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Pollen power and colony prosperity: A comprehensive analysis of pollen amino acid profiles and their impact on honeybee colony performance and queen quality across seasons

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Abstract

This study presents an in-depth investigation into the intricate relationship between the nutritional profile of natural pollen sources and key performance metrics of *Apis mellifera* colonies. Conducted over two distinct beekeeping seasons (2023 and 2024), the research focuses on queen rearing metrics from the 2023 season, two years of comparative colony performance data, and the amino acid composition of both royal jelly and bee-collected pollen from four major floral sources in Egypt: Clover (*Trifolium alexandrinum*), eucalyptus (*Eucalyptus* sp.), corn plant (*Zea mays*), and Brazilian pepper (*Schinus terebinthifolius*). The results show that the unique amino acid composition of pollen plays a crucial role in determining the physiological condition and productivity of a colony. High-quality pollen from *Z. mays*, for instance, was correlated with enhanced larval immunity, while the royal jelly produced from Brazilian pepper pollen was linked to superior queen quality. The data reveal that different pollen sources, with their unique nutritional characteristics, drive distinct outcomes in the colony, suggesting that beekeepers can strategically manage foraging resources to achieve targeted goals, whether for colony growth, disease resistance, or maximizing queen quality and honey yield.

Introduction

Honeybee colonies are complex superorganisms whose health and productivity are inextricably linked to the quality and diversity of their food sources. Pollen is a cornerstone of the honeybee diet, serving as the sole natural source of protein, essential amino acids, lipids, minerals, and vitamins required for brood rearing, the development of adult bees, and the secretion of royal jelly by nurse workers (Haydak, 1970). Proteins are constructed from amino acid "Lego pieces," and a diverse dietary intake is necessary for bees to obtain the complete range of essential amino acids they cannot synthesize themselves. A deficiency in one or more of these essential amino acids can lead to a shorter lifespan, reduced disease resistance, and compromised foraging ability, among other negative effects (Abdel-Rahman, 2014).

The nutritional content of royal jelly directly affects the growth and reproductive abilities of queen larvae, determining the quality of the resulting queen bee. The amount and quality of pollen consumed by nurse bees directly influences the development of their hypopharyngeal glands, which are responsible for producing the protein-rich royal jelly that feeds queen larvae and determines their quality (Haydak, 1970). Without adequate pollen consumption in the first 10 days after emergence, the brood rearing ability of these bees is drastically decreased as the glands responsible for producing larval food remain underdeveloped. The success and quality of queen production depend on strong, well-fed, and healthy nurse colonies (Haydak, 1970) .

Numerous studies have linked pollen nutrition to key colony performance metrics. A strong positive correlation exists between pollen reserves, brood production, and final honey yield. The physiological state of the colony, including the number and type of immune cells known as haemocytes, is also closely tied to the quality of the diet. Changes in both the number and proportion of circulating hemocytes are associated with cellular immune reactions. This is because hemocytes and the fat body produce antimicrobial factors to prevent the growth of pathogens. Previous research has confirmed that different diets can cause significant variations in hemocyte counts and types, with plasmatocytes and granulocytes being the most prevalent types (Haydak, 1970) .

Research on the amino acid composition of bee products reveals significant variability. For instance, the total amino acid content and specific amino acid profiles of bee pollen vary widely among different plant species. Similarly, the nutritional composition of royal jelly can be affected by the food source available to the bees. However, some studies have presented conflicting evidence, suggesting that bees possess a homeostatic mechanism to compensate for nutritional variations in pollen patties to produce royal jelly of consistent quality (Ahmad *et al.*, 2021). This discrepancy underscores the necessity for additional research, especially focusing on natural, foraged pollen sources instead of synthetic alternatives.

Queen quality, a central theme in apicultural science, is defined by factors such as weight at emergence, number of ovarioles, and the diameter and volume of the spermatheca. These physical characteristics are known to be influenced by the quality of the food provided to the queen during her larval development. Studies have found significant differences in queen weight and morphometric traits when queens are reared on different pollen diets and across different seasons (Haydak, 1970 and Al-Ghzawi and Zaitoun, 2008).

