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# EVALUATION OF THE POSSIBILITY OF BREEDING QUEEN BEES FROM LARVAE GRAFTED ON DIFFERENT SUBSTRATES

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**ABSTRACT**

The study compared different food substrates in queen cells on the effectiveness of their rearing. Experiments were carried out during the nectar flow in 2013 year in a breeding apiary. Larvae were grafted on the following substrates: fresh royal jelly diluted with demineralized water at a 1:1 ratio (CON), royal jelly with the addition of vitamins A and E (VIT), freeze-dried royal jelly diluted with deionized water (LIOF) and royal jelly with the addition of ApiBioFarma probiotic (PROB). In the preliminary study (EXP1), 36 bee queens were obtained from 144 larvae, whereas 150 ones were obtained from 288 larvae in the proper study(EXP2). Larvae grafted on fresh diluted royal jelly were accepted the most by bees in colonies. In this group; the large number of obtained queens was observed in both parts of the study. In the case of the body mass, statistically significant differences were observed between groups CON and PROB.

**Key words:** *Apis mellifera*, queen rearing, royal jelly, probiotic, vitamins

## INTRODUCTION

In reasonably maintained apiary goods, in order to maintain a high standard of the family, it is recommended to exchange bee mothers every 2 seasons [Roman 2006]. Therefore, in beekeeping practice, there is a high demand for bee mothers with a well-known descent, whose descendants inherit characteristics useful in specific production conditions, which in turn determines the level of productivity of the apiary [Wilde et al. 2002]. The regular exchange of mothers determines the proper development of the bee colonies, reduces their tenderness and improves productivity [Ratnieks et al. 2007]. In breeding apiaries one of the basic tasks is to develop a plan for the breeding of bee mothers and to select suitable breeding material [Trzybiński 2012]. Any shortcomings or mistakes in education can adversely affect the number and quality of developing and biting mothers. The mother larvae feed during their rearing is royal jelly, which fully covers the need for the developing larvae for nutrients. Hence, most often bringing up the mother as a substrate in the nursing cups, which translates to 1-day larvae, is used as a royal jelly [Abassi et al. 2015]. However, often the weather conditions are not conducive to its acquisition or it is not enough. That is why many breeders try to limit the use of royal jelly, dilute it with water, or translate mother larva onto other substrates or without substrate. Nutritional supplements incorporated into the media can stimulate the various processes that occur during preimaginal development. They can also cause modifications and restrictions related to larval development. On the other hand, this may be influenced by the frequency of larval visit by the feeder.There is a great influence on the acceptance of the larvae subjected to the substrate or its absence and on the external conditions under which the procedure of submitting the nursing home to the family [Skowronek and Skubida 1998].

Lyophilized lyophilized milk is a vacuum-dried milk in a deep freeze at –40°C. Composition and properties similar to fresh royal jelly [Rybak-Chmielewska and Szczęsna 1998]. Probiotics is a microbiological preparation, which consists of living cultures of bacteria. Lactic acid, photosynthetic bacteria and their metabolites, as well as herbal extracts, which may have beneficial effects on the organism to which they are administered, and improve their attractiveness [Trafalska and Grzybowska

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2004]. With regard to bees most attention is paid to the ability of organisms and probiotic substances to colonize the digestive tract of an individual, thus improving the digestive processes and reducing the development of pathogenic microorganisms. They strengthen the condition of families weakened, prolong the service life and additionally improve the sanitary conditions in the nest.

Vitamin A (retinol), is soluble in fats. Stimulating protein synthesis processes is a factor for epithelial regeneration, thus protecting against pathogenic microorganisms, restoring the epithelial structures of the gastrointestinal tract, reproductive organs [Harris et al. 1963]. Vitamin E (tocopherol, like vitamin A, is a fat soluble vitamin.) This vitamin is the main antioxidant that protects cells against oxidant action, and is involved in the delivery of nutrients to cells as it is responsible for the bioregulatory function of the body. In addition, it plays the role of antioxidant, which protects cells against changes in their genetic structure [Burton and Ingold 1981].

