age can

be easily regulated by beekeepers who plan to breed queen bees. The optimal number of grafted larvae is the one that allows growing the maximum number of high-quality queen bees. If the number of grafted larvae is greater than optimal, the quality of queen bees begins to decrease [17; 19]. The quality of queens decreases with the increasing age of grafted larvae, and vice versa. Queens grown from one-day-old larvae are known to be heavier than queens grown from two- and three-day-old larvae, just as queens grown from two-day-old larvae are heavier than queens grown from three-day-old larvae [20]. Researchers have found that bees are able to take an active part in the rearing of queen larvae only from the age of five days, and the best results were obtained in the presence of worker bees of 9-12 days of age [21]. Under conditions of older age (13-20 days), bees were raised next to full-fledged queens, transitional forms, and even older nurses – dwarf queens [22]. It has been experimentally found that strong colonies with a large number of nurse bees provide better conditions for growing queen larvae. An approximate sign of the number of young bees can serve as the number of brood in the queen-rearing colony [23].

To obtain high-quality queens, it is important that the age composition of the queen-rearing colony and the presence of open and sealed brood are optimal. The first reports on this issue are found in the study by S.C. Jay [24]. The researcher pointed out that the amount of larval food depends on the general condition of the queen-rearing colony, and especially on the presence of open brood in its nest. The absence of open brood in the queen-rearing colony significantly increases the amount of food for larvae. Consequently, an increase in the number of open brood in the queen-rearing colony leads to a decrease in the intensity of larval feeding. Later, researchers investigated that the amount of royal jelly in the queen cell depends more on the state of the colony (the number of nurse bees, the availability and reserves of food in the nest, honey collection, etc.) than on the presence or absence of open brood [4; 21].

In previous studies, the optimal composition of the queen-rearing colony was determined by the ratio of the age groups of bees (75% of nurse bees and 25% of flying bees) and open to sealed brood (1 to 5) [23]. However, it was important to find out how many grafted larvae such colonies can grow without reducing the quality of the resulting queen bees.

Materials and Methods

The study was conducted on the premises of the Holosiivskiy educational and experimental apiary of the National University of Life and Environmental Sciences of Ukraine in the period from May to July 2022.

A control and experimental group of queen-rearing colonies were developed, which had 10 kilograms of carbohydrate feed each and tightly seeded 12 frames, of which 8 were with brood. The difference between them was the different ratio of working individuals, the number of open and sealed brood. Each group consisted of three bee colonies. The ratio of the age groups of bees and brood remained unchanged (natural for this period of time). Experimental colonies were formed from 75% of young and 25% of flying bees, this ratio of bees was achieved by shifting the hive approximately 1 m relative to its original position, then the intensity of the flight of bees was visually observed, and if

necessary, the hive was shifted to achieve the required ratio of flying bees in it. The general scheme of the method of pre-

paring a queen-rearing colony, considering the age ratio of bees, is shown in Figure 1.

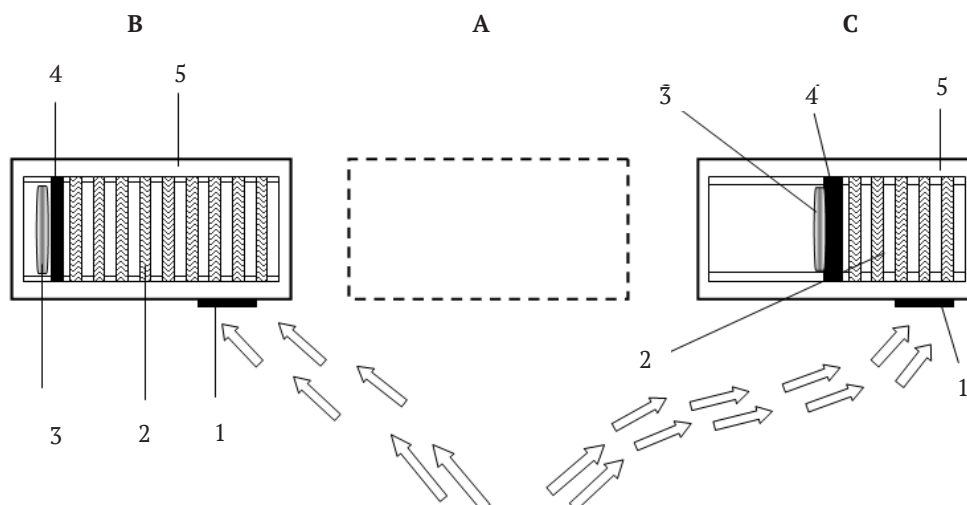


Figure 1. Diagram of moving hives when establishing a queen-rearing colony with the required number of flying bees, (top view): A – location of the bee colony hive before the formation of nucleus and nurse; B – hive of the queen-rearing colony moved to the left after forming a nucleus; C – location of the beehive with nucleus; 1 – hive opening; 2 – honeycomb; 3 – insulation cushion; 4 – partition; 5 – hive body. Arrows indicate the direction of the bees' flight