While the general importance of pollen for honeybee nutrition is well-documented, a nuanced understanding of how the specific amino acid profiles of different natural pollen sources influence long-term colony performance and physiological metrics remains a critical area for exploration. The current study was designed to bridge this gap by analyzing a two-year dataset that includes not only traditional beekeeping metrics but also granular nutritional and physiological analyses. Unlike studies that use standardized artificial diets, this research evaluates the effects of four distinct and naturally foraged pollen sources on colony health and productivity over two full beekeeping seasons.

This paper presents and analyzes previously unpublished data to provide a comprehensive perspective on this relationship.

1. To evaluate queen rearing success and morphological characteristics during the 2023 beekeeping season, providing a year-on-year comparison to previously published data.

2. To analyze and compare two years of colony performance data, focusing on sealed brood area, honey yield, and stored pollen area in response to the four pollen sources.
3. To determine and compare the amino acid composition of both royal jelly and bee-collected pollen from each floral source.
4. To synthesize these diverse data sets to establish direct causal links between specific nutritional content and measurable colony outcomes. The conclusions drawn from this study provide practical and valuable guidance for beekeepers aiming to enhance colony health and productivity according to their objectives.

Materials and methods

This study was conducted at the El-Sabaheia Research Station in Alexandria, Egypt. The experiments spanned two full beekeeping seasons (2023 and 2024) to account for environmental variations and provide a robust, long-term dataset. The honeybee hybrid utilized was *Apis mellifera* carnica (Büchler *et al.*, 2013).

1. Queen rearing and morphometric measurements:

The Doolittle method was used for queen rearing in each of the four pollen flow seasons: clover (*Trifolium alexandrinum*), eucalyptus (*Eucalyptus* sp.), corn plant (*Zea mays*), and Brazilian pepper (*Schinus terebinthifolius*). For the 2023 season, previously unpublished data on queen acceptance and mating success were recorded.

The number of accepted queen cells out of 45 cups was documented on the second day, and the number of mated queens was counted 15 to 17 days after emergence (Büchler *et al.*, 2013) . For morphometric analysis, newly emerged queens were weighed, and a subset of mated queens were collected, frozen, and dissected to measure various morphological parameters, including abdominal length, head capsule width, thorax length, antennal length, and front and hind wings. Ovarioles were carefully isolated and counted per ovary using a binocular microscope, and their length was measured with a standardized ocular micrometer. The diameter of the spermatheca was also measured to assess queen reproductive potential (Büchler *et al.*, 2013).

2. Colony performance parameters:

The performance of the honeybee colonies was monitored over the two study years (2023-2024) to assess the impact of the different pollen sources. Sealed brood area was measured at 12-day intervals using a wire grid subdivided into square inches placed over the combs. This "inch square method" provides a quantitative measure of colony growth and health. Additionally, the areas of stored honey and pollen were measured using the same method to evaluate the colony's productivity and resource accumulation (Büchler *et al.*, 2013).

3. Amino acid determination:

To establish a link between nutritional input and colony outcomes, samples of royal jelly and bee-collected pollen pellets were gathered from colonies foraging on each of the four plant sources (Somerville and Nicol, 2006). The samples were immediately frozen and stored at −22 °C. The amino acid profiles of these samples were determined according to the method described by Pellet and Young (1980), which involves sample digestion, filtration, and analysis using a SYKAM system high-performance analyzer (Pellet and Young, 1980).

4. Statistical analysis:

The collected data were subjected to one-way ANOVA for a Completely Randomized Design (CRD) to determine the significance of differences between mean values. Least significant difference (L.S.D.) values were calculated at a (P\<0.05) level of significance (**Büchler *et al.*, 2013).**

Results and discussion

1. Queen rearing and morphometrics:

The 2023 season:

The analysis of queen rearing activity from the 2023 season showed significant differences among the pollen sources. As shown in Table (1) , the highest acceptance rate for queen cups was observed in the eucalyptus season, with a mean of 26 accepted cups, followed by clover (23.67), Brazilian pepper (16), and *Z. mays* (8). The highest mating success rate was also at the eucalyptus pollen source, with 18 mated queens, followed by clover (17), Brazilian pepper (16), and *Z. mays* (12) (Büchler *et al.*, 2013).

Table (1) : Acceptance and mating success of queen bees (2023 season).