The purpose of the study was to evaluate the possibility of raising bee mothers from larvae in nursery mats on various substrates and to determine whether the number of raised mothers and their weight can be influenced by dietary modification at the 1 day larval stage. The age of the larvae translates into the quality of the mother bee, her weight, the number of oviducts, and the capacity of the seed reservoir. There is a positive correlation between the weight of the biting bee mothers and the number of oviducts and the volume of the seed reservoir. According to Woyke and Jasinski [1978], mothers raised from eggs are weighing an average of 209 mg and have the highest number of ovarian tubes (317 in both ovaries) and the volume of the seed reservoir (1.182 µl), which in turn translates into the number of spermatozoa accumulated in Seed hopper after artificial insemination.

## MATERIAL AND METHODS

The study was conducted in a reproductive apiary stationary from the second half of May to the end of July 2013. The experience was divided into two phases. In the first stage (EXP1) 144 bee larvae were used, Caen breed (from Austria). In the second stage (EXP2), 288 larvae were used. Families raising Daddy type beetles (frame 435×300 mm). Wychow was conducted in unmatched families, orphaned just before the larvae were fed, fed on open bees collected from bee colonies of the same breeding line. Thanks to that, throughout the breeding period mothers in the nest were bee workers of all ages, capable of secretion of royal jelly and feeding mother larvae. This has allowed standardization of the families of the parents. Families were fed with sugar syrup 1 mol · $dm^{-3}$ if needed. Each family raised 2 breeding frames. There were two battens on each frame, fixed to a bee's wax with 18 beeswax wax cups with a diameter of 9 mm. All in all, there were 72 larvae in one family. For the study, they were grouped into four groups according to the medium used: group 1 (control – CON) – 50 ml of fresh royal jelly diluted with demineralised water in a ratio of 1:1, which was 100 ml total; Group 2 (VIT) – 50 ml of fresh royal jelly diluted with demineralized water in a ratio of 1:1 – up to 100 ml of the prepared medium were added 0.04 ml (one drop) of each vitamin A and E; Group 3 (PROB) – 50 ml of fresh royal jelly diluted with demineralised water in a ratio of 1:1 – 1 ml of the ApiBioFarma probiotic was added to 100 ml of the prepared medium; Group 4 (LIOF) – 10 mg lyophilized royal jelly was diluted in 90 ml demineralised water. Fresh royal jelly used for making substrates was taken from the swabs at the day of the test. Dried lyophilized bee was purchased from the manufacturer – Apipol Farm, in the form of capsules. One capsule contained 100 mg of lyophilisate, equivalent to 300 mg of fresh royal jelly. The prepared substrates are placed in a wax cup with 1 drop of pipette. Then a day-long honey bee-litter was transplanted into a bowl with a sterile metal spoon. The larval handling was done taking into account the required environmental conditions: air temperature about 25–26°C, relative humidity approx. 75–80% [Kruk and Skowronek 2002]. After transplanting larvae into bowls, they are immediately placed in prepared places in the families of the nursery.

Two parenting families were used in parenting studies (EXP1), while four (4) families were tested in EXP2 trials. On each breeding frame were nursery cups with larvae on all four substrates – 9 larvae from each group. There were four combinations of each group so that the larval bowls on the given substrate (from a given group) were in different arrangements on the breeding boxes (the numbers were labeled 1-CON, 2-VIT, 3-LIOF, 4-PROB):
First frame in ul. 1:
1 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 – top edge
3 3 3 3 3 3 3 3 3 4 4 4 4 4 4 4 4 – bottom stripe
Second frame in ul. 1:
2 2 2 2 2 2 2 2 1 1 1 1 1 1 1 1 1 – top strip
4 4 4 4 4 4 4 4 3 3 3 3 3 3 3 3 3 – bottom strut
Frame 1 in hive 2:
3 3 3 3 3 3 3 3 4 4 4 4 4 4 4 4 4 – top edge
2 1 2 1 1 1 1 1 1 1 – bottom strip
Second frame in hive 2:
4 4 4 4 4 4 4 4 3 3 3 3 3 3 3 3 3 – top edge
1 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 – bottom rail

Such an arrangement ensured that the nurseries with different substrates (from different groups) were located at different places in the breeding frame, reducing the impact of the internal environment in the larval nest.

