Research paper

Accessible Queen Rearing Under Practical Conditions: Preliminary Findings from a Student Project

Marc Hillen

Master Beekeeping Certificate Program, University of Montana, 2025 · Eikely Honning AS, Mandal, Norway

Abstract

This study investigated the feasibility of producing newly emerged honeybee queens exceeding 210 mg in body weight using accessible queen-rearing methods available to beekeepers. The experiment employed homemade wax cell cups and grafted larvae of approximately 12 hours old, ensuring each cell cup received a standardized amount of royal jelly harvested from a separate batch of queen cells, interrupted three days after grafting. Three cell-starter hives, derived from robust colonies, were prepared using a brood-lifting technique eight days prior to the experiment and relocated to the test apiary. Over three consecutive grafting rounds, each hive was provided with 24 cell cups containing grafted larvae per round, totalling 72 grafted larvae per colony. These colonies were subjected to varying feeding protocols: two received supplemental protein sources — one with liquid amino acids and the other with pollen/candy patties — while the control group was fed exclusively with sugar syrup. Because each treatment was applied to a single starter colony, treatment effects cannot be fully separated from colony-level differences; the study is therefore a pilot providing a foundation for larger multi-colony trials. The research was timed to coincide with a dearth period in early July, reducing the influence of natural nectar flows. The ultimate aim was to develop a replicable, straightforward method enabling beekeepers of all experience levels to cultivate high-performing queens.

Introduction

Honeybee queen quality is critical for colony health, productivity, and overall resilience. Although the ultimate population of worker bees and subsequent colony productivity depend on numerous external factors, Yu et al. (2023) demonstrate that a queen's intrinsic reproductive potential is directly linked to physical quality traits, such as ovariole number and egg-laying capacity. A healthy queen is essential for each hive, as she produces the worker bees and drones necessary for passing on the colony's genetic information (Copeland et al., 2024). Consequently, the success of beekeeping relies on maintaining robust and vigorous bee colonies, as weak colonies lead to unproductive outcomes and increased management challenges (Amiri et al., 2017). Ultimately, the goal is to foster resilient bee colonies that effectively forage for pollen and nectar while demonstrating resistance to prevalent pests. Therefore, identifying accessible methods for producing such high-quality queens is of utmost importance. The quantitative and qualitative reproductive potential of a queen represents her overall “quality”, which is determined by her genome, developmental conditions, and adult environment (Amiri et al., 2017). Furthermore, her mating success with multiple drones is critical, as high-frequency mating enhances the colony's genetic diversity and overall fitness (Delaplane et al., 2021).

Queen weight at emergence is widely used as a practical proxy for these traits, as heavier queens tend to be better developed; values around 210 mg recur in the literature as an indication of a well-developed queen.

The rearing system used in this study was learned at Gospodarstwo Pasieczne “Pasieka Szeligów” in Wielkie Drogi, Poland, during three periods of practical training: a study visit in 2022, a return visit in 2023 accompanying Norwegian students learning instrumental insemination, and a ten-day working period in the apiary in 2024. The system is built around wax cell cups, priming with diluted royal jelly, and grafting of very young larvae. The queens produced there were visibly large and well developed, which prompted the question addressed here: whether comparable results could be obtained with equipment and materials available to an ordinary beekeeper, and whether supplemental protein feeding of the cell starter is necessary to achieve them.

Materials and Methods

Wax cell cups

Wax cell cups produced from wax cappings were used in this study, with an internal diameter of 8.8 mm and an internal height of 8.5 mm. Wax cell cups offer a practical advantage as they are a readily available resource in beekeeping. Additionally, the choice of material enhances the initial acceptance rate of the grafted larvae (Lashari et al., 2022). Beyond the material itself, the physical dimensions of the cell cup critically influence queen development; Mattiello et al. (2022) demonstrated that larger cup diameters (9 mm compared to 8 mm) result in significantly bigger queens with higher body weights. An internal diameter of 8.8 mm was therefore selected to maximise the queen's growth potential (see also Ruttner, 1988). The cell cups were mounted on a plastic cell base and then onto a strip holding up to 12 cell cups, with each cell-building frame accommodating up to 24 cups (Büchler et al., 2024). These frames were placed in a strong bee colony for 12 to 24 hours prior to grafting, depending on colony activity (Büchler et al., 2024). This pre-treatment allowed the bees to clean and polish the cells, further raising the acceptance rate of the grafted larvae.

