SVU-International Journal of Agricultural Sciences
Volume 5 Issue (2) pp.: 29-41, 2023
Print ISSN 2636-3801 | Online ISSN 2636-381X
Doi: 10.21608/svuijas.2023.211972.1286

RESEARCH ARTICLE

Artificial feeding of honey bee colonies by adding nutritional supplements to pollen substitutes and its effect on the development of the hypopharyngeal gland stages of honeybee workers *Apis mellifera* L.

Fatma-Elzahraa R. Mohamed *, K.M. Mohanny and Ghada S. Mohamed

Plant protection Department, Faculty of Agriculture, South Valley University, 83523 Qena, Egypt.

Abstract

This experiment was conducted to study the effect of new alternative supplements on the development of the hypopharyngeal glands in worker honey bees. The main ingredients for each diet (A, B) were inactive yeast, powdered sugar and water. The diet (A) consisted of chamomile, caraway, and sesame powder. While diet (B**)** consisted of anise, Laura paper and ginger. The results showed that the development of the hypopharyngeal glands reached a maximum when feeding on diet A at advanced stages of hypopharyngeal glands, and the lowest development of the pharyngeal gland was in the control bees, while the bees feeding on the diet B had moderate growth in the development of the gland within the same periods of time. It could be concluded that the nutrition of the bees with anise, Laura paper and ginger could play an important role in the development of hypopharyngeal glands according to the nutritional value of the diet.

Keywords: Artificial feeding; Honeybees; Hypopharyngeal glands; Nutritional supplements; Phytogenic

1. Introduction

Nutrition greatly affects the health of the bees and thus on their productivity. Due to the presence of certain times when the bees are not fed due to the scarcity of pasture, pollen supplements are used as a substitute for the protein that the bees get from pollen. The hypopharyngeal glands of worker bees located in the head; consist of thousands of bicellular units composed of a secretory cell and channel cell arranged in groups of about 12 along the length Collection tube. The glands are involved in the production of Royal Jelly fed on queens and larvae. Bees provide nature with priceless gifts. The pollination process of plants is also highlighted, as is the production of honey, Royal Jelly and Propolis. Despite their adaptability and ability to exist in different habitats, climatic conditions and flora, honey bees cannot meet the increasing demands (Aizen and Harder, 2009; Ruttner, 2013; Ahmad *et al*., 2020; Hristov *et al*., 2020; Jagdale *et al*., 2021**)**. Honey bee colony losses are greater today than at any time in recent history (Van Engelsdorp *et al*., 2007, 2008). The causes of colony deaths have been attributed to parasitic mites (Amdam *et al*., 2004) and disease, but the causes of other losses, such as). Perhaps the main factor in colony loss is inadequate nutrition. A constant supply of pollen ensures colony growth as it provides adult bees with protein and stimulates brood production. Food-poor colonies have limited brood rearing (Keller *et al.*, 2005; Mattila and Otis, 2007; DeGrandi-Hoffman *et al*., 2008). Honey bees depend on pollen as a source of proteins, lipids, sterols, vitamins, minerals and some carbohydrates (Todd and Betherick,1942). Nectar is primarily a source of carbohydrates,

.***Corresponding author: Fatma-Elzahraa R. Mohamed**
Email: fatmaalzahraa071@gmail.com
Received: May 10, 2023; Accepted: May 28, 2023;
Published online: May 29, 2023.

but it can contain some amino acids and lipids (Percival, 1961; Baker and Baker, 1975).

Pollen is the most important source of protein for adult bees, and its content and quality influence colony longevity and productivity (Brodschneider and Crailsheim, 2010). The protein content of the pollen can be used as an indicator of the nutritional value and quality of the pollen (Nicolson, 2011). Honey bees collect pollen grains from many different plant species, and the nutrient composition of pollen varies considerably between plant species (Schmidt, 1982; Schmidt 1984) found that bee preferences for pollen type were not based on protein content but on the Cook *et al*. (2003) showed that food preferences can reflect pollen quality. The nutritional value of pollen can be more accurately determined from its amino acid composition than from its total protein content, since its nutritional value decreases with a deficiency in essential amino acids (De Groot, 1953). Under natural conditions, honey bees *Apis mellifera* L. depend solely on the food available in the field for their physiological needs, with nectar serving as a source of energy and pollen serving as a source of proteins, vitamins and minerals (Potts *et al.,* 2016). Proteins make up 10% to 36% of pollen, depending on botanical origin (Estevinho *et al*., 2012). These proteins play an important role in bee nutrition, particularly during the first six days of adult life when worker bees consume large amounts of pollen protein to support the development of the mandibular and pharyngeal glands (Degrandi-Hoffman *et al.,* 2010). The hypopharyngeal glands (HPGs) of lactating bees produce a high-protein food called royal jelly. This food is fed to the queen, larvae, drones and other workers (Crailsheim, 1991). For optimal HPG development, newly formed bees should be fed a protein-rich diet (Kleinschmidt and Kondos 1978; Crailsheim 1990). Pollen consumption has been shown to be positively correlated with glandular development (Crailsheim and Stolberg, 1989; Hrassnigg and Crailsheim, 1998; Corby-Harris *et al.,* 2015). Therefore, HPG growth and development can be seen as important criteria that can be used to assess the suitability of natural pollen feed or protein supplement feed for young bees (Maurizio, 1954; Standifer *et al.*, 1960).