In the relevant study (EXP2), the arrangement of framed bowls and the number of breeding frames in the hive

was identical to the fact that there were 2 times more, ie 4 families raising.

On the second day after larval shedding, families were surveyed to remove unwanted "wild" nurseries and assess the number of larvae taken. After 5 days the larvae were withdrawn from raising families, the nurseries were removed, the undersized or deformed pups were removed, and the culture boxes were placed in a greenhouse for six days (from the 6th to the 11th day including the larval spread). Conditions in the hothouse: temperature 34°C, relative humidity of about 70% [Williams et al. 2013]. At eleven o'clock, larvae were isolated in Zander's cages, and re-housed in a greenhouse. On the fourteenth day after larval shedding, ie on the first day after biting, mothers were weighed using a torsion scale.) Mother masturbation checks were made every 8 hours. The size of the mottled mothers and their appearance were assessed to identify lesions. They were then sent to the custom mailboxes with bees. After 48 hours, the mothers were weighed again

Atmospheric precipitation was assessed each day during the experiment, temperature and humidity of the electric thermometer and hygrometer placed in the apiary were also read (2 times per day).

The results obtained were analyzed statistically. Calculated mean, standard deviation, differences between groups were estimated using one-way analysis of variance ANOVA.

## RESULTS

During the study, the resources in the vicinity of the apiary were moderate, weather conditions varied in successive days and depending on the time of day. During the pre-natal period, usually in the morning, rainfall was observed, afternoons clear. Mean daily air temperature (during mating and pre-mating) was about 18°C and relative humidity was about 70%. In the case of proper rearing, the weather was more stable, mainly due to the predominance of sunny days and brighter than the meteorological conditions during the pre-breeding period.

During EXP1 144 families were raised, from which only 36 mothers were raised, resulting in a mere 25% of total mastitis (Table 1). However, in EXP2, 288 larvae raised 150 bee colonies, which means that the rearing efficiency was 52.1%, and was more than twice as high as in the preliminary study (Table 2). The best results of raising bee mothers in both stages were obtained in the CON group, where the larvae were transferred to a medium diluted with demineralized fresh royal jelly in a ratio of 1: 1. In EXP1 31% of rearing efficiency was obtained (Table 1), while EXP2 was more than twice as high and 74.8% (Table 2). In the CON group also received the best quality mothers, with the highest body weight. It was also shown that in both study groups, the difference in maternal body weight was highest after bite and 2 days (Table 3 and 4). In the LIOF and VIT group, there were no differences in the percentage of mothers who were exposed to larvae (Table 1). On the other hand, in EXP2, the water was diluted with lyophilized milk, since the larvae in this group (LIOF) had 52.5% rearing efficiency. Compared with the group (VIT) where the vitamin supplemented medium was 10.1% higher (Table 2). These results indicate that royal jelly contains all the compounds necessary for the correct development of larvae, including vitamins, and does not need to be enriched with vitamins A + E. In the VIT group, there was also a lower body weight of the mothers who were on average ($\pm$SD) 202.0 ($\pm$21.25) and 200.7 ($\pm$15.5) mg (EXP1 and EXP2 respectively). On the other hand, larvae exposed to medium of lyophilized royal jelly (LIOF group) bred mothers with mean weight ($\pm$SD) 194.4 ($\pm$11.11) mg in EXP1 and 196.6 ($\pm$10.12) mg in EXP2 (Tables 3 and 4). In both study stages, the worst parental outcomes were obtained in the group (PROB), in which the larvae were translated into a diluted bee milf with probiotics. In the first stage of the study, the percentage of bite mothers was only 19%, while in the second stage, 38.4% of the larval mothers (Tables 1 and 2). The cause of such results was most likely that the royal jelly had bactericidal properties and in the mixed probiotic it could be the effect of closing or deactivating the microflora contained in it. However, in this group, higher body weight was observed in mothers than in vitamins (VIT) and lyophilized milk (Tables 3 and 4). These masses were similar to those obtained in the control group (CON). The number of sealed (isolated) nurseries was smaller than the number of larvae taken from all used media (Tables 1 and 2). None of the substrates used in EXP1 had satisfactory maternity efficacy at least 50% (Table1). In all groups, maternal rearing outcomes were very weak and statistically significant differences in the number of raised mothers between the substrates used were not shown. However, during the period of EXP2, the weather conditions improved, which was reflected in the higher maternal efficacy in all groups, on average to 52.1%, and in the group with royal jelly (CON) even 74.8% (Table 2). Despite the differences, there was no statistically significant variation. No maternal morphological defects were observed during mothers' assessment. All individuals were well educated and highly motivated.