Royal jelly

To standardize cell cup preparation, each cup received a measured 10 µl aliquot of diluted royal jelly via micropipette (Sharma et al., 2020). This ensured uniformity and leveraged royal jelly's capacity to enhance the acceptance of grafted larvae. The royal jelly was diluted with approximately 30 % sterile water to achieve a consistency conducive to larval movement (Sharma et al., 2020). This royal jelly was collected from a separate batch of queen cells initiated earlier in the season and interrupted three days after grafting for jelly collection. This timing is important because the chemical composition, nutritional quality and bioactive profile of royal jelly are highly sensitive to environmental and temporal variation (Alkindi et al., 2024). To preserve this biochemical integrity and prevent the degradation of heat-sensitive proteins and enzymes, the collected jelly was stored at −18 °C until grafting commenced, as deep freezing is required to maintain long-term stability (Büchler et al., 2024; see also Messia et al., 2005). Caste fate in honeybees is determined by the quantity and duration of the larval diet: queen-destined larvae are fed royal jelly continuously and in excess throughout larval development, whereas worker-destined larvae receive it only during the first days.

Larval grafting — Doolittle method

In preparation for grafting, royal jelly and sterile water were pre-heated to 35 °C and combined. A precise aliquot of 10 µl of diluted royal jelly was then administered to sets of 12 cell cups. The frame containing the grafting material was moved from the mother colony to the cell starter 12 hours in

advance to ensure the larvae would be floating in larval food. For grafting, the frame was removed from the cell starter and kept in an insulated container to preserve brood temperature, then examined to select appropriate grafting material. The age of the larvae is a key factor in the acceptance rate and the quality of the resulting queen; larvae of around 12 hours of age were selected, and only larvae immersed in royal jelly were grafted (Rehman et al., 2024). The Doolittle method was employed given its established efficacy in the transfer of substantial quantities of larvae. The grafting pen was inserted along the inner wall of a worker cell until positioned beneath the larva, which was then elevated and deposited into a prepared queen cell upon a substrate of royal jelly. Upon completion of a set of 12 cell cups, the row was protected with a slightly moistened, warm cloth to avert desiccation. This procedure was repeated until each queen cell contained a single larva. The grafted cells were then transferred to a cell-starter colony. To maximise larval acceptance, the grafting environment must be carefully managed; hygienically clean tools and a sanitised work area prevent contamination that can negatively affect queen productivity (Dodoloğlu & Emsen, 2007). All tools in contact with the larvae and royal jelly, as well as storage glassware, were disinfected using heat and/or UV light in accordance with standard management protocols (Büchler et al., 2024).

Cell starters

Three cell-starter colonies were established using robust hives, with brood frames elevated above a queen excluder eight days prior to cell introduction. After one week, these brood boxes were relocated to the designated test apiary and placed on individual bottom boards. Each starter colony comprised two boxes with frames, totalling 20 frames populated with bees and a minimum of 4 frames containing both sealed and unsealed brood, with the brood positioned in the upper box (Ruttner, 1988). Prior to introducing the cell-builder frame, a frame was removed from the periphery of the hive to create space in the middle of the top box.

On day eight, each starter colony received 24 cell cups containing grafted larvae (Ruttner, 1988). Grafting was repeated in three successive rounds in each colony, giving a total of 72 grafted larvae per starter colony.

Each starter colony was provided with sugar syrup at a concentration of 1:1.3 by weight (1 kg sugar to 1.3 L water), supplied ready-mixed as 0.5 L every second day, beginning the day before the first grafting and ending when the cells of the third round were capped — approximately 4,5 L per colony in total. Colony A received a commercial liquid amino-acid and vitamin supplement (AMINO-VET, VET-ANIMAL, Starogard Gdański, Poland; batch 060824) mixed into the syrup at the manufacturer's recommended rate of 5 ml per litre. The product declares 8 % crude protein and 118.2 g of free amino acids per litre, so this dose delivered approximately 0.59 g of free amino acids, equivalent to about 0.4 g of crude protein, per litre of syrup. Colony B received pollen/candy patties (Candipolline Gold; declared 3 % sterilised pollen and 1.38 % crude protein, with caseinate and albumin as additional protein sources) ad libitum, a fresh 1 kg pouch being supplied whenever the previous one was consumed. An estimated 2–3 kg was consumed over the experiment, corresponding to approximately 28–42 g of crude protein. The control colony, C, received sugar syrup only. The two supplemented treatments therefore differed not only in protein source but by more than an order of magnitude in the quantity of protein supplied, and in whether it was offered at a fixed dose or ad libitum.