In particular, several studies have examined the structural changes that occur in the larynx as worker bees age and/or take on other tasks (Knecht; Kaatz, 1990; Richter *et al*., 2016). However, few studies have examined gland development in bees based on the food provided to them. Hypopharyngeal glands located in the head of the worker bee, produces a protein-based substance that is responsible for caste differentiation and is also used to feed young larvae, drones and the queen (Feng *et al*., 2009; Kamakura, 2011). HG activation occurs in the presence of protein-rich food (Al-Ghamdi *et al*., 2011). HG size is related to gland production and generally increases from 6 to 18 days of age in nursing mothers (Deseyn and Billen, 2005), Glandular thickening size (Hrassnigg and Crailsheim, 1998; Deseyn and Billen, 2005; Feng and Fang, 2009) or protein content (Knecht and Kaatz, 1990; Heylen *et al*., 2017). The HPG in bees are age-dependent pairs of glands associated with different social behaviors through different secretions (Liu *et al*., 2013; Ueno *et al*., 2015). Little oval structures, which make up each gland, are joined by axial and terminal secretory ducts. Because to the highly efficient secretion, active secretions reach the lower pharynx of the glands by the time new worker bees (6–13 day old bees) attain full development, therefore HPG has primarily been examined in worker bees (Lass and Crailsheim, 1996; Rahman *et al*., 2014). Once the bee begins feeding, these glands begin to deteriorate. Cluster size, which fluctuates with age and reflects workers' age polytheism, is the primary factor influencing HPG activity (Robinson, 1992; Deseyn and Billen, 2005; Johnson, 2010). These glands are also susceptible to various stresses such as starvation, heat, and Varroa infestations, which can lead to a decrease and glandular degeneration (Khalil, 1992; Yousef *et al*., 2014).

Poor pollen may result in inferior weight gain, lower protein or nitrogen content, shorter lifespans, and an incomplete development of the HPG, which in turn results in less Royal Jelly production, which could impede the growth of the larvae and queen. It is essential to assess the availability and nutritional value of specific pollen substitutes and to take into account how this diet may affect the morphological and physiological functions of honey bees of various ages. The aim of the current investigation was to determine the Influence of some pollen substitutes on the development in stages of the hypopharyngeal glands in the honeybee *Apis mellifera* L.

2. Materials and methods

2.1. Honeybee source

The present study was carried out in Plant Protection Department, Faculty of Agriculture, South Valley University during 2021 and 2022. The hybrid of the Carniolan bee race was chosen to start the planned experiment. For the experiment three honeybee colonies of equal strength nearly each colony was fed with twenty-one supplemental diets, supplemental diets were placed in two different seasons. The consumption rates of diets were calculated by the difference between the weight of the diets before and after then choose the best diets depending on the rate of consumption. Number of six diets of the mixture was the highest consumption was chosen. Two groups of these six diets were made; each group was divided into three types, where the selection was made based on the percentage of protein in each of them. Three diets were formed from each of the two groups (A) and (B) in three different proportions.

Three honey bee colonies representing three replicates of each treatment of A, B and Control (C) were made and nine colonies of equal strength were chosen. Diets feeding was provided to the colonies throughout the experimental period for each colony in treatments (A, B), while the control colonies (C) untreated.

2.2. Date experimental period

The beginning of the experiment was from the first of March (1/3) until the end of June (30/5), with an interval of 10 days between the readings during two seasons 2021/2022.

2.3. Components of diets

2.3.1. Pollen substitutes

a. Inactive dry brewer's yeast
b. Can sugar
c. Distilled water

2.3.2. Nutritional supplements

a. Chamomile *Matricaria chamomilla*, Caraway *Carum carvi*, and Sesame *Sesamum indicum*.
b. Anise *Pimpinella anisum*, Laura leaves *Laurus nobilis* and Ginger *Zingiber officinale.*

All nutritional supplements used in the experiment were provided in powder form and mixed with pollen substitutes at the rates mentioned below in the preparation of the diets.

2.4. Diets preparation

Diet (A): Consists of both (Chamomile, Caraway and Sesame) were mixed in equal rates (15 g. of each) and this mixture was added to paste from inactive dry brewer's yeast (45 g.) and sugar (90 g.) and distilled water (90 cm.)

Diet (B): Consists of both (Anise, Laura leaf, and ginger) were mixed in equal rates (5g. of each) and this mixture was added to paste from inactive dry brewer's yeast (30g.) and sugar (90g.) and distilled water (60 cm).

2.5. Place the diets in the beehives

Both first and second treatment replicates (A and B) were received the diets described above continuously and the nutrients placed directly inside the hives on Top bar of combs throughout the experiment period.

2.6. Bee sampling

Samples were taken from nurse bees (25 nurse bees from each colony) randomly at different ages from the middle brood nest. And that was done every ten days by taking samples from the different hives. Several steps were carried out to determine the developmental stages as follows: The bees were taken and immobilized by temporarily placing them in a freezer. The head capsule of each worker was removed (dissected) slightly and gently until the hypopharyngeal glands became observed. Each gland was pulled out gently and placed on a glass slide.

2.7. Morphometric studies

Samples were taken from the workers and the heads were dissected according to the method of Hrassnigg and Crailsheim (1998) in NaCl solution (7.5 g/L) under a stereomicroscope. The HPGs were removed and spread on a glass slide for each bee separately. The external chitinous exoskeleton of the facial region of the head was removed between the compound eyes. An assessment for each gland was carried out to determine different developmental stage according to (Maurizio, 1954). The experiment was conducted from April to May two consecutive seasons.

