A SUPPLEMENTAL PROTEIN DIET SIGNIFICANTLY IMPROVES MORPHOMETRIC AND REPRODUCTIVE TRAITS OF HONEY BEE QUEENS DURING LATE WINTER

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Abstract. A harsh winter poses a critical challenge for honey bee colonies, leading to substantial losses. This work aimed to improve the morphometric and reproductive features of the queens reared in the late winter. This study examined the morphometric and reproductive characteristics of queens reared in colonies supplemented with a high-protein diet during late winter, compared to unfed colonies. The queens were artificially reared in queen-less colonies supplemented with a high-protein diet (diet 1) consisting of brewer's yeast (30%) + defatted soybean flour (30%) + skimmed milk (10%) + cotton honey (20%) + sugar powder (10%) and also were reared in other unfed colonies. The assessment of 4 pollen substitutes during winter showed significant superiority of diet 1 in colony performance. Therefore, diet 1 was selected to feed colonies used for queen rearing. Workers from the fed colonies exhibited significantly higher of body weight, 2nd wax mirror area, number of acinus/mm, acini area, and the mandibular gland area compared to workers from the unfed colonies. Compared to the unfed colonies, the fed queen-rearing colonies showed significantly higher acceptance percentage, number of emerged queens, royal jelly (RJ) yield/queen cell, size of a queen cell, and the morphometric and reproductive features of the queen. It can be concluded that feeding colonies a protein-rich diet improves queen morphometric and reproductive features and colony productivity during winter.
Keywords: *Apis mellifera, Brewer's yeast, environment, season, spermatheca*

Introduction

The queen bee manages all reproductive tasks, while facultatively sterile workers handle other colony functions, including queen care. Because of its function of laying eggs, the queen bee is the most important individual in the colony. Worker bees rear new queens in three cases: 1) replacement of dead or missing queens (emergencies), 2) replacement of old, inferior, sick, or injured queens (supersedure), and 3) in swarms (Taha et al., 2024). Queen rearing is influenced by many factors, including the age of grafted larvae (Dhaliwal et al., 2018; Okuyan and Akyol, 2018; Cengiz et al., 2019; Ustadi, 2022), rearing techniques (Dhaliwal et al., 2018; Adgaba et al., 2019), honey bee subspecies (Elenany and Abdallah, 2016), rearing season (Elenany and Abdallah, 2016; Shawer et al., 2021), proteinaceous feeding of colonies (Cengiz et al., 2019), and comb age (Taha et al., 2024).

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Beekeepers can rear the honey bee queens in limited numbers for their use from the emergency queen cells. The artificial queen-rearing method has been commercially used for rearing honey bee queens in comparatively larger numbers (Dhaliwal et al., 2018; Adgaba et al., 2019). The body size of the queen has been reported as an indicator of the quality of the queen (Kahya et al., 2008; Delaney et al., 2011; Shawer et al., 2021; Taha et al., 2024). However, the queen's body size has been influenced by the availability of nectar and pollen flora (Gabka et al., 2014), queen-rearing techniques (Adgaba et al., 2019), supplemental feeding (Cengiz et al., 2019), and comb age (Taha et al., 2024).

The assessment of the morphometric and reproductive features of a queen can be used to predict its fitness and subsequently colony fitness (Delaney et al., 2011; De Souza et al., 2019). Several studies have suggested that the quality of the queen is related to the genotype, rearing season, colony strength, rearing method and technic, feed supplying, larval age, the number of grafted larvae, and comb age (Elenany and Abdallah, 2016; Adgaba et al., 2019; Taha et al., 2024). The quality of a queen can be determined by measuring her morphological and reproductive characteristics (Kahya et al., 2008; Tarpy and Mayer, 2009; Gabka et al., 2014; Taha et al., 2024).

Feeding bee colonies is a widely applied technology. However, applications of feed mixtures are a conventional way of improving the conditions of bee colonies (Irandoust and Ebadi, 2013; Taha, 2015b; De Souza et al., 2019; Kumari and Kumar, 2020; Topal et al., 2022; Elwakeil et al., 2025). The present study focus on using proteinaceous feeding on improving the early queen rearing during late winter.

Egypt is considered the most significant beekeeping nation in both the Arab world and Africa (Al-Ghamdi et al., 2016). There are more than 1,576,542 bee colonies in Egypt (Fao, 2016), and about 1,025,000 package bees are yearly exported (Kamel et al., 2021). A large number of bee colonies have annually been lost during cold winters. To compensate for this loss, a large number of package bees and bee nuclei are produced in late winter and early spring, and queen rearing is needed to lead such colonies. For good queen rearing, the colonies should be provided with dietary components rich in protein, carbohydrates, mineral elements, and vitamins (Avni et al., 2009; Estegamat and Gholami, 2010). The quality of the grafted larvae and, consequently, the quality of the queen bees have greatly improved when additional nutrients are given to the rearing colonies (Mahbobi et al., 2012). We may hypothesize that supplemental high-protein feeding may raise the number of reared queens in late winter and improve the characteristics related to the queen's quality. Also, reaching to feed formula nearly similar to bee pollen may provide a bee colony with the requirements of its nutritional feeding. The present study aimed to investigate the impact of supplemental high-protein feeding on early queen rearing during late winter and the morphometric and reproductive characteristics of the queens.

Materials and method

Study area

This work was carried out at the apiary of the College of Agriculture, Kafrelsheikh University (31° 5′ 54″ N, 30° 57′ 0″ E), Kafrelsheikh, Egypt, during the late winter of 2023.