## DISCUSSION

The results of our own research show that the best effects of raising bee mothers were obtained in the group where the substrate was fresh royal jelly. Similar results were previously obtained by Zielony et al. [2012], who had the highest percentage of successful mothers (62.2%) repor-

**Table 1.** The results of breeding queen bees – preliminary study (EXP1)

**Tabela 1.** Wyniki wychowu matek pszczelich – badania wstępne (EXP1)

| Group<br>Grupy | Number of larvae<br>Liczba przełożonych larw | Larvae accepted<br>Larwy przyjęte | | Isolated queen cells<br>Zasklepione (lub zaizolowane mateczniki) | | Queen emerged<br>Matki wygryzione | |
|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % |
| CON (royal jelly diluted of water)<br>CON (mleczko pszczele rozcieńczone woda) | 36 | 23 | 35 | 19 | 34 | 11 | 31 |
| VIT (royal jelly with vitamins A and E)<br>VIT (mleczko pszczela z witaminą A i E) | 36 | 16 | 24 | 14 | 25 | 9 | 25 |
| PROB (royal jelly with probiotic)<br>PROB (mleczko pszczele z probiotykiem) | 36 | 13 | 20 | 10 | 18 | 7 | 19 |
| LIOF (lyophilized royal jelly diluted with water)<br>LIOF (mleczko pszczele liofilizowane rozcieńczone wodą) | 36 | 14 | 21 | 13 | 23 | 9 | 25 |
| Altogether – Ogółem | 144 | 66 | 45.8 | 56 | 38.9 | 36 | 25.0 |

**Table 2.** The results of breeding queen bees – proper study (EXP2)

**Tabela 2.** Wyniki wychowu matek pszczelich – badania właściwe (EXP2)

| Group<br>Grupy | Number of larvae<br>Liczba przełożonych larw | Larvae accepted<br>Larwy przyjęte | | Isolated queen cells<br>Zasklepione (lub zaizolowane mateczniki) | | Queen emerged<br>Matki wygryzione | |
|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % |
| CON (royal jelly diluted of water)<br>CON (mleczko pszczele rozcieńczone woda) | 72 | 59 | 81.8 | 57 | 79.8 | 54 | 74.8 |
| VIT (royal jelly with vitamins A and E)<br>VIT (mleczko pszczela z witaminą A i E) | 72 | 37 | 51.5 | 34 | 47.5 | 31 | 42.4 |
| PROB (royal jelly with probiotic)<br>PROB (mleczko pszczele z probiotykiem) | 72 | 35 | 48.5 | 31 | 43.4 | 28 | 38.4 |
| LIOF (lyophilized royal jelly diluted with water)<br>LIOF (mleczko pszczele liofilizowane rozcieńczone wodą) | 72 | 43 | 59.6 | 41 | 56.6 | 38 | 52.5 |
| Altogether – Ogółem | 288 | 174 | 60.42 | 164 | 56.94 | 150 | 52.1 |