Following a 24-hour period post-grafting, all cell starters were inspected to determine larval acceptance. Unaccepted cells were discarded, and the remaining accepted cells were consolidated to

maintain a uniform cell density across all colonies. The accepted cells then remained in the same cell starter until day 5, by which point they had been capped by the bees. This precaution mitigates confounding variables that may arise from redistributing cells to different hives.

On day 5, the frame containing the queen cells was removed and transported to the laboratory, where each cell was examined, measured and weighed. Cells were weighed on a precision balance with milligram resolution. Weighing was performed with the cell on its plastic base and holder, and a constant tare of 1.26 g was subtracted to obtain the net cell weight. Cell length was measured with a vernier calliper to the nearest 0.1 mm. The cells were then transferred to an incubator maintained at 34.5 °C, regulated by a P.I.D. thermostat with a precision of 0.1 °C, and a relative humidity of 70 % achieved through automatic humidity control.

Following emergence

Queens were weighed on the day of emergence on the same precision balance. Acceptance, capping, emergence and weight data were recorded separately for each grafting round and each colony.

Statistics

Cell weight and cell length at day 5, and queen weight at emergence, were compared between colonies using two-sample t-tests in Microsoft Excel (unequal variances). Tests were two-sided with $\alpha = 0.05$.

Individual cells and individual queens were treated as the unit of observation. Because each feeding treatment was applied to a single starter colony, and observations within a round within a colony are not independent, these tests describe differences between the colonies as observed and cannot be interpreted as treatment effects.

Timing and location

The study was carried out within a five-week student project window, during the first three weeks of July 2025, at 58°04’22.4”N 7°31’00.1”E. Given the timing, immediately following the raspberry harvest and prior to any subsequent nectar flow, the outcomes are unlikely to have been strongly influenced by the bees' natural foraging activities, reducing the influence of natural nectar flows. To maintain comparability and experimental control, the colonies were situated in close proximity to one another. The single-season, three-round design follows from this project window.

Results

This project aimed to establish a standardized, easily mastered methodology accessible to all beekeepers to maximize queen bee emergence weight, thereby supporting apiary stability and reducing management strain. The study successfully produced queens with an average weight well exceeding the predefined 210 mg threshold, demonstrating the viability of utilizing resource-efficient rearing techniques. Following emergence, the overall average weight of the queens was 250 mg (n = 101), with individual weights ranging from 178 mg to 287 mg. Pronounced variations were observed between the groups: queens from Colony A averaged 221.0 mg (n = 6), Colony B averaged 247.65 mg (n = 41), and the control group (Colony C) yielded the heaviest individuals with an average of 254.1 mg (n = 54).

Colony A was significantly lighter than both Colony B and Colony C ($P < 0.05$), while queens from Colony B and Colony C did not differ significantly ($P = 0.105$).

These findings challenge the assumption that supplementary feeding is a prerequisite for producing heavy queens, given that the control group matched or exceeded the emergence weights of the supplemented groups. This indicates that the intrinsic strength and health of the cell-starter colony exert a more decisive influence on physical queen development than supplemental feeding during the rearing phase (Voinalovych et al., 2022). Consequently, these results underscore the necessity of using rigorous, multi-variable methodologies when evaluating commercial beekeeping practices.