2.8. Statistical Analysis

The experimental data were analyzed using SAS statistical software (SAS, 2009). Response parameters were analyzed using two ways analysis of variance (ANOVA) according the following model:

$$Yijk = \mu + Dj + Tj + DTk + \varepsilon ijk$$

Where : Yijk was the dependent variable, μ was the overall mean, Dj was the diets, Tj was the effect of times, DTk was the interaction effect between diet and time and εijk was the residual error term.

Treatment means were compared using Duncan's Multiple Range Test, and P values less than 0.001 will be expressed as '<0.001' rather than the actual value.

3. Results and discussion

3.1. Diets and date periods and its effect on the growth of the hypopharyngeal glands of honey bee workers during season 2021

3.1.1. The effect on the growth of stages of hypopharyngeal glands of honey bee workers

The data presented in the Table (1) showed the mean values of the Hypopharyngeal gland stages in honeybees fed with three different diets (A, B), Control (C) and the corresponding p-values and standard error of the mean (SEM) for each stage. The Hypopharyngeal glands of honeybees are responsible for producing Royal Jelly, which is an important food source for the queen bee and developing larvae. The gland's development is a critical process that influences the quality and quantity of Royal Jelly produced, which ultimately affects the overall health and productivity of the bee colony.

The current results revealed that the offered diets A and B significantly improved (P< 0.001) the development of the hypopharyngeal glands at all four stages compared to control group. The mean values of hypopharyngeal glands of honeybees for each diet also varied considerably between development stages. In the first stage of hypopharyngeal glands, diet C had the highest mean value (4.53), which was significantly different from diets A and B. In the second stage of hypopharyngeal glands, diet C still had the highest mean value (7.1), but this time, it was significantly different from all other diets. In the third stage of hypopharyngeal glands, diet A had the highest mean value (9.53), which was significantly different from diet C. Finally, in the fourth stage, diet A had the highest mean value again (10.83), and it was significantly different from diets B and C. It appears that diet A was the most effective in promoting the development of the hypopharyngeal glands in honeybees, particularly in the later stages. This may suggest that the composition of diet A is more suitable for providing the necessary nutrients for the bees to produce high-quality Royal Jelly.

Table 1. Effect of feeding honey bee colonies with diets of pollen supplements and alternatives and date periods on growth of hypopharyngeal glands of workers during 2021.

Treatments	Hypopharyngeal glands stages			
	1st St.	2nd St.	3rd St.	4th St.
Diets				
A	1.90b	2.73c	9.53b	10.83a
B	1.76b	3.80b	10.16a	9.26b
C (control)	4.53a	7.10a	7.50c	5.90c
SEM	0.251	0.249	0.623	0.555
P-value	0.001	0.001	0.001	0.001
Dates				
1(1-Mar)	3.00bc	3.00e	12.67a	6.33d
2(11-Mar)	2.67bc	4.33cd	11.33c	6.67cd
3(21-Mar)	2.33bc	3.67de	11.67bc	7.33cd
4(31-Mar)	2.67bc	5.67ab	9.11d	7.56c
5(10-Apr)	2.44bc	6.00a	7.00e	9.56b
6(20-Apr)	1.33d	4.78bc	12.33ab	6.67cd
7(30-Apr)	2.11cd	4.33cd	11.22c	7.33cd
8(10-May)	3.33b	3.67de	5.00f	13.00a
9(20-May)	3.11bc	4.67bcd	5.00f	12.22a
10(30-May)	4.33a	5.33abc	5.33f	10.00b
SEM	0.210	0.281	0.405	0.471
P-Value	0.001	0.001	0.001	0.001

A The diet (A), B The diet (B), C mean control group and the date ten reading from 1/3 to 30/5/2021.

The presented data shows the mean values of hypopharyngeal gland stages in honey bees for different months during the year. The data also includes the results of statistical analysis, represented by the standard error of the mean (SEM) and the p-value. Overall, it can be observed that the values for the 1st, 2nd, and 3rd stages of hypopharyngeal glands are highest in the first three months, with a peak in the 1st stage in 1st date and a peak in the 2nd and 3rd stages in 3rd date. The values for the 4th stage of hypopharyngeal glands, on the other hand, are highest in 8th date and 9th date. The statistical analysis shows that there is a significant difference (P< 0.001) between the mean values for all stages of hypopharyngeal glands across the different dates, for each stage. These results suggest that the hypopharyngeal gland activity in honey bees is influenced by the dates (time period), and there may be seasonal variations in their development and functions.

3.1.2. Effect of interaction between different treatments and date periods on growth of hypopharyngeal glands

Data in Table 2 shows the interaction between diets and months on the hypopharyngeal gland stages of honey bees. The results indicate that there is a significant interaction effects (P< 0.001) between diets and months in terms of their effect on the hypopharyngeal gland stages. A closer examination of the data shows that some diets have a more significant effect on the hypopharyngeal gland stages than others. For instance, in the 1st stage, diets 1A and 1C show higher values than other diets, while in the second stage, diets 5A and 5C show lower values than others. In the 3rd stage, diets 1A, 1C, and 3B show higher values, while in the 4th stage, diets 1B and 8B show higher values. The data also indicate that there is a significant effect of times on the hypopharyngeal gland stages. For instance, in the 1st stage, the values are highest in 1st date, while in the 2nd stage, the values are highest in 5th date. In the third stage, the values are highest in 3rd date, while in the 4th stage, the values are highest in 8th date. The interaction between diets and dates suggests that different diets have different effects on the hypopharyngeal gland stages at different dates.