ted in the group where larvae were transplanted to this type of substrate. Nevertheless, the same results (54.4%) were obtained by the same authors [Zielony et al. 2012] using diluted royal jelly. Bin et al. [2008] have demonstrated by laboratory experiments that mother larvae for normal growth need extra water. In their study, they observed that the larvae that received water-diluted colostrum were characterized by higher body weights compared to larvae fed with fresh bee milk. According to Pidek [1999], larvae fed on royal jelly are always more likely to be taken than on other media, as confirmed in their own tests (Table 1 and 2). Comparable results of mothers' parental education were obtained by Zielony et al. [2012] when used as a substrate to translate larvae of lyophilized royal jelly diluted with water – 60.0 and 61.1% (two concentrations of milk). The mean body weight of raised bee mothers was 188.4 mg (159 to 241 mg) and showed no significant difference in maternal weight of larvae transferred to different substrates. Likewise lyophilized royal jelly was used in Bin et al. [2008]. Pidek [1999] believes that before transferring the larvae to the wax cups, nectar (honeycomb), plucked from honeycomb diluted with honey or boiled water, can also be added. Bobrzecki and Prabucki [1975] have shown that there is no difference in the acceptability of larvae on water and honey or bud. Significant influence on the rearing of mothers was the weather conditions and profitable during the rearing. The results obtained during the initial tests could have been due to poor weather conditions and the use of the records during this period. Rainfall hindered bees harvesting benefits. In turn, the lack of food in the nest reduced the interest in bees raising mothers and influenced the poor results of raising mothers, despite the feeding of bee colonies during this period did not get satisfactory improvement of the results of raising. Therefore, in pre-testing in all groups, maternal rearing outcomes were very weak and statistically significant differences in the number of raised mothers between the substrates used were not shown. The above achievements are confirmed by Zielony et al. [2012], who led mothers during periods of weather-favorable conditions achieved 54.4 to 62.2% of success. Similar conclusions were reached by Genc et

**Table 3.** Body weight emerged queen – preliminary study (EXP1)

**Tabela 3.** Masa ciała matek – badania wstępne (EXP1)

| Group<br>Grupa | Mean (± SD) body weight in mg<br>Średnia (±SD) masa ciała matek w mg | |
|---|---|---|
| | after emerged<br>po wygryzieniu | on day 2 of life<br>w 2. dniu życia |
| CON (royal jelly diluted of water)<br>CON (mleczko pszczele rozcieńczone wodą) | 212.9 (±17.23)** | 198.9 (±11.90)** |
| VIT (royal jelly with vitamins A and E)<br>VIT (mleczko pszczela z witaminą A i E) | 202.0 (±21.25) | 190.7 (±15.08) |
| PROB (royal jelly with probiotic)<br>PROB (mleczko pszczele z probiotykiem) | 209.2 (±17.00) | 201.9 (±20.53) |
| LIOF (lyophilized royal jelly diluted with water)<br>LIOF (mleczko pszczele liofilizowane rozcieńczone wodą) | 199.4 (±11.11)** | 189.0 (±10.93)** |

** Statistically significant differences between measurements at $P \leq 0.01$.
** Różnice statystycznie istotne między pomiarami na poziomie $P \leq 0,01$.

**Table 4.** Body weight emerged queen – proper study (EXP2)

**Tabela 4.** Masa ciała wygryzionych matek – badania właściwe (EXP2)

| Group<br>Grupa | Mean (± SD) body weight in mg<br>Średnia (±SD) masa ciała matek w mg | |
|---|---|---|
| | after emerged<br>po wygryzieniu | on day 2 of life<br>w 2 dniu życia |
| CON (royal jelly diluted of water)<br>CON (mleczko pszczele rozcieńczone wodą) | 208.8 (±12.76)** | 197.4 (±10.49)** |
| VIT (royal jelly with vitamins A and E)<br>VIT (mleczko pszczela z witaminą A i E) | 200.7 (±15.50)** | 190.3 (±12.08)** |
| PROB (royal jelly with probiotic)<br>PROB (mleczko pszczele z probiotykiem) | 206.9 (±13.05)** | 198.0 (±14.49)** |
| LIOF (lyophilized royal jelly diluted with water)<br>LIOF (mleczko pszczele liofilizowane rozcieńczone wodą) | 196.6 (±10.12)** | 186.0 (±10.08)** |