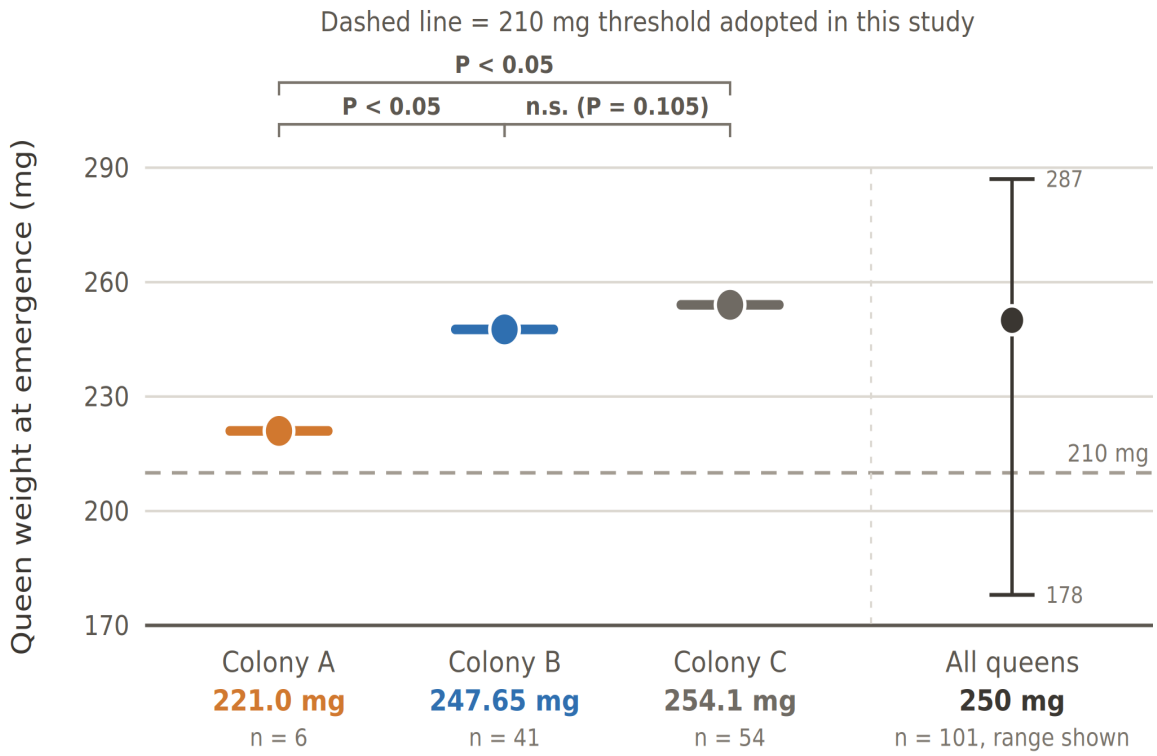

Fig. 1. Queen weight at emergence per cell-starter, with the 210 mg criterion marked. Data collected July 2025, Mandal, Norway.

Cell length and cell weight at day 5 differed significantly among the cell-starters; cell weight values for Colony A were markedly lower (1.74 g in R1, 1.69 g in R2) than those for Colonies B and C (approximately 1.8–2.0 g), which did not differ substantially from each other. This underperformance in Colony A was consistent from the first round onward. In grafting round 3, Colony A produced no capped cells at all, and therefore no emergence data for that cohort, as shown in the subsequent tracking data (Fig. 2 and Fig. 3).

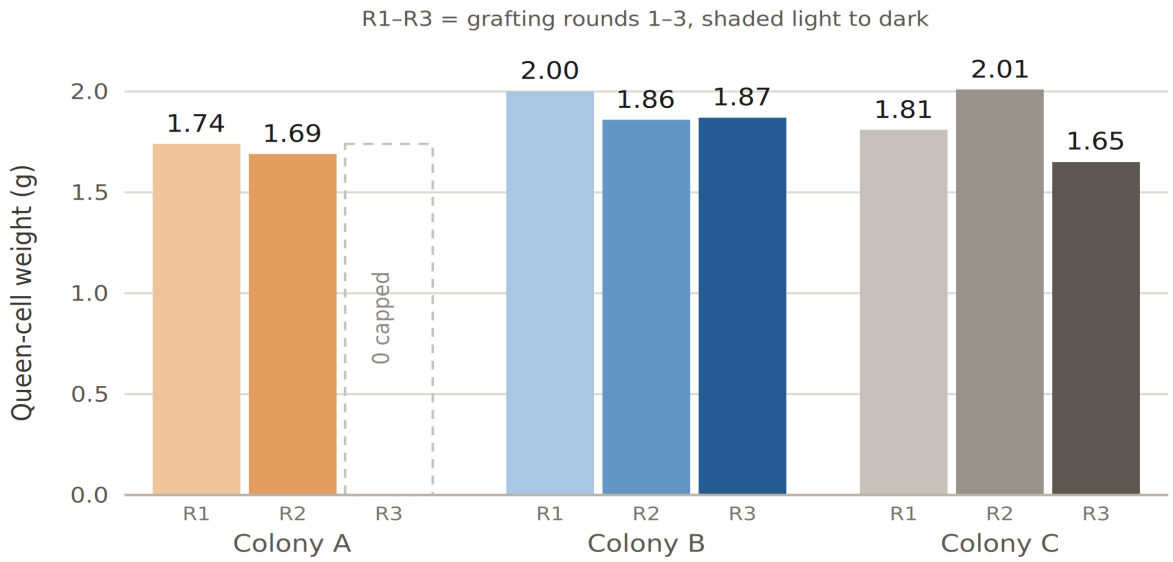

Fig. 2. Queen-cell weight (g) at day 5 of the cell-building phase, by starter colony and grafting round (R1–R3). Colony A produced no capped cells in round 3. Note that cell weight is a distinct measure from queen weight at emergence. Data collected July 2025, Mandal, Norway.