Experimental colonies

On 15 November 2022, the ectoparasite varroa, *Varroa destractor* (Anderson and Trueman) was controlled in all apiary colonies using the method of oxalic acid

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vaporization. On 21 December 2022, 51 Carniolan hybrid honey bee (*Apis mellifera carnica* Pollmann × *A. m. lamarkii* Cockerell) colonies of the same population size (7 combs for each), brood area, and storage food and headed by sister open-mated queens were used in this experiment. The colonies were divided into five groups (10 colonies for each), and the remaining colony was later used as a breeder colony. The first 4 groups were fed high-protein diets; meanwhile, the 5th group was left without proteinaceous feeding (control). All the colonies were fed with one liter of sugar syrup (1: 1) every 7 days until the end of the experiment.

Experimental diets

A high-protein diet (diet 1) consisting of 30% brewer's yeast (*Saccharomyces cerevisiae*) + 30% defatted soybean (*Glycine max*) flour + 10% skimmed milk + 20% cotton (*Gossypium barbadense*) honey + 10% sugar powder was compared with other diets in a preliminary experiment during winter 2023. The other diets consist of 25% brewer's yeast + 35% defatted soybean flour + 10% skimmed milk + 20% cotton honey + 10% sugar powder (diet 2), 20% brewer's yeast + 40% defatted soybean flour + 20% cotton honey + 10% sugar powder (diet 3), and 15% brewer's yeast + 45% defatted soybean flour + 10% skimmed milk + 20% cotton honey + 10% sugar powder (diet 4). The ingredients of each diet were mixed to make a paste, and placed on waxed paper to prevent moisture loss. The paste was offered freshly to the colony directly over the brood nest at 100 g/colony/week. Diet 1 exhibited superiority during the preliminary experiment, so it was used in the current study.

Proximate analysis of the experimental diet (diet 1)

A sample of the selected diet (diet 1) was used to determine the chemical composition of the diet. Moisture content was determined by drying a sample of 2 g at 105°C until the constant weight. Crude protein content, ash content, and crude lipid content were determined using AOAC standard methods (AOAC, 2000). A sample of 0.5 g was used to determine the nitrogen content using the Kjeldahl method, and the 6.25 factor was used to convert the total nitrogen to crude protein. A sample of 2 g was used to extract lipid content using the Soxhlet apparatus, petroleum ether (40–60°C) for 6 h. A sample of 2 g was incinerated in a muffle furnace at 550°C to determine ash content. Available carbohydrates were calculated by subtracting crude proteins, moisture, ash, crude fibers, and crude lipids from 100. All determinations were conducted in triplicate.

Colony activities

The activities of a colony were determined during the period from 21 December 2022 until 9 March 2023. The numbers of foragers and pollen foragers were counted as the total number of incoming workers without and with pollen loads, respectively to a colony within 1 min. The counts were performed at 1200–1300 h once a week. The areas (square inches) of stored pollen and worker and drone sealed broods were measured in 12-day intervals using a plastic sheet divided into square inches, and the overall period areas were counted. The colony population size was estimated as the number of bees in a colony by counting the number of combs covered with bees/colony at the end of the experimental period.

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Queen rearing procedure

The assessment of 4 pollen substitutes preliminary experiments during winter resulted in the superiority of colonies fed diet 1 in colony performance. Therefore, diet 1 was selected to feed colonies used for queen rearing. On 9 March 2023, colonies fed diet 1 and control colonies were used in queen rearing experiment. The breeder colony was used for providing larvae for grafting, and the 20 colonies were used as queen cell builder colonies (QCPC). The queens were removed from QCPC brood chambers to be queen-less. Forty wax cups were grafted with larvae aged 24 h, and the cups were introduced into the colony. After 72 h of grafting, the percentage of accepted queen cells was calculated, and royal jelly (RJ) was harvested (Al-Kahtani and Taha, 2020a) from 5 queen cell cups in previously weighed small bottles. The mean yield of RJ (mg/queen cell) was calculated by subtraction (Taha and Al-Kahtani, 2020a). The accepted queen cells in each colony were recounted after 10 days of grafting to estimate the number of the ripped queen cells, then they were caged on a comb. The number of emerging queens was recorded. The queen cell size was measured using distilled water and a medical syringe by calculating the amount of water used for filling the queen cell. The depth of the queen cell was measured using a digital caliper.

Morphometric and reproductive features

Ten newly emerged queens from each colony were weighed to determine their fresh body weight using a TN-Series digital electronic scale (20 g × 0.01 mg). A Photoshop software program (Adobe Photoshop CS5) was used to measure the antenna length, head area, mandibular gland area, fore and hind wings areas, the number of hamuli on the right hind wing, abdomen length, and diameter using the Scan Photo technique (Shawer et al., 2021). The 10 newly emerged queens from each colony were dissected to determine the number of ovarioles/ovary, ovariole length and diameter, and diameter of spermatheca. The size of the spermatheca was measured (*Eq. 1*).

$$\text{Size} = 4/3\ \pi r^3 \qquad \text{(Eq.1)}$$

where $\pi = 3.14$ and r = 1/2 diameter of spermatheca.

Royal jelly and wax glands of nurse worker

Ten nurse workers were collected from the middle queen cell bar of each colony. The workers were weighed to determine their fresh body weight using a TN-Series digital electronic scale. The heads of workers were dissected to remove the mandibular and hypopharyngeal glands. The number of acinus/mm of the hypopharyngeal gland, acini area, and mandibular gland area were determined. Also, the 2nd wax gland was dissected to determine the area of the wax mirror using the Scan Photo technique.