Table 2. The effect of interaction between different treatments and the date periods on growth of hypopharyngeal glands in honey bee workers during 2021.

Interaction	Hypopharyngeal glands stages			
	1st St.	2nd St.	3rd St.	4th St.
1A	2	3	14	6
1B	3	1	13	8
1C	4	5	11	5
2A	2	2	11	10
2B	2	5	13	5
2C	4	6	10	5
3A	2	3	10	10
3B	0	5	15	5
3C	5	3	10	7
4A	1	1	9.3	13
4B	2	5	13	5
4C	5	11	5	4
5A	0	4	5	15
5B	3	6	10	6
5C	4	8	6	7
6A	0	2	15	7
6B	3	5	12	5
6C	0	7	10	8
7A	1	2	10	12
7B	0	5	14.67	5
7C	5	6	9	5
8A	3	2	10	10
8B	1	1	2	21
8C	6	8	3	8
9A	3	3	5	14
9B	0	3	4	17.67
9C	6	8	6	5
10A	4	5	6	10
10B	3	2	5	15
10C	6	9	5	5
SEM	0.577	0.577	0.577	0.577
P-Value	0.001	0.001	0.001	0.001

For instance, in the first stage, diets 1A and 1C have a stronger effect at 1st time than at other times. Similarly, in the second stage, diet 5C has a stronger effect at 3rd time than at other times. Overall, the results suggest that both diets and times have a significant effect on the hypopharyngeal gland stages of honey bees.

These results confirmed previous research on honey bee colonies conducted by (Maurizio, 1954; Haydak, 1957; El-kloely, 1966; kropacova *et al*., 1970; Landiren and Regfina, 1977; Abou-lila, 1990; Sgymas, 1994; Sherif *et al*., 2022) which demonstrated the significance of protein nutrition for the growth of the HPG. Their 5-day-old acini were larger than those of newly emerged bees because these bees consumed protein-rich diets shortly following emerging in order to appropriately develop their HPG, and for the bees, the acini got smaller after reaching their maximum size between 2 and 9 days (Hagedorn and Moeller, 1968). For the bees, the acini became smaller after reaching their maximum size between 2 and 9 days. This confirms earlier findings made in cages or colonies (Altaye *et al*., 2010). The preparation of bees for guarding tasks, which require venom for colony defense, can be explained by age-related increases in AG sac volumes (Nenchev, 2003). These findings imply that there may be seasonal fluctuations in the growth and function of the hypopharyngeal glands, which are regulated by the diets of various phytogenic substances consumed at various times. (Abdel-Rahman *et al*., 2021) have evaluated how the hypopharyngeal gland changed as workers ages and diets changed. This explained the idea that feeding has a great influence on how hypopharyngeal gland develops according to diet nutritional values.

3.2. Diets and date periods and its effect on the growth of the hypopharyngeal glands of honey bee workers during season 2022

3.2.1. The effect on the growth of stages of hypopharyngeal glands of honey bee workers

According to data in Table (3), the development of the hypopharyngeal glands is greatly influenced by the type and composition of the diets. The diet A showed significantly lower values in the first and second stages of gland development compared to diets B and C, indicating that this diet may have a negative impact on the early stages of gland development. However, in the third and fourth stages, diet A had significantly higher values than the other two diets, suggesting that it may have a positive impact on the later stages of gland development. Furthermore, diet B showed relatively consistent values across all stages of gland development, except for the second stage where it had significantly lower values compared to diet A. This finding suggests that diet B may not have a major impact on the development of the hypopharyngeal glands. On the other hand, the control group showed significantly higher values in the first and second stages of gland development compared to diets A and B, indicating that it may have a positive impact on the early stages of gland development. However, in the third and fourth stages, control group had significantly lower values compared to diets A and B, suggesting that it may have a negative impact on the later stages of gland development. Also, the data presented in the table showed the effect of different months on the hypopharyngeal gland stages of bees. The results indicate that there is a significant variation in the gland stages among the different months.

Table 3. Effect of feeding honey bee colonies with diets of pollen supplements and alternatives and date periods on growth of hypopharyngeal glands in workers during 2022.

Treatments	Hypopharyngeal glands stages			
	1st St.	2nd St.	3rd St.	4th St.
Diet				
A	2.10b	2093c	10.10a	9.87a
B	2.50b	4.20b	10.00a	8.30b
C (control)	3.78a	7.15a	9.22b	4.85c
SEM	0.232	0.283	0.460	0.554
P-value	0.001	0.001	0.001	0.001
Dates				
1(1-Mar)	2.00cd	4.67bcd	12.33a	6.00e
2(11-Mar)	3.30ab	5.67ab	11.22b	4.78f
3(21-Mar)	3abc	3.67de	10.33bc	8.00c
4(31-Mar)	1.70d	5.78a	10.33bc	7.11cd
5(10-Apr)	3.00abc	4.33cd	12.33a	5.33ef
6(20-Apr)	2.00cd	4.67bcd	11.33b	7.00d
7(30-Apr)	3.67a	5.67ab	10.00c	5.67ef
8(10-May)	2.33bcd	5.33abc	6.33e	11.00ab
9(20-May)	3.67a	4.67bcd	5.00f	11.00a
10(30-May)	3.17abc	3.17e	8.00d	10.00b
SEM	0.185	0.301	0.342	0.415
P-Value	0.0001	0.0001	0.0001	0.0001

A The diet (A) , B The diet (B) , C mean control group and The date ten reading from 1/3 to 30/5/2022.