** Statistically significant differences between measurements at $P \leq 0.01$.
** Różnice statystycznie istotne między pomiarami na poziomie $P \leq 0,01$.

al. [2005] who examined the morphological parameters of mothers raised during various periods of the season and weather conditions in eastern Turkey. According to them, the best time to raise mothers under the climatic conditions is April and July, because in these periods, raised mothers were more massy. The weight of the mothers' bees is a very important parameter for evaluating the quality of mothers raised. The minimum body weight of a Caucasian mother immediately after biting should not be less than 185 mg (Industry Standard BN-77/9148-02). There is a justified claim that the larger the mother of the bee, the more well-developed ovaries [Woyke 1978, Szabo et al. 1987]. In turn, the more oviparous ovaries in the ovary, the more eggs can be lodged within 24 hours. So, the mass of the bee's body translates into redness, which affects the power of the family and their productivity. Various observations have been presented in the work of Jackson et al. [2011] who performed histopathological preparations from mothers' ovaries from different sources. Thanks to such comparison he could accurately determine the number of ovarian tubes and compare it with their mass. Particular attention should be paid to the age of the larvae used when determining the differences in breeding performance. One-day larvae were used in their own studies, according to the results of the study. Woyke [1971] and Hamdan [2014], who showed that the most valuable mothers were obtained from day-old larvae. The same conclusions are presented by Mahbobi et al. [2012].

## SUMMARY

The results of the study show that enrichment of the royal jelly into a variety of additives to improve its composition does not improve the results of raising colostrum. Placing the larvae on the diluted royal jelly provides the best results for their rearing, indicating that the milk itself is the best balanced food, providing all the larvae necessary for the development of nutrients. It is not advisable to use probiotics as additives to enhance the composition of the substrate to raise bee mothers.

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Additional information

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# OCENA MOŻLIWOŚCI WYCHOWU MATEK PSZCZELICH Z LARW PRZEKŁADANYCH NA RÓŻNE PODŁOŻA

## STRESZCZENIE

W pracy porównano efektywności wychowu matek pszczelich z larw przekładanych na różne podłoża do sztucznych miseczek matecznikowych. Badania przeprowadzono w sezonie pożytkowym 2013 roku, w pasiece reprodukcyjnej. Jednodniowe larwy przekładano na: świeże mleczko pszczele rozcieńczone wodą demineralizowaną 1:1 (CON), mleczko pszczele z dodatkiem witamin A i E (VIT), mleczko pszczele liofilizowane rozcieńczone wodą demineralizowaną (LIOF) oraz mleczko pszczele z dodatkiem probiotyku ApiBioFarma (PROB). W badaniach wstępnych (EXP1) ze 144 larw poddanych rodzinom wychowującym uzyskano 36 matek pszczelich. Natomiast w badaniach właściwych (EXP2) z poddanych 288 larw uzyskano łącznie 150 matek. Pszczoły najlepiej akceptowały larwy przekładane na podłoże z rozcieńczonego świeżego mleczka pszczelego. Na tym podłożu uzyskano największą efektywność wychowu matek w obu etapach badań odpowiednio 31 i 74,8%. Wykazano różnice istotne statystycznie pod względem masy ciała matek wychowywanych z larw przekładanych na świeże mleczko pszczele i mleczko z dodatkiem probiotyku.

**Słowa kluczowe:** *Apis mellifera*, wychów matek pszczelich, mleczko pszczele, probiotyk, witaminy