The ratio of open and sealed brood in the experimental colonies was 1:5, honeycombs with extra open brood were moved to other colonies, and the required amount of sealed brood was taken from donor colonies. In total, there were 7 honeycombs with brood in the colony. Due to the use of the method of shifting the hive of the future queen-rearing colony, some of the flying bees move from its nest to another hive or department with a separate opening, but the nurse bees of the queen larvae remain in the queen-rearing colony. 12 hours before the larvae were grafted for queen rearing, the queen was selected in colonies and a space was left in the middle of the nest for setting up a grafting frame with larvae. Experimental colonies received open brood from donor colonies every 6 days, and after 12 days – sealed to maintain a constant ratio between them. Colonies in the control group were replenished with only two frames of sealed brood after 12 days.

Grafting of larvae was carried out in artificially made wax bowls, which were fixed on the grafting frame in three rows. 24-hours old larvae were taken for grafting. During the research, 30 to 60 larvae were grafted in the queen-rearing colony at intervals of 5 days. Two days after the transfer of larvae, the percentage of their reception by queen-rearing colonies was checked. The quality assessment of infertile queen bees was carried out based on the

determination of their weight in the first 4 hours after their exit from the queen cells using WALCOM lb202 laboratory scales. A total of 862 queens were evaluated during the study.

Results and Discussion

The process of establishing a queen-rearing colony is a defining stage in the technology of breeding queen bees. That is why many researchers have tried to determine the optimal way to establish them [21; 22; 24]. However, the vast majority of researchers describe methods that are based on general recommendations for the preparation and establishment of queen-rearing colonies [17; 19]. Based on previous studies, it was found that the best results in growing queens are obtained from queen-rearing colonies formed with a certain ratio between nurse bees and flight bees, as well as open and sealed brood [23].

As a result of the study to determine the optimal number of grafted larvae in queen-rearing colonies, which are formed considering the ratio between the age groups of bees and the number of open and sealed brood (experimental group), it was established (Table 1) that the percentage of larval reception in the 30-piece grafting variant was 88.1%, and no significant difference was found with the colonies of the control group.

Table 1. Influence of the method of establishing a queen-rearing colony on the reception of larvae and the weight of infertile queen bees, $n = 862$

Indicators	Given larvae, units	Groups	
		Experimental	Control
Percentage of larval reception, %	30	88.1 ± 2.65	87.7 ± 2.53
	40	86.2 ± 2.41	81.6 ± 2.34
	50	84.3 ± 1.40***	71.3 ± 0.90
	60	80.5 ± 0.79***	69.7 ± 0.94

Table 1, Continued

Indicators	Given larvae, units	Groups	
		Experimental	Control
Received queens, units	30	26.4 ± 0.66	26.3 ± 0.63
	40	34.4 ± 0.71	32.6 ± 0.71
	50	42.1 ± 0.49***	35.6 ± 0.32
	60	48.3 ± 0.32***	41.8 ± 0.37
Weight of queens, mg	30	197.3 ± 1.14	193.6 ± 0.85
	40	195.0 ± 1.00	185.4 ± 1.06
	50	193.7 ± 1.25***	176.3 ± 1.54
	60	190.3 ± 1.25***	172.4 ± 1.21

Note:*** – $P < 0.001$ significantly relative to the control group

With a further increase in the number of larvae that were grafted in the queen-rearing colony, there was a tendency to gradually decrease the percentage of larval reception. Thus, under the conditions of grafting 40 larvae in the queen-rearing colony, the bees provided 86.2% rearing, which is 5% more than in the control group, and when grafting 50 larvae, the percentage of admission in the colonies of the experimental group was 84.3%, which is 18.2% more compared to the control group. In the case of transmission of 60 larvae to queen growing, the percentage of admission in the experimental group decreased by 5% with the previous variant (50 larvae), but in the control group there was still a significant decrease in the percentage of admission to the level of 65.7%, this, in turn, is 22.5% less compared to the indicators of the experimental group, the difference is highly significant ($P < 0.001$).