The first hypopharyngeal gland stage showed significant differences among the months, with the highest value recorded in the fifth month and the lowest in the fourth month.

The second hypopharyngeal gland stage also showed significant differences among the months, with the highest value recorded in the 5th date and the lowest in the 3rd date. The highest value was obtained in the 1st date and the lowest in the eighth for the third hypopharyngeal gland stage, showing substantial changes across the dates. The fourth hypopharyngeal gland stage also showed significant differences among the dates, with the highest value recorded in the 8th date and the lowest in the 2nd date.

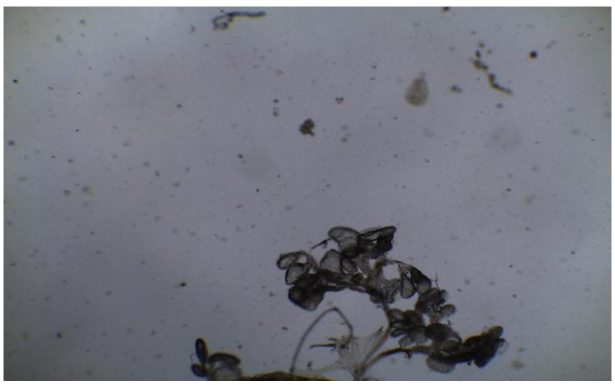
3.2.2. Effect of interaction between different treatments and date periods on growth of hypopharyngeal glands

Data in Table (4) showed the interaction between diets and months on the hypopharyngeal gland stages of honey bees. The results indicate that there is a significant interaction between diets and months in terms of their effect on the hypopharyngeal gland stages. The examination of the data revealed that some diets are significantly more effective than others at influencing the stages of the hypopharyngeal gland.

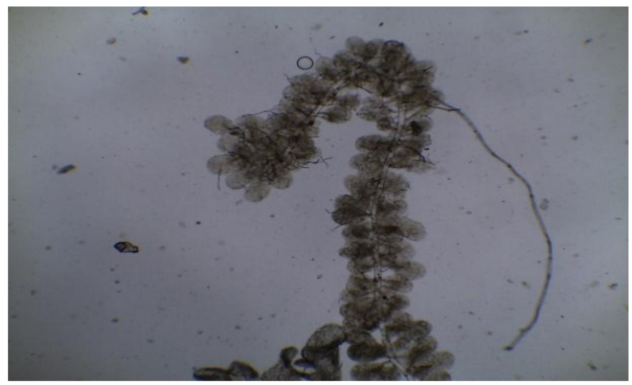
Table 4. The interaction between the treatments and the date periods on the development of the hypopharyngeal glands stages of honey bee workers during the years 2022.

Interaction	Hypopharyngeal glands stages			
	1st St.	2nd St.	3rd St.	4th St.
1A	3	4	13	5
1B	1	6	8	10
1C	2	4	16	3
2A	1	3	10	11
2B	3	4	15	3
2C	6	10	8	0.33
3A	3	2	967	11
3B	3	7	9	6
3C	3	2	13	7
4A	2	0.33	11	11.67
4B	3	6	10	6
4C	0.33	11	10	3.67
5A	2	2	15	6
5B	2	4	12	7
5C	5	7	10	3
6A	1	4	9	11
6B	4	2	14	5
6C	1	8	11	5
7A	4	5	9	7
7B	2	6	11	6
7C	5	6	10	4
8A	1	4	10	10
8B	1	3	6	15
8C	5	9	3	8
9A	2	2	6	15
9B	3	2	5	15
9C	6	10	4	5
10A	2	3	9	11
10B	3	2	10	10
10C	4	4	6	9.50
SEM	0.577	0.577	0.577	0.577
P-Value	0.001	0.001	0.001	0.001

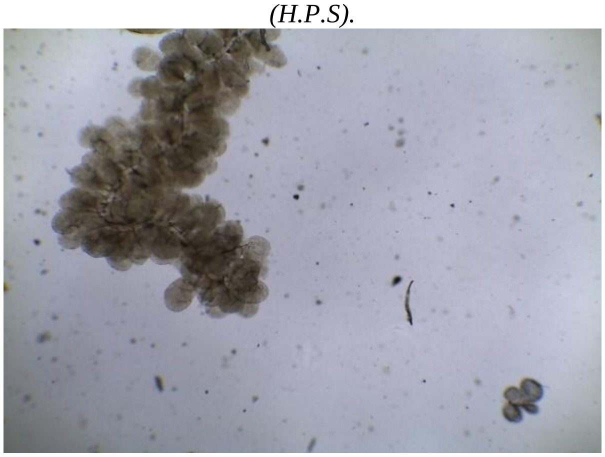
For instance, in the first stage, diets 9C and 6C show higher values than other diets, while in the second stage, diets 2C and 4C show lower values than others. In the third stage, diets 1C, 5A, and 2B show higher values, while in the fourth stage, diets 9A and 9B show higher values. The data also indicate that there is a significant effect of dates on the hypopharyngeal gland stages. For instance, the values were highest in the first stage at 9th and 6th dates, whereas they were highest in the second stage at 2nd and 9th dates. In the third stage, 1st date had the greatest values, while 8th and 9th date in the fourth stage had the highest values. The relationship between diets and dates showed that various diets have varying impacts on the stages of the hypopharyngeal gland at certain times. Overall, the findings support previous findings from studies on honey bee colonies, including those by Maurizio (1954), Haydak (1957), El-kloely (1966), Kropacova *et al* (1970), Landiren and Regfina (1977), Abou-lila (1990), Sgymas (1994) and Zahra and Talal (2008), Zhao Hongyu *et al*. (2019) and which found that a protein-rich diet is crucial for the development of the hypopharyngeal gland.