Colony A exhibited consistently poorer performance from the outset, whereas cell-starters B and C performed similarly.

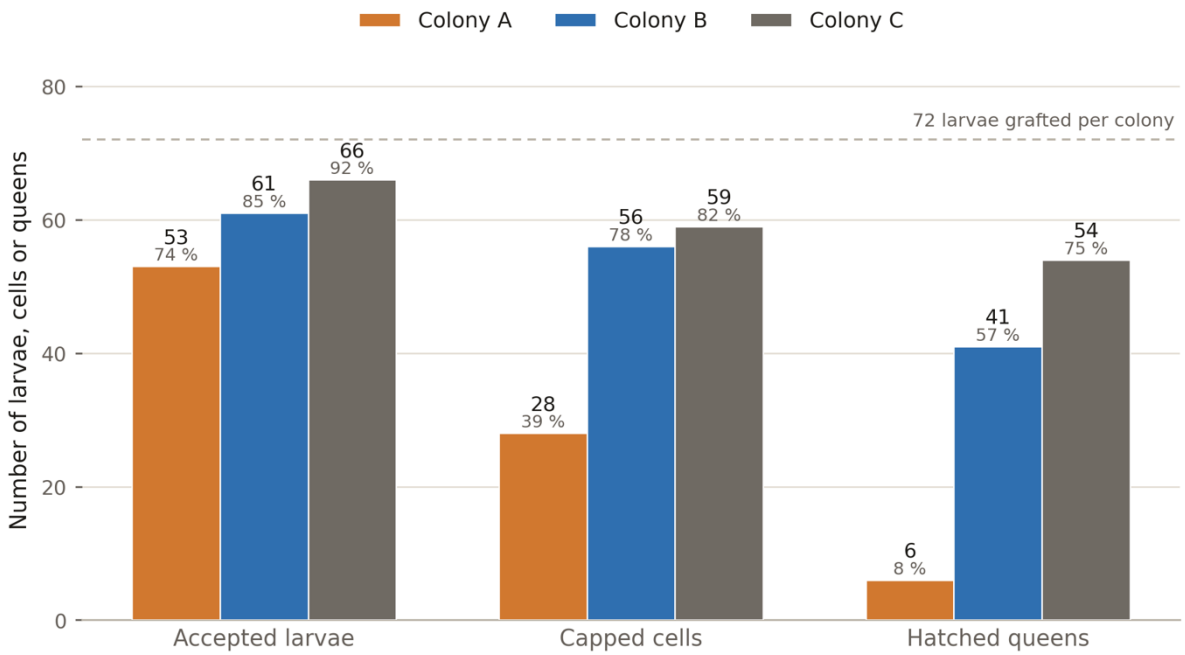

Fig. 3. Number of accepted larvae, capped cells and emerged queens per cell-starter, expressed as counts and as a percentage of the 72 larvae grafted into each colony. Data collected July 2025, Mandal, Norway.

The data reveal substantial variation in larval acceptance and cell development across the three colonies. The control, Cell-starter C, showed the most favourable results, with the highest proportion of grafted larvae progressing through to emerged queens (accepted 92 %, capped 82 %, emerged 75 % of 72 grafted). Colony A performed poorest at every stage (accepted 74 %, capped 39 %, emerged 8 %), and Colony B was intermediate (accepted 85 %, capped 78 %, emerged 57 %).

Discussion

This study investigated the feasibility of producing heavy honeybee queens using accessible rearing methods. The findings indicate that achieving a high average queen emergence weight (>210 mg) is possible without additional protein feeding, offering a practical and resource-efficient approach for beekeepers to optimize queen size at emergence. In this study, queen quality was operationally assessed using body weight at emergence; reproductive performance, mating success, and longevity were not evaluated.

