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Determination of some Effects of Different Feeding Treatments on Honeybee Drones (*Apis Mellifera* l.).

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Abstract

Honeybee drones are known to only have a reproductive function. In the current study, the drone'sactivities as affected by feeding with pollen substitutes were studied during two successive years 2020/2021 and 2021/2022. The effect of seven pollen substitutes patties on broad rearing and body weight (larvae and adult) as well as fertility (semen volume and sperm count) of *Apis mellifera* droneswas studied. Changes in these activities across months and seasonsof the two years of study observed. During the autumn and summer the bee drones showed highly broad rearingboth years. Whereas, during September the honeybee colonies were found to be more active in consumingall used patties than other months in both years. Finally, regarding the drone's fertility, the patty contained glycerol was the best one for all fertility parameters, semen volume was 1.28 mL, sperm count/ mL was 2.77 ($\times10^{9}$)and sperm count/ one drone was 3.10 ($\times10^{6}$)which confirm our hypothesis that the artificial feeding with pollen substitute may affect the drone's activities.

Keywords: Honeybee, drone's rearing, drone's weight, drone's fertility.

1. Introduction

Honeybee drones have an important role in the reproduction of honeybee colonies during the mating season. Drones exit the hive and conduct orientation flights to find congregation areas for mating. During these flights, drones may lose the way to return their colony or simply get lost predation. But they do not care, to perform their function in the colony **(Heinze, 2016)**. During each mating flight, between 6-17 drones go to mate eachvirgin queen, but only one drone success to arrive and mate the queen. After mating, the drones are either driven out of the colony or killed to maintain the community **(Choi, 2021).**In commercial apiaries, obtaining appropriately mated queen bees is one of the important processes for producing queen bees with a long fertile life span**(Khodairy *et al.*, 2003)**,this requires careful management by the beekeepers in providing enough drones of suitable age in the same area while virgin queens are on mating flights. Drones do not feed themselves because they have less direct pollen consumption and lower levels of digestive enzymes than workers, although their nutritional requirements are quite high. Therefore, adult drones depend on an ample supply of nurse bees, which feed them with pre-digested food via proteinaceous secretions from hypopharyngeal gland, pollen, and honey through trophallaxis**(Schneider 2012).**During the first 6 days of life, the consumption of pollen by drones is significant, and once they start flying, they depend on carbohydrates from honey as their energy source **(Fathi *et al.*, 2018).**Reproductive quality is affected by having an adequate pollen supply, as the reproductive capacity of drones reared in colonies with limited access to pollen is impaired due to low semen volume and reduced ejaculatory ability **(Rangel and Fisher, 2019).**However, other parameters of reproductive quality, including sperm viability, number, and concentration, were apparently not affected by pollen deficiency **(Czekonska *et al.*, 2015).**

The aims of our studywere to test the influence of food alternativeson honeybee drones (brood rearing, weight, and semen).

2. Material and methods

2.1. Honeybee colonies

Twenty-four *A. mellifera* colonies headed with queens of similar ages of relatively medium and similar strength (five combs, three of them sealed brood and two honey with bee pollen covered by bees) were used.

These colonies were divided into eight groups, each group containing three replicates seven of them that were fed on seven different protein diets, and one not supplied with protein as a control. Throughout the trial, all colonies were received 50% sugar syrup (1 L/week/colony) and managed in accordance with the best beekeeping techniques and management practices.

2.2. Preparation and chemical analysis of diets

Seven different protein substitute was prepared in honeybee lab at the Faculty of Agriculture, Benha University as follows (g/100g):

Diet1: (5mL Glycerol, 25soya bean flour, 20brawer yeast, 15honey and 35sugar powder).

Diet2: (25germinated chickpea brawer, 20 brawer yeast, 15honey, 25sugar powder and 15soya bean flour).

Diet3: (25germinated faba bean, 20brawer yeast, 15honey, 25sucrose powder and 15soya bean flour).

Diet4: (5mLlactic acid, 25soya bean flour, 20brawer yeast, 15honey and 35sugar powder).

Diet5: (2mLsodium chloride, 25soya bean flour, 20brawer yeast, 18honey and 35sugar powder).

Diet6: (25germinated fenugreek, 20brawer yeast, 15honey, 25sugar powder and 15soya bean flour).

Diet7: (25soya bean flour, 20brawer yeast, 20honey and 35sugar powder).

Control group was fed on sugar syrup (50%) only.