Meteorological factors

Some meteorological factors (relative humidity, air temperature, rainfall, and wind velocity) during the experimental period were gotten from meteorological station of Rice Research and Training Center, Sakha, Kafrelsheikh, Egypt (*Table 1*).

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Table 1. *The mean values of some meteorological factors in Kafrelsheikh province, Egypt during experimental months in 2022/2023*

Months	Air temperature (°C)		Relative humidity (%)	Wind velocity (km/hr.)	Precipitation/rainfall (mm)
	Maximum	Minimum			
December 2022	23.07	14.6	72.09	2.04	2.00
January 2023	17.72	9.78	69.83	2.60	3.00
February 2023	19.29	10.78	60.7	3.39	4.00
March 2023	20.10	13.49	59.51	4.10	3.50

Statistical analysis

The differences between the fed and unfed colonies for the studied aspects were tested by one-way analysis of variance (ANOVA) via the PROC GLM function in SAS version 9.1 (SAS Institute, 2003). Tukey's HSD post-hoc test was used to compare the treatment means. Pearson correlation coefficients between queen cell size, RJ yield/queen cells, body weight, and morphometric characteristics were determined.

Result

The chemical analysis of the diet 1 (*Table 2*) shows the contents of crude protein (24.01%), moisture (19.35%), crude lipids (5.31%), crude fiber (4.64%), ash (4.06%), and nitrogen-free extract (42.63%).

Table 2. *Chemical composition of diet 1*

Component	Concentration (%)
Moisture	19.35
Crude protein	24.01
Crude lipids	5.31
Crude fiber	4.64
Ash	4.06
NFE	42.63

NFE = nitrogen-free extract (available carbohydrates)

Foraging activity, gathering and storing pollen, worker and drone-sealed brood areas, and colony population size if fed colonies significantly ($P < 0.01$) improved during the winter season (*Fig. 1*). The colonies fed diet 1 [brewer's yeast (30%) + defatted soybean flour (30%) + skimmed milk (10%) + cotton honey (20%) + sugar powder (10%)] recorded the highest significant ($P < 0.01$) levels of foraging activity, gathering and storing pollen, worker and drone sealed brood areas, and colony population size in comparison with colonies fed other diets or the unfed colonies.

The statistical analysis of the obtained data shows that the body weight, 2nd wax mirror area, number of acinus/mm, acini area, and the mandibular gland area of nurse workers from the fed colonies were significantly ($P < 0.05$) higher than those in workers from the unfed colonies (*Table 3*).

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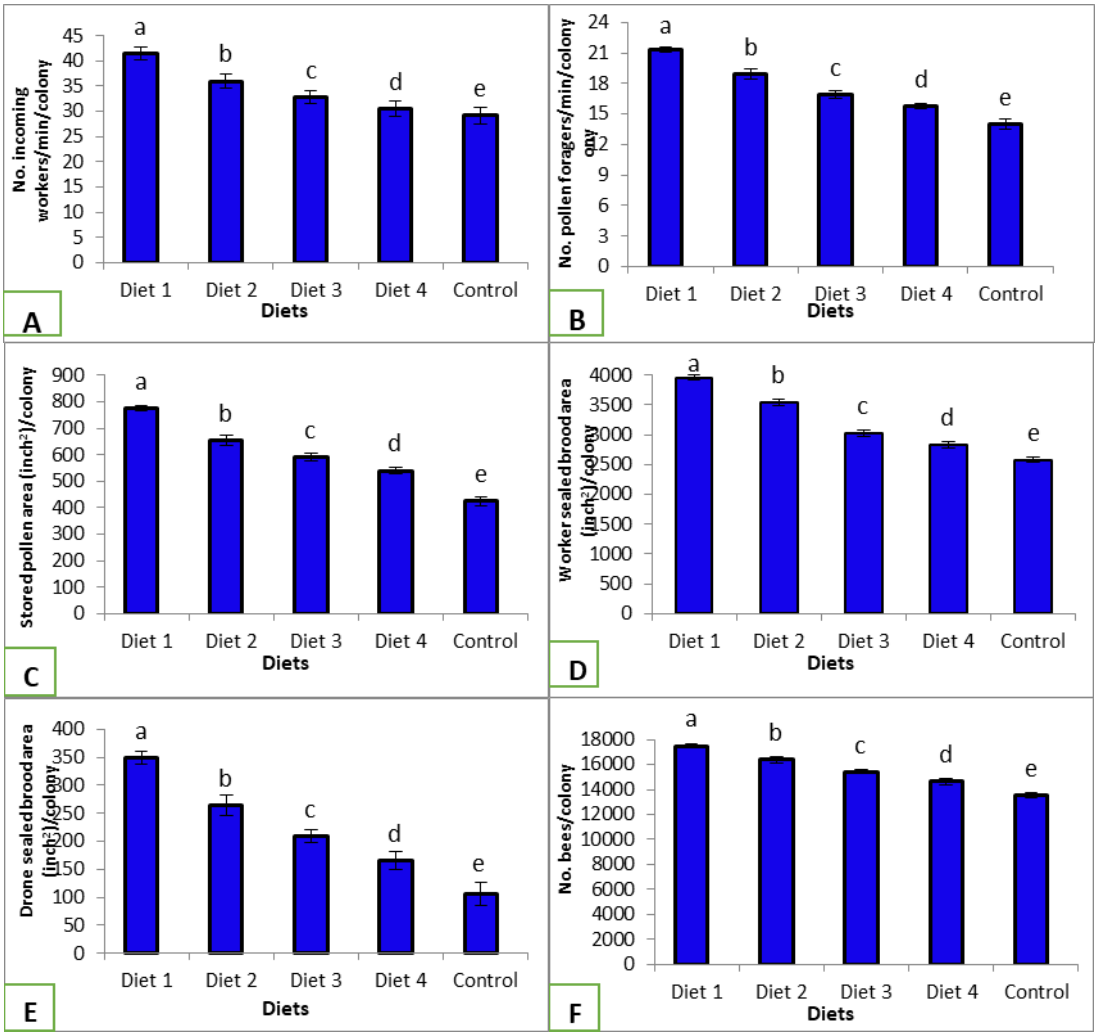