As a result of the study, there is a tendency to gradually decrease the percentage of larval reception with an increase in the number of grafted larvae. Thus, when growing queen larvae in the amount of 30 to 40 units by queen-rearing colonies, there was no significant difference in the reception of larvae by the colonies of the control and experimental group, only when 40 to 60 larvae were grafted, there was a predominance of 18-22.5% in the reception of larvae by the colonies of the experimental group. The output of infertile queens also corresponded to this trend.

Queens with the highest weight were obtained under the conditions of grafting 30 larvae in the queen-rearing colony, their average weight was 197.3 mg, which is 2.0% more than the same indicators of the control group. There was no significant difference between these variants compared to the control group. In the future, with an increase in the number of grafted larvae, this indicator gradually decreased, so when 40 larvae were transferred to growing, the weight of infertile queens in the colonies of the experimental group was 195 mg, which is 5.2% more than in the control group. When grafting 50 larvae, the queens of the experimental group had an average weight of 193.7 mg and prevailed in this indicator by 10% over the queens of the control group, which did not meet the breed standard by average weight. With a further increase in the load of queen-rearing colonies with larvae for growing queens, there was a further decrease in the weight of infertile queens received, in particular, in the experimental group it was 190.3 mg, which is 10.4% more compared to the weight of queens received from the colonies of the control group. The difference between the weight of queens raised

in queen-rearing colonies with a load of 50 to 60 larvae is significant ($p < 0.001$).

According to the standard of the Ukrainian steppe bee breed, the weight of infertile queens should be at least 185 mg. These requirements were met by the queens of the control group of colonies under growing conditions from 30 to 40 larvae. At the same time, when establishing queen-rearing colonies, considering the ratio of the age composition of bees, and the ratio of open to sealed brood, it was possible to obtain infertile queens, which even with an increase in grafted larvae to 60 units met the requirements of the standard for this breed of bees, since they had an average body weight of 190.3 mg.

Artificial breeding of bees is a responsible process in which many factors must be considered to achieve the desired result. Thus, the period of the season affects the physiological state of colonies, which, in turn, affects the tendency of bees to raise queens [7]. Since the preparation of the queen-rearing colony is an important technological element in the growing of queen bees, therefore, many researchers put forward similar requirements for the state of the queen-rearing colony, and note that it must be healthy, have a force of at least 12 compartments with bees of different ages, 7-9 honeycombs with different ages of brood, and feed supply at the level of 10-12 kg [17; 18]. The researchers also paid much attention to the investigation of the influence of the general condition of the queen-rearing colony on the quality of the queens received, but the researchers did not give specific ratios for different ages of bees in colonies. When studying the influence of working individuals on the cultivation of brood in a queen-rearing colony, researchers found that the level of feeding of queen larvae depends on the presence of young bees in its composition [4; 21; 22]. However, there are no uniform recommendations among researchers regarding the number of larvae that are transferred for growing to the queen-rearing colony. Some researchers claim that the queen-rearing colony, which is established by the method of complete orphanhood, can be grafted with 60 larvae or more [19], others, on the contrary, prove the opposite, that their number should not exceed 20-30 units [18]. Analysis of the vital activity of bee colonies throughout the spring-summer season showed that even under optimal conditions in the nests of bee colonies, there was no condition that 75% of young bees were in their nests, and the ratio between open and sealed brood was 1:5 [23]. At the same time, in some cases, bee colonies create conditions in their nests that are similar to those

that were in the colonies of experimental groups when conducting these studies. This applies to swarming. Admittedly, swarm queens are characterised by high quality if bees raise them in strong colonies, in the presence of honey collection and favourable weather [6; 7; 10]. Given these conditions, as a result of the conducted research, it was first established that when establishing queen-rearing colonies in the nests of which mainly young bees are concentrated and the share of open brood is reduced, it is possible to increase the number of larvae grafted on queen growing to 60 units. At the same time, the yield of queen bees and their weight is ensured at a high level.