The study examined the impact of supplemental feeding to cell builders and found no significant difference in queen weight between the control colony and the colony that received pollen patties in addition to sugar syrup. It should be emphasized that each feeding treatment was applied to a single starter colony (n = 1 colony per treatment). The three grafting rounds provide within-colony replication but not causal separation of feeding effect from colony effect. Colony B and Colony C did not differ significantly in queen weight ($p = 0.105$). This is a pilot-scale study; its results provide a foundation for larger, multi-colony trials rather than definitive causal conclusions.

The results suggest that pollen patties offered no significant benefit to the cell-builder colony. The patties declared 1.38 % crude protein and 3 % sterilised pollen, giving roughly 14 g of crude protein per kilogram, and a substantial part of that protein was caseinate and albumin rather than pollen. Milk and egg proteins are poorly utilised by honey bees compared with pollen protein. A further consideration is that the specific amino-acid profiles and micronutrient content of commercial patties may not align with the dietary requirements of developing queen larvae at each stage.

The colony supplemented with liquid amino acids (Colony A) produced the lightest queens and the lowest emergence rate. However, because each treatment was applied to a single starter colony, this outcome cannot be attributed to the amino acids rather than to underlying differences between the colonies themselves. Colony A underperformed from the first round, consistent with a weaker starter rather than a treatment effect. In addition, the liquid supplement delivered on the order of 0.4 g crude-protein equivalent per litre of syrup at the label dose, far less than the patties supplied to Colony B. Amino-acid supplementation therefore warrants cautious interpretation, but this study cannot establish a causal effect.

The strong performance of the control colony is notable. It may indicate that the environmental conditions and resources within the control colony were already favourable for brood rearing, potentially limiting the additional benefit from supplemental feeding (Mansor et al., 2021; Sakla & El-shafeiy, 2022). Alternatively, the nutritional requirements necessary to maximize queen emergence weight may have been adequately met by the colony's natural pollen stores and the standard sugar syrup, rendering additional protein supplementation unnecessary.

Limitations and future work

- Treatment was confounded with starter colony: each feeding treatment was applied to a single colony (n = 1 colony per treatment), so amino-acid and pollen effects cannot be separated from underlying colony differences. The three rounds provide within-colony replication, not causal separation.

- Colony A yielded only six emerged queens and underperformed on acceptance, capping and emergence from the first round onward; its lower weights are best read as a weak-starter signal, not evidence against amino acids.
- The two supplemented treatments were not comparable in the quantity of protein supplied, nor in the feeding regime: Colony B received an estimated 28–42 g of crude protein ad libitum, while Colony A received a fixed label dose delivering roughly 0.4 g crude-protein equivalent per litre of syrup.
- Single season and single site (Mandal, Norway); results may not generalise across climates, seasons, or nectar-flow conditions. Colony B and C did not differ significantly on queen weight ($p = 0.105$).
- Cup material (wax, 8.8 mm) and the overall rearing system were adopted as fixed choices and not evaluated as variables here.
- Future work: replicate across multiple colonies per treatment and several seasons; test lower amino-acid concentrations and alternative delivery forms; compare wax versus plastic cups within the same system. This requires a longer time frame than the project allowed.

Conclusion

The findings demonstrate that queen bees exceeding the study's predefined 210 mg weight threshold can be produced through the application of limited resources and uncomplicated techniques, making this approach feasible for beekeepers of all experience levels. Critical factors for achieving this target weight include precise timing and a robust cell-starter colony. Within this study, supplemental protein feeding conferred no advantage in emergence weight over sugar syrup alone. The queen-rearing methodology detailed here presents an accessible strategy that can be implemented without significant financial investment, underscoring the viability of utilizing readily available resources and simple techniques to maximize queen emergence weight.

References

Alkindi, F. K. S. A., El-Keblawy, A., Ridouane, F. L., & Mirza, S. B. (2024). Factors influencing the quality of Royal jelly and its components: a review. Cogent Food & Agriculture, 10(1). https://doi.org/10.1080/23311932.2024.2348253

Amiri, E., Strand, M. K., Rueppell, O., & Tarpy, D. R. (2017). Queen quality and the impact of honey bee diseases on queen health: potential for interactions between two major threats to colony health. Insects, 8(2), 48. https://doi.org/10.3390/insects8020048