Figure 1. *Impact of feeding on number of incoming workers/min/colony (A), number of pollen foragers/min/colony (B), stored pollen area (inch^2)/colony (C), worker sealed brood area (D), drone sealed brood area (inch^2)/colony (E), and number of bees/colony (F) during winter season. Different letters above the bars indicate a significant difference ($P < 0.01$) according to Tukey's test*

Table 3. *Effect of feeding a high-protein diet on worker body weight and the areas of wax and RJ glands*

Parameters	Fed colonies	Unfed colonies	Significance
Worker body weight (mg)	106.33 ± 1.96	94.00 ± 3.45	**
2nd wax mirror area (mm^2)	2.96 ± 0.04	2.72 ± 0.08	*
No. acinus/mm	47.22 ± 0.62	34.00 ± 0.30	**
Acini area (mm^2)	0.049 ± 0.002	0.038 ± 0.003	*
Mandibular gland area (mm^2)	1.44 ± 0.03	1.26 ± 0.06	*

The values are the mean ± standard error. ** and * indicate $P < 0.01$ and $P < 0.05$, respectively

The obtained data statistically show that the acceptance rate, amount of RJ/queen cell, the number of ripped queen cells, number of emerged queens, queen cell size,

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queen cell depth, and queen cell diameter were substantially ($P < 0.01$) improved by feeding the colonies (*Table 4*). The body weight and the 12 examined morphometric and reproductive characteristics of honey bee queens were significantly ($P < 0.01$) affected by feed supply (*Table 4*). Compared to those from the unfed colonies, queens from fed colonies exhibited significantly higher values of body weight, antenna length, head area, area of the mandibular gland, fore wing area, hind wing area, number of hamuli, abdomen length, abdomen diameter, number of ovarioles/ovary, ovariole length, ovariole diameter, and size of spermatheca.

Table 4. *Impact of feeding a high-protein diet on queen rearing, RJ production, body weight and the morphometric and reproductive characteristics of the newly emerged queen of the honey bee*

Parameters	Fed colonies	Unfed colonies	Significance
No. grafting queen cells	40.00 ± 0.00	40 ± 0.00	NS
No. accepted queen cells	36.22 ± 0.76	28.30 ± 0.96	**
Acceptance rate (%)	90.55 ± 2.15	70.75 ± 3.26	**
Weight of royal jelly (mg)/cell	403.33 ± 9.75	345.50 ± 10.60	**
*No. accepted queen cells	31.22 ± 0.76	23.30 ± 0.96	**
No. ripped queen cells	31.00 ± 0.72	21.80 ± 0.88	**
No. emerged queens	29.66 ± 0.85	21.30 ± 0.87	**
Queen cell size (cm^3)	0.88 ± 0.01	0.79 ± 0.02	**
Queen cell depth (mm)	20.22 ± 0.32	17.80 ± 0.44	**
Body weight (mg)	184.66 ± 2.46	161.60 ± 1.24	**
Length of the antenna (mm)	4.29 ± 0.03	4.01 ± 0.02	**
Area of the head (mm^2)	11.40 ± 0.06	10.79 ± 0.05	**
Area of mandibular gland (mm^2)	3.43 ± 0.05	2.95 ± 0.04	**
Area of the fore wing (mm^2)	18.52 ± 0.09	17.61 ± 0.07	**
Area of the hind wing (mm^2)	10.67 ± 0.05	10.20 ± 0.04	**
No. hamuli	21.44 ± 0.24	18.80 ± 0.39	**
Abdomen length (mm)	11.75 ± 0.16	10.16 ± 0.13	**
Abdomen diameter (mm)	4.97 ± 0.02	4.75 ± 0.02	**
No. ovarioles/ovary	175.14 ± 2.92	145.74 ± 2.33	**
Length of ovariole (mm)	6.03 ± 0.11	4.95 ± 0.09	**
Diameter of ovariole (mm)	0.066 ± 0.002	0.050 ± 0.001	**
Size of spermatheca (mm^3)	0.256 ± 0.003	0.220 ± 0.002	**

The values are the mean ± standard error. ** and NS indicate $P < 0.01$ and $P > 0.05$ (insignificant differences), respectively

*No. accepted queen cells after using 5 queen cells to determine RJ yield/queen cell on the 3rd day post-grafting

As shown in *Table 5*, the queen cell size, queen cell depth, and RJ yield/queen cell were significantly correlated (r = 0.43 to 0.66, $P < 0.05$ to 0.001) with the queen's body weight and all tested characteristics. The queen's body weight was significantly correlated (r = 0.77 to 0.84, $P < 0.001$) with all tested characteristics. The number of ovarioles/ovary, ovariole length, ovariole diameter, and size of the spermatheca were significantly correlated (r = 0.71 to 0.99, $P < 0.001$) with body weight and all morphometric characteristics.