2.3. Determinations

2.3.1. Estimate of sealed brood area

In all experimental colonies,sealed brood surface ofdroneswas measured every 15 days, by an empty Langstroth frame divided into square inches **(Nowar*et al.*, 2018).**

2.3.2. Weight of drone's larvae

Five larvae were carefully taken from their cells for each replicate. After taking the larvae, they were washed with distilled water to remove food residues, then dried on filter paper and the larvae were weighed and the weight in grams for each larva was recorded according to **Human *et al.* (2013).**

2.3.3. Weight of drone's adults

Fifteen new emerging drones were taken from the brood comb (five drones per replicate), then placed in a Benton cage and the cage was weighted with bees and after removing the bees, the cage was weighted empty and the fresh weight of bees according to **Human *et al*., (2013),** and recording the weight in gram per drone by the following equation:

$$\text{Weighing of adult (g)} = W1 - W2$$

Where: W1= Weight of cage and drones
W2= Weight of cage without drones

2.3.4. Drone's semenproduction

2.3.4.1. Semen collection

From five drones, semen was collected by manual inversion of the genitalia**(Woyke 2008)** in a glass capillary tube (from each treated colony seven pooled samples were obtained).Firstly,with the thumb and forefinger, pressure was applied to the head of the drone to produce partial reversal of the internal canal and then pressure to the posterior end of the abdomen to cause full reversal of the genitalia. The seminal fluid is cream-colored in mature drone and is located at the tip of the genitals on a mass of white mucus.Secondly, by means of a large capacity Harbo syringe the semen was collected.

Only 0.2 µL of semen (the minimum required for a syringe) was collected from the drones, then the volume was recorded to the nearest 0.2 mL.

2.3.4.2. Sperm count

A Cell-Vu sperm counting chamber was used for sperm count. 1 µl were added from each of the collected semen samples in a sterile Eppendorf tube containing 1.5 mL of modified KIEV solution (0.3g D-Glucose, 0.21g $NaHCO_3$, 0.41g KCl, and 2.43g sodium dihydrate, in 100mL water supplemented with 0.05% $C21H41N7O12$;diluted = 1:500) and mixed by vortex at the lowest speed.From the initial dilution of semen 4µlwere withdrawn from each Cell-Vu chamber and the number of sperm in 100 squares (of the 1 × 1 mm grid) was counted using a phase-contrast light microscope at 400x magnification. Then obtain the average number of sperm per ml as follows:

$$\text{Sperm count/ml} = \frac{\text{average number of sperm cells per 100 square}}{11500\ (\text{dilution factor}) \times 50000}$$

2.4. Statistical analysis

All the data of experiments were analyzed using ANOVA tables by MSTAT-Cversion 1.41 according to **Snedecor& Cochran (1980).**Also, significance between means was determinedbyDuncan's multiple range tests at 0.05 probability levelaccording to **Steel & Torrie (1980).**

3. Results and discussion

3.1. Drones sealed brood rearing.

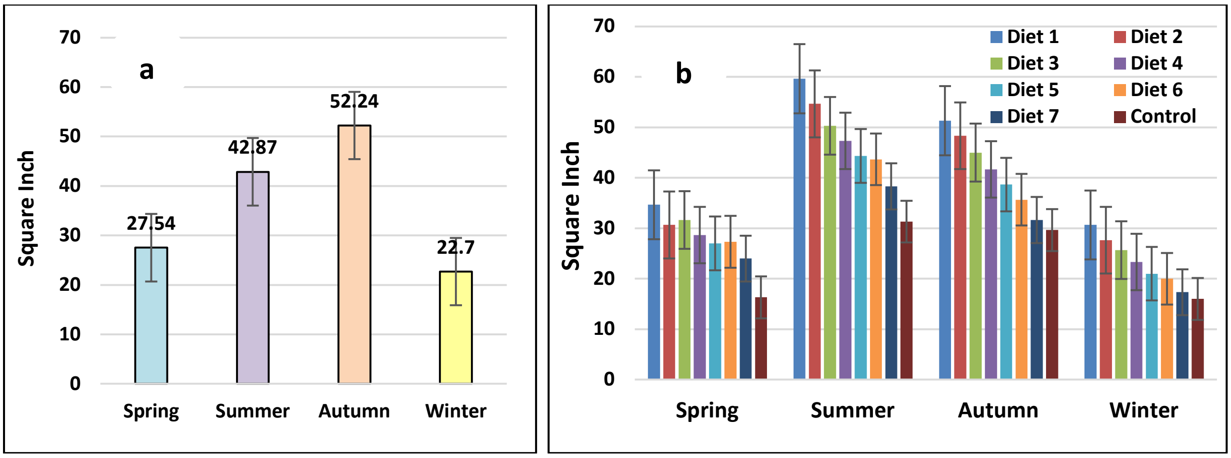
3.1.1.The first year 2020-2021

Results presented in **Fig. (1a)** showed that the highest drones brood rearingactivity was during Autumn followed by Summer with a means of 52.24 and 42.87 inch^2/colony, respectively, with significant differences. On the other hand, the lowest drone'sbrood rearing activity was obtained during spring and winter with a means of 27.54and 22.70-inch^2/colony, respectively, with significant differences.Also, results graphically illustrated by**Fig. (1b)** indicated that higher sealed brood area of droneswas obtained when honeybee colonies were fed on protein diets compared with control.Highestsignificant drone'ssealed brood area was observed in the colonies provided with glycerol patty (Diet 1) than other patties with a mean of 44.077 inch^2/colony.This might be because glycerin has three hydrophilic hydroxyl groups, which are responsible for its water solubility and hygroscopic nature as well as it was known to cause no honeybee mortality. Furthermore, it is a natural component of honey, and it can be used as a food additive without a specific value for daily consumption**(Rademacher *et al*., 2013)**.