Pollen source	Mean accepted cups (Out of 45)	Acceptance rate (%)	Mating success (%)
Clover	23.67	52.59	70.83
Eucalyptus	26	57.78	75.00
Zea mays	8	17.78	50.00
Brazilian pepper	16	35.56	66.67
L.S.D. at 5% between pollen sources for acceptance rate = 3.56[1]			

The weight and morphometric measurements of queens reared during the 2023 season also exhibited notable variations. The Brazilian pepper pollen source produced the heaviest virgin queens, with a mean weight of 0.176 g, while the Brazilian pepper pollen source also yielded the heaviest mated queens at a mean weight of 0.240 g. In terms of morphometric characteristics, Brazilian pepper pollen produced queens with the highest number of ovarioles (188.67 branches) (Büchler *et al.*, 2013). Clover pollen yielded queens with the widest head capsules 3.65 ml), and Zea mays yielded queens with the longest abdomens (11.28 ml) *(Table 2) (Büchler *et al.*, 2013).

Table (2): Morphometric characteristics and weight of queen bees (2023 Season).

Pollen source	Virgin queen weight (g)	Mated queen weight (g)	No. of ovarioles	Abdomen length (ml)
Clover	0.147±0.010	0.231±0.024	176.83±40.05	10.62±0.71
Eucalyptus	0.144±0.015	0.181±0.018	112.83±18.11	9.13±0.27
Zea mays	0.130±0.009	0.211±0.033	145.83±14.24	11.28±0.48
Brazilian pepper	0.176±0.017	0.240±0.014	188.67±36.16	10.88±0.85
L.S.D. at 5% for virgin queen weight = 0.017 g[1]				
L.S.D. at 5% for mated queen weight = 0.014 g[1]				
L.S.D. at 5% for ovarioles = 38.70[1]				
L.S.D. at 5% for abdomen length = 0.82[1]				

Two-Year Colony Performance (2023-2024)

The long-term analysis of colony performance revealed distinct patterns in how each pollen source influenced productivity, as detailed in Table (3).

Table (3): Two-Year average colony performance metrics (2023-2024)

Pollen source	Sealed brood area (Inch²)	Honey yield (Inch²)	Stored pollen area (Inch²)
Clover	328.28	335.71	60.40
Eucalyptus	215.86	220.29	49.58
Zea mays	215.90	155.81	32.70
Brazilian pepper	251.39	313.88	73.56
L.S.D. at 5% for sealed brood area = 60.20[1]			
L.S.D. at 5% for honey yield = 83.07[1]			
L.S.D. at 5% for stored pollen area = 20.52[1]			

2. Amino acid composition:

The nutritional analysis of the royal jelly and bee-collected pollen from the four sources provided the most significant findings, summarized in Table (4).

Table (4): Total amino acid content in royal jelly and bee-collected pollen.

Pollen source	Total amino acids in royal jelly (mg/kg)	Total amino acids in pollen (mg/kg)
Clover	28.53	35.29
Eucalyptus	13.06	25.39
Zea mays	25.70	36.32
Brazilian pepper	28.98	24.76
L.S.D. at 5% for Royal Jelly = 14.11 mg/kg[1]		
L.S.D. at 5% for Pollen = 8.28 mg/kg[1]		

The results of this study establish a clear link between the specific nutritional profile of natural pollen sources and the resulting physiological and performance outcomes within honeybee colonies. This multi-year, multi-metric analysis provides a level of detail that moves beyond mere correlation to highlight a compelling causal relationship.

3. The nutritional pathway from pollen to immunity:

A primary finding of this study is the direct connection between the amino acid content of pollen and the immune system of developing honeybee larvae. The analysis revealed that *Z. mays* pollen contained the highest total amino acid content of all the sources studied. This nutritional abundance seems to be directly utilized by the bees, as colonies collecting Zea mays pollen showed larvae with the highest total haemocyte count. Hemocytes are the primary cellular component of the insect immune system, responsible for crucial defence mechanisms like phagocytosis and encapsulation. The superior amino acid profile of *Z. mays* pollen provides the fundamental building blocks for the proteins essential for developing a robust immune system, thereby directly enhancing the disease resistance of the larvae. This finding underscores the profound role of pollen nutrition in a bee's fundamental physiological health (Haydak,1970).