Stage 1. *Lateral ducts of the hypopharyngeal gland (H.P.S).*



Stage 2. *Lateral ducts of the H.P.S started to vanish while lobes began to enlarge.*



Stage 3. *The lobes appeared to be larger and more crowded with the main duct of the H.P.S still obvious.*

Stage 4. *The main duct disappeared while lobes are clearly crowded.*

Illustrative photos under the microscope of the stages of development of the hypopharyngeal glands in honey bee workers.

4. Conclusion

To be able to produce Royal Jelly in high quantities and quality, we must think about breeding technology and nutrition technology. Royal jelly glands are Flexible member of young honey bee workers and highly responsive to the needs of the colony. In general, the first diets (A) Chamomile, Caraway, and Sesame substitute diets were superior to the second diets (B) Anise, Laura paper, Ginger and are recommended for addition to the diets of honeybee colonies.

Authors' Contributions

All authors are contributed in this research.

Funding

There is no funding for this research.

Institutional Review Board Statement

All Institutional Review Board Statements are confirmed and approved.

Data Availability Statement

Data presented in this study are available on fair request from the respective author.

Ethics Approval and Consent to Participate

Not applicable

Consent for Publication

Not applicable.

Conflicts of Interest

The authors disclosed no conflict of interest starting from the conduct of the study, data analysis, and writing until the publication of this research work

5. References

Abdel-Rahman, M. F., Sayed, R. Q., Moustafa, A. M., Mahbob, M. A. E. M. (2021). 'The Effect of An Alternative Diet Fermented by Bee Bread Microorganisms on Hypopharyngeal Glands Development and Acini Size of Honey Bee Workers, (*Apis mellifera* L.)', *Egyptian Academic Journal of Biological Sciences. A, Entomology,* 14(1), pp. 227-241.

Abou-Lila, M.M.S. (1990). *'Physiological studies on the honey bee',* P.h.D. Thesis faculty of Agric., Alex. University,Egypt.

Ahmad, S., Campos, M.G., Fratini, F., Altaye, S.Z., Li, J. (2020). 'New insights into the biological and pharmaceutical properties of royal jelly', *Int. J. Mol. Sci.*, 21 (2), pp. 382.

Aizen, M.A., Harder, L.D. (2009). 'The global stock of domesticated honey bees is growing slower than agricultural demand for pollination', *Curr. Biol.,* 19, pp.915–918.

Al-Ghamdi, A.A., Al-Khaibari, A.M., Omar, M.O.M. (2011). 'Effect of honey bee race and worker age on development and histological structure of hypopharyngeal glands of honey bee', *Saudi Journal of Biological Sciences,* 18، pp.113–116.

Altaye, S.Z., Pirk, C.W., Crewe, R.M. and Nicolson, S.W. (2010). 'Convergence of carbohydrate-biased targets in caged worker honeybees fed different protein sources', *J. Exp. Biol.*, 213(19): pp. 3311–3318

Amdam, G.V., Hartfelder, K., Norberg, K., Hagen, A., Omholt, S.W. (2004). 'Altered physiology in worker honey bees (Hymenoptera: Apidae) infested with the mite Varroa destructor (Acari: Varroidae): a factor in colony loss during overwintering', *Journal of Economic Entomology,* 97, pp.741–747.

Baker, H.G., Baker, I. (1975). *'Studies of nectar constitution and pollinator-plant coevolution',* In: Gilbert, L.E., Raven, P.H. (Eds.). Coevolution of Animals and Plants. University of Texas Press, Austin, Texas, pp. 100–140.

Brodschneider, R., Crailsheim, K. (2010). 'Nutrition and health in honey bees', *Apidologie,* 41, pp. 278–294.

Corby-Harris, V., Meador, C.A., Snyder, L.A., Schwan, M. R., Maes, P., Jones, B. M., Walton, A., Anderson, K. E. (2015). 'Transcriptional, translational, and physiological signatures of undernourished honey bees *Apis mellifera* suggest a role for hormonal factors in hypopharyngeal gland degradation', *J. Insect Physiol.,* 85, pp. 65–75.

Crailsheim, K. (1990). 'The protein balance of the honey bee worker', *Apidologie,* 21, pp. 417–429.

Crailsheim, K. (1991). 'Interadult feeding of jelly in honeybee (*Apis mellifera* L.) colonies', *J. Comp. Physiol. B.,* 161, pp. 55– 60.

Crailsheim, K., Stolberg, E. (1989). 'Influence of diet, age and colony condition upon intestinal proteolytic 434 E. Omar et al. activity and size of the hypopharyngeal glands in the honeybee (*Apis mellifera* L.)', *J Insect Physiol.,* 35, pp.595–602.