Conclusions

As a result of the conducted studies to determine the optimal number of grafted larvae in the queen-rearing colony, which are established considering the ratio between the age groups of bees and the number of open and sealed brood, it was found that the percentage of larval reception under grafting conditions from 30 to 40 units was 88.1-86.2%, respectively. The average weight of infertile queens obtained within this series of graftings in the experimental group was 195.0-197.3 mg, there was no significant difference with the colonies of the control group. With a further increase in the number of larvae that were grafted in the

queen-rearing colony, there was a tendency to significantly decrease the percentage of larval reception. When grafting 50 larvae, the percentage of reception in the experimental group was 84.3%, which is 18.2% more than in the control group, the average weight of queens was 193.7 mg, which is 10% higher than the weight of queens in the control group. When grafting 60 larvae, the percentage of reception in the experimental group decreased by only 5% with the previous variant (50 larvae), but in the control group there was still a significant decrease in the percentage of reception to the level of 65.7%, which is 22.5% less compared to the indicators of the experimental group, the difference is significant ($P < 0.001$).

Thus, it was found out that when establishing a queen-rearing colony, which includes 75% of nurse bees and 25% of flying bees, the ratio of open to sealed brood is 1 to 5 allows increasing the number of grafted larvae by 20 units compared to the conventional method of establishing a queen-rearing colony and bringing it to 60 larvae, their reception is at the level of 80.5%, and the average weight of queens is 190.3 mg, which corresponds to the breed standard.

The results of the study will contribute to improving the methods of establishing queen-rearing colonies during the artificial breeding of queen bees.

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Вплив якісного і кількісного складу сім'ї-виховательки та кількості прищеплених личинок на масу бджолиних маток

Микола Володимирович Войналович, Валерій Дмитрович Броварський, Ігор Іванович Головецький

Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна

Анотація. Актуальність дослідження зумовлена практичною необхідністю визначення оптимальної кількості прищеплених личинок у сім'ї-виховательки при виведенні бджолиних маток. Метою даного дослідження було вивчення впливу якісного і кількісного складу бджіл та розплуду в сім'ї-виховательці на прийом маточних личинок і масу вирощених бджолиних маток. Дослідження проводили з використанням бджіл раси *Apis mellifera sossimai*. Для виконання поставлених завдань було сформовано контрольну і дослідну групи по три бджолиних сім'ї в кожній. Різниця між контрольною і дослідною групою полягала у різному віковому співвідношенні бджіл та кількості відкритого і запечатаного розплуду. У кожную групу сімей по черзі прищеплювали 30, 40, 50, 60 личинок за один раз з інтервалом 5 діб, визначали відсоток їх прийому, вихід ділових маток та їх масу шляхом підрахунку кількості личинок які бджоли годували, та з яких вийшли матки, визначення маси маток здійснювали на лабораторних вагах впродовж перших чотирьох годин після виходу з маточника. Всього було виведено та досліджено 862 бджолині матки. Під час проведення досліджень з'ясовано, що в сім'ях дослідної групи, за умови прищеплення їм від 30 до 40 личинок, відсоток їх прийому становив 88,1-86,2 % відповідно. Аналізуючи показники маси маток, одержаних за даної кількості прищеплених личинок, встановлено, що вона становила 195,0-197,3 мг, варто відмітити, що вірогідної різниці з відповідними показниками контрольної групи не виявлено. Однак з подальшим навантаженням сімей-вихователюк дослідної групи 50-ма личинками, відсоток їх прийому переважав на 18,2 %, а маса маток на 10 % аналогічні показники контрольної групи. Варто відміти, що середня маса маток у контрольній групі становила 176,3 мг, що на 8,7 мг менше за встановлені стандартом породи вимоги до маси неплідних маток цієї раси. При подальшому збільшенні одночасно прищеплених личинок у сім'ї-виховательки до 60 штук, показник їх прийому в дослідній групі знизився на 5 % і становив 80,5 %. В той же час, у контрольній групі прийом личинок та маса маток знизилися до неприйнятних рівнів і становили 67,5 % та 172,4 мг відповідно. Отже за рахунок збільшення чисельності бджіл годувальниць та зменшення кількості відкритого розплуду в сім'ї-виховательці створюються кращі умови для вирощування якісних бджолиних маток, це в свою чергу дозволяє одночасно прищеплювати на 48 % більше личинок порівняно з традиційним способом. Результати дослідження становлять практичну цінність для удосконалення способів формування сімей-вихователюк при штучному виведенні бджолиних маток

Ключові слова: *Apis mellifera sossimai*, бджолиний розплід, прийом личинок, якість маток, маточне молочко, маточні личинки