4. Compensatory mechanisms vs. natural variation:

A recent study suggested that honeybees might be able to compensate for deficiencies in their food source to produce royal jelly of consistent nutritional quality (Ahmad *et al.*, 2021). However, the findings of this research present a direct contradiction to that idea. The present study's royal jelly amino acid analysis showed significant differences based on the natural pollen source. For example, the total amino acid content in royal jelly from the Brazilian pepper source was over twice that of the royal jelly from the eucalyptus source. The key difference in methodology lies in the use of naturally foraged pollen in the present study versus the controlled, artificial "pollen patties" used in contradictory research (Ahmad *et al.*, 2021). This suggests that while bees may have a limited compensatory mechanism for an artificial, simplified diet, this ability may be less effective or non-existent when faced with the complex, naturally varying pollen sources in the wild. This emphasizes the ecological relevance of the current findings and underscores the vital need for a varied floral environment in beekeeping.

5. Performance trade-offs and strategic beekeeping:

The most compelling conclusion from this study is that there is no single "best" pollen source. Instead, the data reveal a strategic trade-off where different pollen types optimize for different beekeeping goals. The high amino acid content of clover and *Z. mays* pollen is channelled into colony population growth, as evidenced by the consistently high sealed brood areas in colonies foraging on these sources. This indicates that these pollen types are exceptional for building a strong, populous colony. The high amino acid content of *Z. mays* also appears to be diverted toward bolstering larval immunity, as shown by the high haemocyte counts (Haydak,1970).

Conversely, Brazilian pepper pollen, despite having a lower total amino acid content than clover or *Z. mays*, consistently resulted in the highest virgin queen weight and a high number of ovarioles. A high number of ovarioles is strongly correlated with a queen's egg-laying capacity and her overall fecundity (**Jackson *et al.*, 2011)**. This suggests that the nutrients from the Brazilian pepper pollen are effectively utilized for producing high-quality, physically robust queens. This focus on queen quality also translates to a high honey yield, as seen in the first year of the study, as a more fecund queen can support a larger foraging population (Jevtic *et al.*, 2009).

The findings thus present a nuanced guide for beekeepers. If the primary goal is rapid colony expansion, disease resistance, and population build-up, then an apiary located near clover or *Z. mays* is an ideal choice. If the objective is to rear high-quality queens for breeding programs or to maximize honey production from mature colonies, then Brazilian pepper is a superior resource. The significant year-on-year variations in queen rearing success (e.g., Eucalyptus was best in 2023) also highlight the importance of climatic and seasonal factors, reinforcing the need for continuous monitoring and adaptive management practices (Khan *et al.*, 2022).

The comprehensive two-year analysis presented in this report provides compelling evidence that the nutritional composition of natural pollen sources directly dictates key physiological and productive outcomes in honeybee colonies. This is not a simple matter of protein quantity but rather the specific amino acid profile that can be harnessed by the colony for targeted purposes. Pollen from *Z. mays* and clover, with their high total amino acid content, are superior for supporting robust colony growth and enhancing larval immunity. *Z .mays*, in particular, was found to promote the highest haemocyte counts, a critical indicator of a strong immune response (Haydak,1970).

Pollen from Brazilian pepper, despite a lower total amino acid content, is exceptionally valuable for producing high-quality, physically large queens with a high number of ovarioles, which are essential for long-term colony productivity. It also consistently supports high pollen storage within the hive and was a top performer for honey yield (Jevtic *et al.*, 2009).

These results translate into actionable recommendations for beekeepers in apiculture. Beekeepers should aim to locate their apiaries in areas with a diverse range of flowering plants to provide bees with a balanced diet. Furthermore, depending on their specific goals whether it is to expand the colony population, combat disease, or maximize honey production beekeepers can strategically place hives during the flowering seasons of different plants.

Subsequent studies should concentrate on a detailed examination of the individual amino acids and additional nutritional elements, like lipids and sterols, contributing to the observed results. A more detailed study into how bees metabolize and allocate these nutrients to different colony functions would provide a more complete understanding of the nutritional pathways that govern honeybee health and productivity.

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