Büchler, R., Andonov, S., Bernstein, R., Bienefeld, K., Costa, C., Du, M., Gabel, M., Given, K., Hatjina, F., Harpur, B. A., Hoppe, A., Kezić, N., Kovačić, M., Kryger, P., Mondet, F., Spivak, M., Uzunov, A., Wegener, J., & Wilde, J. (2024). Standard methods for rearing and selection of Apis mellifera queens 2.0. Journal of Apicultural Research, 64(2), 555–611. https://doi.org/10.1080/00218839.2023.2295180

Copeland, D., Ricigliano, V., Mott, B. M., Kortenkamp, O. L., Erickson, R. J., Gorrochategui-Ortega, J., & Anderson, K. E. (2024). A longitudinal study of queen health in honey bees reveals tissue specific response to seasonal changes and pathogen pressure. Scientific Reports, 14(1). https://doi.org/10.1038/s41598-024-58883-1

Delaplane, K. S., Given, K., Menz, J., & Delaney, D. A. (2021). Colony fitness increases in the honey bee at queen mating frequencies higher than genetic diversity asymptote. Behavioral Ecology and Sociobiology, 75(9). https://doi.org/10.1007/s00265-021-03065-6

Dodoloğlu, A., & Emsen, B. (2007). Effects of larvae transfer conditions on queen bee productivity. Journal of Applied Animal Research, 31(2), 181–182. https://doi.org/10.1080/09712119.2007.9706658

Lashari, M. A., Ghramh, H. A., Ahmed, A. M., Mahmood, R., Rafique, M. K., Ahmad, S., Al-Shehri, B. M., Mohammed, M. E. A., & Khan, K. A. (2022). Aptness of diverse queen cup materials for larval graft acceptance and queen bee emergence in managed honey bee (Apis mellifera) colonies. Journal of King Saud University – Science, 34(4), 102043. https://doi.org/10.1016/j.jksus.2022.102043

Mansor, M. S., Al-Bajari, H. A. J., & Ibrahim, M. K. (2021). Effect of feeding with vitamins, sugars and pollen on the biology of honey bee Apis mellifera L. (Hymenoptera: Apidae) during the autumn season and the use of various wintering methods. IOP Conference Series: Earth and Environmental Science, 923(1), 012018. https://doi.org/10.1088/1755-1315/923/1/012018

Mattiello, S., Rizzi, R., Cattaneo, M., Martino, P. A., & Mortarino, M. (2022). Effect of queen cell size on morphometric characteristics of queen honey bees (Apis mellifera ligustica). Italian Journal of Animal Science, 21(1), 532–538. https://doi.org/10.1080/1828051x.2022.2043790

Messia, M. C., Caboni, M. F., & Marconi, E. (2005). Storage stability assessment of freeze-dried royal jelly by furosine determination. Journal of Agricultural and Food Chemistry, 53(11), 4440–4443. https://doi.org/10.1021/jf0404647

Rehman, N. U., Anjum, S. I., Qureshi, N. A., Khan, M. H., Albasher, G., Kaleem, M., & Kamal, A. (2024). The effect of larval age, and wet and dry grafting, on the rearing of queen bees using the Doolittle grafting method. *Entomological Research*, *54*(1), e12700. https://doi.org/10.1111/1748-5967.12700Ruttner, F. (1988). Breeding techniques and selection for breeding of the honeybee. British Isles Bee Breeders' Association.

Sakla, R., & El-shafeiy, S. (2022). Evaluation of royal jelly quality and queens production by using natural food supplements in honeybee colonies. Egyptian Journal of Agricultural Research. https://doi.org/10.21608/ejar.2022.141398.1237

Sharma, A., Rana, K., Sharma, H. K., & Sharma, A. (2020). Evaluation of priming media and queen cup material on larval graft acceptance and queen emergence in Apis mellifera L. Journal of Entomology and Zoology Studies, 8(4), 1089–1097. https://doi.org/10.22271/j.ento.2020.v8.i4q.7268

Voinalovych, M., Brovarskiy, V., & Golovetskyi, I. (2022). Influence of the qualitative and quantitative composition of the queen-rearing colony and the number of grafted larvae on the weight of queen bees. Animal Science and Food Technology, 13(3). https://doi.org/10.31548/animal.13(3).2022.14-19

Yu, L., He, X. J., Shi, X., Yan, W., & Wu, X. (2023). Honey bee maternal effects improve worker performance and reproductive ability in offspring. Frontiers in Cell and Developmental Biology, 11, 1156923. https://doi.org/10.3389/fcell.2023.1156923