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Table 5. *Pearson correlation coefficients for queen cell, RJ/queen cell, body weight and morphometric traits of the queen of honey bee*

Characteristics	Queen cell size	Queen cell depth	RJ yield/queen cell	Body weight	Antenna length	Mandibular gland area	Head area	Fore wing area	Hind wing area	No. hamuli	Abdomen length	Abdomen length	Size of spermatheca	No. ovarioles/ ovary	Ovariole length
Queen cell size															
Queen cell depth	0.52**														
RJ yield/queen cell	0.58**	0.63**													
Body weight	0.66**	0.63**	0.51**												
Antenna length	0.53**	0.54**	0.48*	0.77**											
Mandibular gland area	0.53**	0.54**	0.48*	0.77**	0.99**										
Head area	0.53**	0.54**	0.48*	0.77**	0.99**	0.99**									
Fore wing area	0.53**	0.54**	0.48*	0.77**	0.99**	0.99**	0.99**								
Hind wing area	0.53**	0.54**	0.48*	0.77**	0.99**	0.99**	0.99**	0.99**							
No. hamuli	0.42*	0.54**	0.42*	0.81**	0.71**	0.71**	0.71**	0.71**	0.71**						
Abdomen length	0.54**	0.57**	0.53**	0.84**	0.96**	0.96**	0.96**	0.96**	0.96**	0.80**					
Abdomen diameter	0.53**	0.54**	0.48*	0.77**	0.99**	0.99**	0.99**	0.99**	0.99**	0.71**	0.96**				
Size of spermatheca	0.53**	0.54**	0.48*	0.77**	0.99**	0.99**	0.99**	0.99**	0.99**	0.71**	0.96**	0.99**			
No. ovarioles/ovary	0.53**	0.54**	0.48*	0.77**	0.99**	0.99**	0.99**	0.99**	0.99**	0.71**	0.96**	0.99**	0.99**		
Ovariole length	0.54**	0.57**	0.54**	0.84**	0.96**	0.96**	0.96**	0.96**	0.96**	0.80**	0.99**	0.96**	0.96**	0.96**	
Ovariole diameter	0.53**	0.54**	0.51**	0.77**	0.99**	0.99**	0.99**	0.99**	0.99**	0.71**	0.96**	0.99**	0.99**	0.99**	0.96**

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed)

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Discussion

The contents of crude protein, moisture, crude lipids, crude fiber, ash, and nitrogen-free extract in the experimental diet were nearly similar to those in bee pollen for crude protein, crude lipids, ash, and crude fiber (Taha, 2015; Al-Kahtani and Taha, 2020b; Pascoal et al., 2023).

The growth and vitality of a colony may be impacted by the good feeding resulting from the abundance of pollen and nectar flora (Helal et al., 2003; Keller et al., 2005; Taha and Al-Kahtani, 2013, 2019, 2020b; Shawer et al., 2003, 2021). Because of this, beekeepers can provide their colonies with pollen substitutes or supplements to make up for pollen shortages. However, these supplements must be tasty and nourishing for bees (Mattila and Otis, 2006; Taha, 2015). In the current study, a significant increase in foraging activity, stored pollen, and brood rearing was observed after feeding the colonies on diet 1. In comparison with the unfed colonies, the colonies fed on a protein diet in winter recorded significant higher levels of the number of forager bees (42.34, 23.52, 12.71, and 8.17%), number of pollen foragers (52.06, 34.95, 20.18, and 12.34%), stored pollen area (82.28, 53.55, 39.21, and 26.75%), worker-sealed brood area (53.47, 37.25, 17.69, and 9.70), drone-sealed brood area (228.47, 148.47, 96.70, and 55.29%), and colony population size (28.91, 20.80, 13.57, and 7.82%) for diet 1, diet 2, diet 3, diet 4, respectively. In comparison with the colonies fed diet 1, the colonies fed diet 2, diet 3, and diet 4, in winter recorded lower levels of the number of forager bees (15.23, 26.29, and 31.59%), number of pollen foragers (12.68, 26.53, and 35.36%), stored pollen area (18.68, 30.91, and 43.78%), worker-sealed brood area (11.82, 30.40, and 39.90), drone-sealed brood area (32.20, 66.98, and 111.51%), and colony population size (6.72, 13.51, and 19.65%) for diet 2, diet 3, diet 4, respectively. These results agree with Pande and Karnatak (2014)'s results. According to Kumari and Kumar (2020), Topal et al. (2022), and Elwakeil et al. (2025), the colonies provided with pollen substitutes or supplements reared more workers brood and had a greater bee population size than the unfed colonies. The amount of brewer's yeast was decreased from 30% in diet 1 to 20% in diet 4 which caused the differences among the diets fed colonies.

From the obtained results, body size, RJ and wax glands of workers in colonies fed a high-protein diet exhibited significantly higher values in comparison with the unfed colonies. The worker's body weight has reportedly correlated with morphometrics linked with the colony productivity (Al-Kahtani and Taha, 2014, 2021) which influences the queen rearing. Feeding the colonies on a high-protein diet leads to a good development of hypopharyngeal and mandibular glands in nurse workers that secret RJ and feed larvae well, resulting in large workers with well-developed glands that play an important role in RJ production and queen rearing. In this context, Kumari and Kumar (2021) noticed that pollen consumption during the nursing phase of honey bee workers was positively linked with the extent of gland growth and RJ production. Meanwhile, bees fed sugar syrup alone resulted in smaller hypopharyngeal glands which secreted RJ with lower protein contents (DeGrandi-Hoffman et al., 2010). The lack of essential proteins needed for constructing the body's organs and glands is the cause of the slight workers' body weight gain when a colony is given sugar syrup as a protein-free diet (Irandoust and Ebadi, 2013).