De Groot, A.P. (1953). 'Protein and amino acid requirements of the honeybee (*Apis mellifica* L.)', *Physiol. Comp. Oecol.*, 3, pp. 1–83.

Degrandi-Hoffman, G., Chen, Y., Huang, E., Huang, M. H. (2010). 'The effect of diet on protein concentration, hypopharyngeal gland development and virus load in worker honey bees (*Apis mellifera* L.)', *Journal of Insect Physiology,* 56(9), pp. 1184–1191. https://doi.org/10.1016/j.jinsphys.2010.03.017

DeGrandi-Hoffman, G., Wardell, G., Ahumada-Segura, F., Rinderer, T., Danka, R., Pettis, J. (2008). 'Comparisons of pollen substitute diets for honeybees: consumption rates by colonies and effects on brood and adult populations', *Journal of Apicultural Research*, 47, pp.265–270.

Deseyn, J., Billen, J. (2005). 'Age-dependent morphology and ultrastructure of the hypopharyngeal gland of *Apis mellifera* workers (Hymenoptera, Apidae)', *Apidologie, Springer Verlag,* 36 (1), pp.49-57.

EL-Kloely, M.K. (1966). *'Some studies on the glands of the adult honey bees Apis mellifera',* M.Se. Thesis, fac., Agric. Alex. Univ.Egypt.

Estevinho, L. M., Rodrigues, S., Pereira, A. P., Feas, X. (2012). 'Portuguese bee pollen: Palynological study, nutritional and microbiological evaluation', *International Journal of Food Science and Technology*, 47(2), pp. 429–435. https://doi.org/ 10.1111/j.1365-2621.2011. 02859.x

Feng, M., Fang, Y., Li, J. (2009). 'Proteomic analysis of honeybee worker (*Apis mellifera*) hypopharyngeal gland development', *BMC Genomics,* 10, pp. 645- 672.

Hagedorn, H., Moeller, F. (1968). 'Effect of the age of pollen used in pollen supplements on their nutritive value for the honeybee. I. Effect on thoracic weight, development of hypopharyngeal glands and brood rearing', *J. Apic. Res.,* 7, pp. 89–95.

Haydak, M.A. (1957). 'Changes with age in the appearance of some internal organs of the honeybee', *Bee world.,* 38(8), pp.197-207.

Heylen, K., Gobin, B., Arckens, L., Huybrechts, R., Billen, J. (2017). 'The effects of four crop protection products on the morphology and ultrastructure', *Frontiers in Zoology,* 14(22), pp. 13-27.

Hrassnigg, N., Crailsheim, K. (1998). 'Adaptation of hypopharyngeal gland development to the brood status of honeybee (*Apis mellifera* L.) colonies', *J. Insect Physiol.,* 44, pp. 929–939.

Hristov, P., Shumkova, R., Palova, N., Neov, B. (2020). 'Factors associated with honey bee colony losses', a mini-review. *Veterin. Sci.,* 7, pp.166. https:// doi.org/10.1051/apido:2004068

Jagdale Y.D., Mahale S.V., Zohra B., Nayik, G.A., Dar, A.H., Khan, K. A., Abdi, G., Karabagias, I. K. (2021). 'Nutritional Profile and Potential Health Benefits of Super Foods', A Review. *Sustainability,* 13 (16), pp. 9240.

Johnson, B.R. (2010). 'Division of labor in honey bees', form, function, and proximate mechanisms. *Behavioral Ecology and Sociobiology,* 64, pp.305–316. https://doi.org/10.1007/ s00265-009-0874-7

Kamakura, M. (2011). 'Royalactin induces queen differentiation in honeybees', Nature, 473, pp.478–483.

Keller, I., Fluri, P., Imdorf, I. (2005). 'Pollen nutrition and colony development in honey bees – part II', *Bee World,* 86, pp.27–34.

Khalil, S.I.Y. (1992). 'Effect of Varroa infestation on the mortality rate, body weight and development of hypopharyngeal glands of honey bee workers', *Zagazig Journal of Agricultural Research,* 19, pp. 901–908.

Kleinschmidt, G., Kondos, A. (1978). 'Effect of dietary protein on colony performance', *Aust. Beekeep.,* 79, pp. 251–25760.

Knecht, D., Kaatz, H.H. (1990). 'Patterns of larval food production by hypopharyngeal glands in adult worker honey bees’, *Apidologie*, 21, pp.457–468.

Kropacova, S., Hasibachova H., Pavel J. (1970). ‘Changes in the hypopharyngeal glandes of bee during the development of colonies’, *Apis. Abst.*,23(1) pp. 109.

Landim, C.D., Regina L.M.S.D. (1977). ‘Degenerative structure in the hypopharyngeal glandsof workers of *Apis mellifera* L’, *Biol. Abst.*, 65(12), pp. 6864.

Lass, A., Crailsheim, K. (1996). ‘Influence of age and caging upon protein metabolism, hypopharyngeal glands and trophallactic behavior in the honey bee *Apis mellifera* L’, *In- sectes Sociaux*, 43, pp. 347–358. https://doi.org/10.1007/bf01258408

Liu, Z., Ji, T., Yin, L., Shen, J., Shen, F., Chen, G. (2013). ‘Transcriptome sequencing analysis reveals the regulation of the hypopharyngeal glands in the honey bee, *Apis mellifera* Carnica Pollmann’, *Plos ONE, 8, pp. e81001* https://doi.org/10.1371/journal.pone.0081001

Mattila, H.R., Otis, G.W. (2007). ‘Dwindling pollen resources trigger the transition to broodless populations of long-lived honeybees each autumn’, *Ecological Entomology*, 32, pp.496–500.