Previous studies have shown a positive correlation between supplemental feeding during queen rearing and larval acceptance percentage (Gamal Eldin et al., 2018;

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Cengiz et al., 2019) where it has improved the acceptance rate of the grafted larvae and fitness features of the reared queens (Eremia et al., 2014). In the current study, the acceptance rate was increased by 27.98% and the number of emerged queens increased by 39.25% in queen-rearing colonies when fed on a high-protein-content diet. The brewer's yeast in the diet raises protein content that activates the mandibular and hypopharyngeal glands which secret RJ fed to the larvae. These findings are consistent with those of Hammad (2007) who observed that colonies fed on a liquid yeast diet (*Candida troicalis*) at 25% produced much more RJ than control colonies. In this study, the cell size and depth in colonies fed on a high-protein diet were significantly higher than those in the unfed colonies. These results agree with those of Zaghloul et al. (2017) who reported that the fed colonies had more queen cells with the longest depth.

In the current study, all colonies were in the same location, so the availability of nectar and pollen was not varied, also, the grafted larvae were from one colony and of the same age, so the differences should be related to the feed supplementation. In comparison to the newly emerged queen from the unfed colonies, the newly emerged queens from fed colonies showed a significant increase (14.27%) in their body weight and we suggest this increase was attributed to the variations in queen cell size that were positively influenced by feed supply. Here we detected a significant positive correlation ($r = 0.66$, $P < 0.001$) between the queen body weight and queen cell size. A similar correlation has been obtained by Taha et al. (2024). In comparison with the unfed colonies, Cengiz et al. (2019) have reported a considerable increase in queen body weight from the fed colonies.

The improvement of body weight, head and thorax appendages, and abdomen length of the honey bee queens may support their productivity (Okuyan and Akyol, 2018; Taha et al., 2024). The obtained results show that feeding colonies on a high-protein diet significantly improved most of the morphological characteristics of the queens. In comparison with the unfed colonies, the fed colonies exhibited a significant increase in antenna length (6.98%), area of the head (5.65%), mandibular gland area (16.27%), fore wing area (5.17%), hind wing area (4.61%), number of hamuli (14.04%), abdomen length (15.65%), and abdomen diameter (4.00%). The obtained results are in agreement with those of Mahbobi et al. (2012) who reported that supplemental feeding significantly improved most of the morphological features of the queens. In addition, Gamal Eldin et al. (2018) noticed that the maximum length of the queen's abdomen was obtained from colonies fed on a corn gluten diet. The queen's abdomen can be used as an indicator of the quality of the queen. Here we detected significant positive correlations ($r = 0.97–0.99$, $P < 0.001$) between abdomen length and diameter and the number of ovarioles/ovary, length and diameter of ovariole, and size of the spermatheca.

The reproductive characteristics including the number of ovarioles/ovary, length and diameter of ovariole, and size of spermatheca were significantly higher in the fed colonies. Compared to those from the unfed colonies, the newly emerged queens from the fed colonies exhibited a significant increase in the number of ovarioles/ovary (20.17%), ovariole length (21.82%), ovariole diameter (32.00%), and spermathecal size (16.36%). These increments may be related to body weight ($r = 0.77–0.85$). The obtained data confirm the findings of Shawer et al. (2007), and Taha et al. (2024) who observed significant positive correlations between body size and the number of ovarioles/ovary, ovariole length, ovariole diameter, and the size of spermatheca. The current findings agree with those obtained by Cengiz et al. (2009), for the size of the spermatheca, and Elaidy et al. (2010) for ovariole length.

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Conclusions

It can be concluded that feeding queen cell builder colonies on a high-protein diet consisting of brewer's yeast (30%) + defatted soybean flour (30%) + skimmed milk (10%) + cotton honey (20%) + sugar powder (10%) significantly improved the worker body size, hypopharyngeal glands development, areas of the mandibular gland and 2nd wax mirror, the number of accepted queen cells, royal jelly yield/queen cell, the number of emerged queens, body size, queen morphometric characteristics, and reproductive quality including the number of ovarioles/ovary, length and diameter of ovariole, and the size of the spermatheca. The queen's body size can be used as an indicator of the queen's quality. Feeding colonies on a high-protein diet nearly similar to bee pollen in its components can increase the outcome of bee colonies.

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REFERENCES

[1] Adgaba, N., Al-Ghamdi, A., Tadesse, Y., Alsarhan, R., Single, A., Mohammed, S., Khan, Kh. A. (2019): The responses of *Apis mellifera jemenitica* to different artificial queen rearing techniques. – Saudi Journal of Biological Sciences 26: 1649-1654.

[2] Al-Ghamdi, A. A., Alsharhi, M. M., Abou Shaara, H. F. (2016): Current status of beekeeping in the Arabian countries and urgent needs for its development inferred from a socioeconomic analysis. – Asian Journal of Agricultural Research 10: 87-98.

[3] Al-Kahtani, S. N., Taha, E.-K. A. (2014): Morphometric studies on dwarf honey bee *Apis florea* F. workers in Saudi Arabia. – Journal of Apicultural Science 58: 127-134.

[4] Al-Kahtani, S. N., Taha, E.-K. A. (2020a): Post grafting time significantly influences royal jelly yield and content of macro and trace elements. – Plos One 15: e0238751.

[5] Al-Kahtani, S. N., Taha, E.-K. A. (2020b): Seasonal variations in nutritional composition of honey bee pollen loads. – Journal of the Kansas Entomological Society 93: 105.

[6] Al-Kahtani, S. N., Taha, E.-K. A. (2021): Morphometric study of Yemeni (*Apis mellifera jemenitica*) and Carniolan (*A. m. carnica*) honey bee workers in Saudi Arabia. – Plos One 16: e0247262.

[7] AOAC (2000): Official Methods of Analysis. 17th Ed. – Association of Official Analytical Chemists, Washington, DC.

[8] Avni, D., Dag, A., Shafir, S. (2009): The effect of surface area of pollen patties fed to honey bee (*Apis mellifera*) colonies on their consumption, brood production and honey yields. – Journal of Apicultural Research 48: 23-28.

[9] Cengiz, M., Emsen, B., Dodoloğlu, A. (2009): Some characteristics of queen bees (*Apis mellifera* L.) rearing in queen right and queenless colonies. – Journal of Animal and Veterinary Advances 8: 1083-1085.

[10] Cengiz, M., Yazici, K., Arslan, S. (2019): The effect of the supplemental feeding of queen rearing colonies on the reproductive characteristics of queen bees (*Apis mellifera* L.) Reared from egg and different old of larvae. – Kafkas Universitesi Veteriner Fakultesi Dergisi 25: 849-855.

[11] 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: 1184-1191.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_25272540

[12] Delaney, D. A., Keller, J. J., Caren, J. R., Tarpy, D. R. (2011): The physical, insemination, and reproductive quality of honey bee queens (*Apis mellifera* L.). – Apidologie 42: 1-13.
[13] De Souza, D. A., De, Huang, M. H., Tarpy, D. R. (2019): Experimental improvement of honey bee (*Apis mellifera* L.) queen quality through nutritional and hormonal supplementation. – Apidologie 50: 14-27.
[14] Dhaliwal, N. K., Singh, J., Chhuneja, P. K. (2018): Effect of rearing method, age of brood and queenliness of cell-builder colony on weight of *Apis mellifera* Linnaeus queen bees. – Journal of Entomology and Zoology Studies 7: 1260-1262.
[15] Elaidy, W. K., Ebeid, A. A., Fathy, H. M., Salama, S. A. (2010): Effect of some food additives on workers and queens of honey bee (*Apis mellifera* L.). – Journal of Plant Protection and Pathology 1: 1013-1021.
[16] Elenany, Y. E., bdallah, A. E. (2016): Rearing honey bee queens in, *Apis mellifera* L. colonies during the activity season of oriental wasps *Vespa orientalis* L. – Journal of Agricultural Technology 12: 667-674.
[17] Elwakeil, N. M., Hassanein, Z. A. E., Taha, R., Al-Kahtani, S. N., Aljabr, A. M., Taha, E.- K. A. (2025): A comparative assessment of three pollen substitutes for honey bee (*Apis mellifera* L.) colonies during winter and spring. – Polish Journal of Environmental Studies https://doi.org/10.15244/pjoes/188232.
[18] Eremia, N., Zagareanu, A., Modvală, S. (2014): Use of additive in bee feeding at queens' growing. – Scientific Papers. Series D. Animal Science 57: 189-195.
[19] Estegamat, O., Gholami, M. (2010): Acceptance of queen transplantation in honey bee colonies influenced with artificial feeding (syrup, nectar or pollen). – Academic Journal of Entomology 3: 52-54.
[20] FAO (2016): FAOSTAT. – Food and Agriculture Organization of the United Nations, Rome.
[21] Gabka, A. J., Zajdel, B., Skorupka, M., Ostaszewska, T., Kamaszewski, M. (2014): Effect of honey flow on acceptance of bee eggs at different age in rearing colonies. – Med. Weter 70(12): 760.
[22] Gamal Eldin, N. K., Ebeid, A. A., Sallam, A. M., Basuny, N. K. (2018): Effect of pollen supplements and substitutes on honey bee queen ovaries and worker hypopharyngeal glands. – Journal of Plant Protection and Pathology 9: 83-91.
[23] Hammad, H. A. (2007): Effect of stimulative feeding with pollen substitutes on the development and production of honey bee colonies. – M.Sc. Thesis, Fac. Agric. Cairo Univ.
[24] Helal, R. M., El-Dakhakhni, T. N., Shawer, M. B., Taha, E.- K. A. (2003): Effect of moving the apiaries on activity of honey bee colonies. 2- Flight activity, gathering of nectar and sugar concentration contents and honey. – Journal of Agricultural Research Tanta University 29: 268−282.
[25] Irandoust, H., Ebadi, R. (2013): Nutritional effects of high protein feeds on growth, development, performance and overwintering of honey bee (*Apis mellifera* L.). – International Journal of Advanced Biological and Biomedical Research 1: 601-613.
[26] Kahya, Y., Gençer, H. V., Woyke, J. (2008): Weight at emergence of honey bee (*Apis mellifera caucasica*) queens and its effect on live weights at the pre and post mating periods. – Journal of Apicultural Research 47: 118-125.
[27] Kamel, S. M., Shafey, A. S., Shebl, M. A., Mahmoud, M. F. (2021): Evaluation of colony parameters for queen rearing under arid ecosystem conditions. – Journal of Applied Plant Protection 10: 69-76.
[28] Keller, I., Fluri, P., Imdorf, A. (2005): Pollen nutrition and colony development in honey bees: part 1. – Bee world 86: 3-10.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_25272540