Maurizio, A. (1954). ‘Pollen nutrition and vital processes in the honey bee’, *Landw. Jb. Schweiz*, 68, pp. 115–182.

Nenchev, P. (2003). ‘Seasonal changes in the venom gland of the honey bee *Apis mellifera* L.’, *in Bulgaria. Zhivotnovdni Nauki*, 40 (5), pp. 87–88.

Nicolson, S.W. (2011). ‘Bee food: the chemistry and nutritional value of nectar, pollen and mixtures of the two’, Review article. *Afr. Zool.*, 46, pp.197–204.

Percival, M.S. (1961). ‘Types of nectar in angiosperms’, *New Phytologist*, 60, pp.235–281.

Potts, S. G., Imperatriz-Fonseca, V., Ngo, H.T., Aizen, M. A., Biesmeijer, J. C., Breeze, T. D., Dicks, L. V., Garibaldi, L. A., Hill, R., Settele, J., Vanbergen, A. J. (2016). ‘Safeguarding pollinators and their values to human well-being’, *Nature*, 540(7632), pp. 220–229. https://doi.org/10.1038/nature20588

Rahman, S., ftangkhiew, I., Hajong, S.R. (2014). ‘Hypopharyngeal gland activity in task-specific workers under brood and broodless conditions in *Apis cerana* Indica (Fab.)’, *Journal of Apicultural Science*, 58, pp. 59–70. https://doi.org/10.2478/jas-2014-0022 21.

Robinson. G.E. (1992). ‘Regulation of division of labor in insect societies’, *Annual Review of En- tomology*, 37, pp. 637–665. https://doi.org/10.1146/annurev.en.37.010192.003225

Roulston, T., Cane, J.H. (2000). ‘Pollen nutritional content and digestibility for animals’, *Plant Syst. Evol.*, 222, pp.187–209.

Ruttner, F. (2013). ‘Biogeography and taxonomy of honeybees’, *Springer Science and Business Media.*

SAS, Institute. (2009). *‘User's Guide: Statistics. Version 9.2.’,* SAS Institute, Inc., Cary, NC, USA.

Schmidt, J.O. (1982). ‘Pollen foraging preferences of honey bees’, *Southw. Entomol.*, 7, pp.255–259.

Schmidt, J.O. (1984). ‘Feeding preferences of *Apis mellifera* L. (Hymenoptera: Apidae): individual versus mixed pollen species’, *J. Kans. Entomol. Soc.*, 57, pp.323–327.

Sgymas, B. (1994). ‘Evaluation of the nutritive value of pollen substitutes of honey bees *Apis mellifera* L.’, *Rocgniki- Akademii-Rolnicgej -w- Pognaniu, -Rogprawy-Naukowa.*, No 2, pp .56-68.

Sherif, A. S. F., Marwa, B. M. G., Konper, H. M. A. (2022). ‘Royal jelly quantity means (Mg/Cups) under different diets and bars level within two successive years’, *Egyptian Journal of Plant Protection Research Institute*, 5(2), pp.184-191. http://www.ejppri.eg.net/pdf/v5n2/8.pdf

Standifer, L., McCaughey, W., Todd, F., Kemmerer, A. (1960). 'Relative availability of various proteins to the honey bee', *Ann. Entomol. Soc. Am.*, 53, pp.618–625.

Todd, F.E., Betherick, O. (1942). 'The composition of pollens', *Journal of Economic Entomology* 35, pp. 312–317.

Ueno, T., Takeuchi, H., Kawasaki, K., Kubo, T. (2015). 'Changes in the gene expression profiles of the hypopharyngeal gland of worker honey bees in association with worker behavior and hormonal factors', *Plos ONE*. 10, pp. e0130206. https://doi.org/10.1371/journal.pone.0130206

Van Engelsdorp, D., Hayes Jr., J., Underwood, R., Pettis, J. (2008). 'A survey of honey bee colony losses in the U.S.', fall 2007 to spring 2008" *PLoS ONE*, 3, pp. e4071.

Van Engelsdorp, D., Underwood, R., Caron, D., Hayes Jr., J. (2007). 'An estimate of managed colony losses in the winter of 2006–2007: a report commissioned by the Apiary Inspectors of America', *American Bee Journal*, 147, pp. 599–603.

Yousef, S.I., Basheir, Z.M., Teleb, S.S., Ibraheem, E.E. (2014). 'Effect of Varroa infestation on the morphological and histological structure of the hypopharyngeal glands of *Apis mellifera* workers', *Journal of American Science*. 10, pp. 69–78.

Zahra, A., Talal, M. (2008). 'Impact of pollen supplements and vitamins on the development of hypopharyngeal glands and on brood area in honey bees', *Journal of Apicultural Science*, 52(2), pp 5-12.

Zhao, H., Xue, X., Wang, H. (2019). 'Effect of pollen substitute on hypopharyngeal gland development and vitellogenin gene expression in *Apis mellifera* L.', *Journal of Apicultural Research.*, 58(1) pp. 86-94.