[29] Kumari, I., Kumar, R. (2020): Pollen substitute diet for *Apis mellifera*: consumption and effects on colony parameters in sub-tropical Himalaya. – Indian Journal of Agricultural Research 54: 147-153.
[30] Kumari, I., Kumar, R. (2021): Effect of stimulative diets fed to honey bees on the acinal size of hypopharyngeal gland in worker honey bees (*Apis mellifera*) during scarce period in western Himalayas. – Uttar Pradesh Journal of Zoology 42: 85-94.
[31] Mahbobi, A., Farshineh-Adl, M., Woyke, J., Abbasi, S. (2012): Effects of the age of grafted larvae and the effects of supplemental feeding on some morphological characteristics of Iranian queen honey bees (*Apis mellifera meda* Skorikov, 1929). – Journal of Apicultural Science 56: 93-98.
[32] Mattila, H. R., Otis, G. W. (2006): Influence of pollen diet in spring on development of honey bee (Hymenoptera: Apidae) colonies. – Journal of Economic Entomology 99: 604-613.
[33] Okuyan, S., Akyol, E. (2018): The effect of age and number of grafted larvae on some physical characteristics of queen bees and acceptance rate of queen bee cell. – Turkish Journal of Agriculture-Food Science and Technology 6: 1556-1561.
[34] Pande, R., Karnatak, A. K. (2014): Germinated pulses as a pollen substitute for dearth period management of honey bee colonies. – Current Biotica 8: 142-150.
[35] Pascoal, A., Chambó, É. D., Estevinho, L. M. (2023): Botanical origin, physicochemical characterization, and antioxidant activity of bee pollen samples from the northeast of Portugal. – Journal of Apicultural Research 62(4): 898-908.
[36] SAS Institute (2003): SAS/STAT User's Guide Release 9.1. – SAS Institute Inc. Cary, NC.
[37] Shawer, M. B., El-Dakhakhni, N. M., Helal, R. M., Taha, E.- K. A. (2003): Effect of moving the apiaries on activity of honey bee colonies. 1- Gathering and storing pollen, brood rearing and wax secretion. – Journal of Agricultural Research Tanta University 29: 250-267.
[38] Shawer, M. B., Taha, E.- K. A., Helal, R. M., El-Dakhakhni, T. N. (2007): Effect of nectar and pollen flora on rearing of honey bee queens. – National Conference of Environment, Protection and Pollution, Faculty of Science Qassim University, Saudi Arabia, 18-20 March.
[39] Shawer, M. B., Taha, E.- K. A., Mousa, K. M., Khan, Kh., Ibrahim, S., Hassan, S., Elnabawy, E. M. (2021): Seasonal variations of colony activities linked to morphometric and glands characterizations of hybrid Carniolan honey bee (*Apis mellifera carnica* Pollmann) workers. – Journal of King Saud University - Science 33(6): 101543.
[40] Taha, E.-K. A. (2015a): Chemical composition and amounts of mineral elements in honeybee-collected pollen in relation to botanical origin. – Journal of Apicultural Science 59(1): 75-81.
[41] Taha, E.-K. A. (2015b): The impact of feeding certain pollen substitutes on maintaining the strength and productivity of honey bee colonies (*Apis mellifera* L.). – Bulletin of Entomological Society of Egypt, Econ. Ser. 41: 63-74.
[42] Taha, E.-K. A., Al-Kahtani, S. N. (2013): Relationship between population size and productivity of honey bee colonies. – Journal of Entomology 10: 163-169.
[43] Taha, E.-K. A., Al-Kahtani, S. N. (2019): Comparison of the activity and productivity of Carniolan (*Apis mellifera carnica* Pollmann) and Yemeni (*Apis mellifera jemenitica* Ruttner) subspecies under environmental conditions of the Al-Ahsa oasis of eastern Saudi Arabia. – Saudi Journal of Biological Sciences 26: 681-687.
[44] Taha, E.-K. A., Al-Kahtani, S. N. (2020a): Effect of harvest time on royal jelly yield and chemical composition. – Journal of the Kansas Entomological Society 93: 132-139.
[45] Taha, E.-K. A., Al-Kahtani, S. N. (2020b): The relationship between comb age and performance of honey bee (*Apis mellifera*) colonies. – Saudi Journal of Biological Sciences 27: 30-34.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_25272540

[46] Taha, E.-K. A., Rakha, O. M., Elnabawy, S. M., Hassan, M. M., Shawer, D. M. B. (2021): Comb age significantly influences the productivity of the honey bee (*Apis mellifera*) colony. – Journal of King Saud University-Science 33: 101436.
[47] Taha, E.-K. A., Shawer, M. B., Taha, R., Elashmawy, A., Gaber, S., Mousa, K. (2024): Comb age significantly influences the emergency queen rearing, morphometric and reproductive characteristics of the queens. – Journal of Apicultural Research 1-8. https://doi.org/10.1080/00218839.2024.2336376.
[48] Tarpy, D. R., Mayer, M. K. (2009): The effects of size and reproductive quality on the outcomes of duels between honey bee queens (*Apis mellifera* L.). – Ethology Ecology and Evolution 21: 147-153.
[49] Topal, E., M˘arg˘aoan, R., Bay, V., Takma, Ç., Yücel, B., Oskay, D., Düz, G., Acar, S., Köso ˘glu, M. (2022): The effect of supplementary feeding with different pollens in autumn on colony development under natural environment and in vitro lifespan of honey bees. – Insects 13: 588.
[50] Ustadi, Ikhsan, N., Junus, M., Suyadi (2022): Improving the larvae acceptance rate and morphometric characteristics of queen *Apis cerana javana* by grafting larvae at different ages. – Journal of Biological Diversity 23: 166-170.
[51] Zaghloul, A. O., El-Sayed, N. A., Hassona, N. M., Mourad, A. K., Abdel-Razek, B. A. (2017): Enhancement of honey production of *Apis mellifera* L. colonies in Egypt. – Alexandria Science Exchange Journal 38: 426-432.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_25272540