Additionally, the colonies fed on chickpea patty (Diet 2) and germinated faba bean patty (Diet 3) showed significant increase in the drones sealed area with a means of 40.324 and 38.162 inch^2/colony, respectively. This trend of results was observed in all seasons except during spring. Moreover, the colonies fed on Diets 4, 5, 6 and7gave significant high sealed area compared with colonies received sugar syrup only by35.245, 32.747,31.662 and 27.83 inch^2/colony. Concerning the monthly changes in drone's sealed area, data illustrated by **Fig. (1c)** indicated that the highest drones sealed brood area was obtained during October followed by July, April and August by means73.12, 46.5, 44.5 and 41.252inch^2/colony, respectively.While the lowest drones sealed brood area was observed during February January and with a means of 15.37 and 13.87 inch^2/colony, respectively.

3.1.2.The second year 2021-2022

Results graphically illustrated in **Fig. (2a)** showed that the highest significant drone's brood rearing activity was during Autumn followed by Summer with means of 95.24 and 46.45 inch^2/colony, respectively. On the other hand, lower significant drone's brood rearing activity was recorded during springand winterwith means of27.04 and 21.49 inch²/colony, respectively. Also, results presented in **Fig. (2b)**indicated that the highest drone's sealed brood area was obtained when honeybee colonies were fed on protein diets, the sealed brood area of the drones was significantly higher than those without protein diets and the mean of drones sealed brood area in the colonies which provided with glycerol patty was significantly higher than other patties with a mean of 44.24 inch^2/colony, while the colonies which fed on chick pea patty came in the second place followed by the colonies which fed on germinated faba bean patty with significant differences with a means of 40.24 and 38.91 inch^2/colony, respectively, followed by the colonies which fed on lactic acid patty, sodium chloride patty , fenugreek patty and Diet 7 with a means 35.99, 32.66,30.16 and 27.42 inch^2/colony. While the lowest workers brood rearing activity was observed with significant differences in control colonies which fed on sugar syrup only with a mean of 20.74 inch^2/colony. Moreover, **Fig. (2c)** indicated that the highest drone's sealed brood area was obtained during June followed by July, April, and August with means of 50.5, 48.0, 44.25 and 42.62 inch^2/colony, respectively. While the lowest drones sealed brood area was observed during January and February with means of 12.75 and 14.12inch^2/colony, respectively.



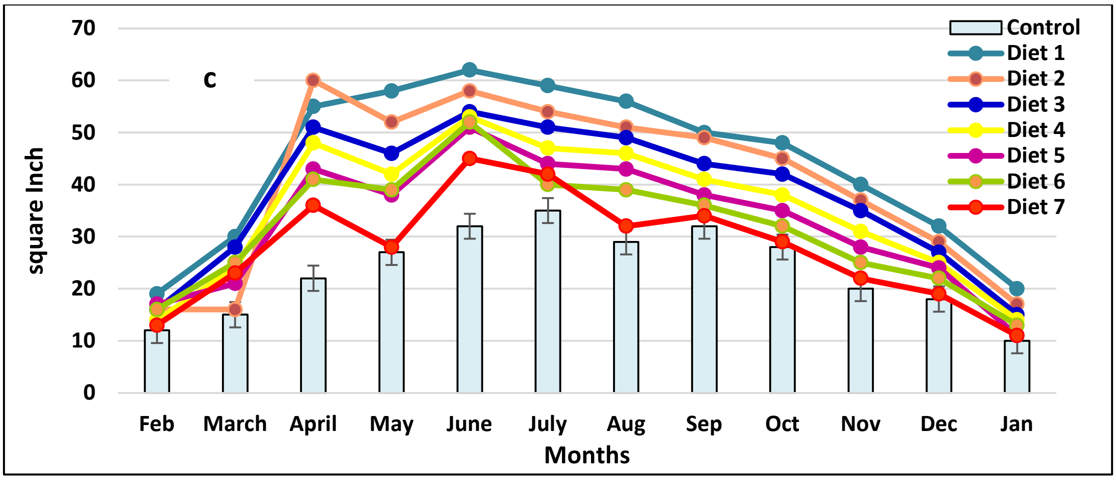


Figure1. Changes in drones sealed brood area as affected by different pollen substitutes during the first year 2020-2021, **a)** mean seasonal changes during the experimental period; **b)** seasonal changes per colony; **c)** monthly changes per colony.

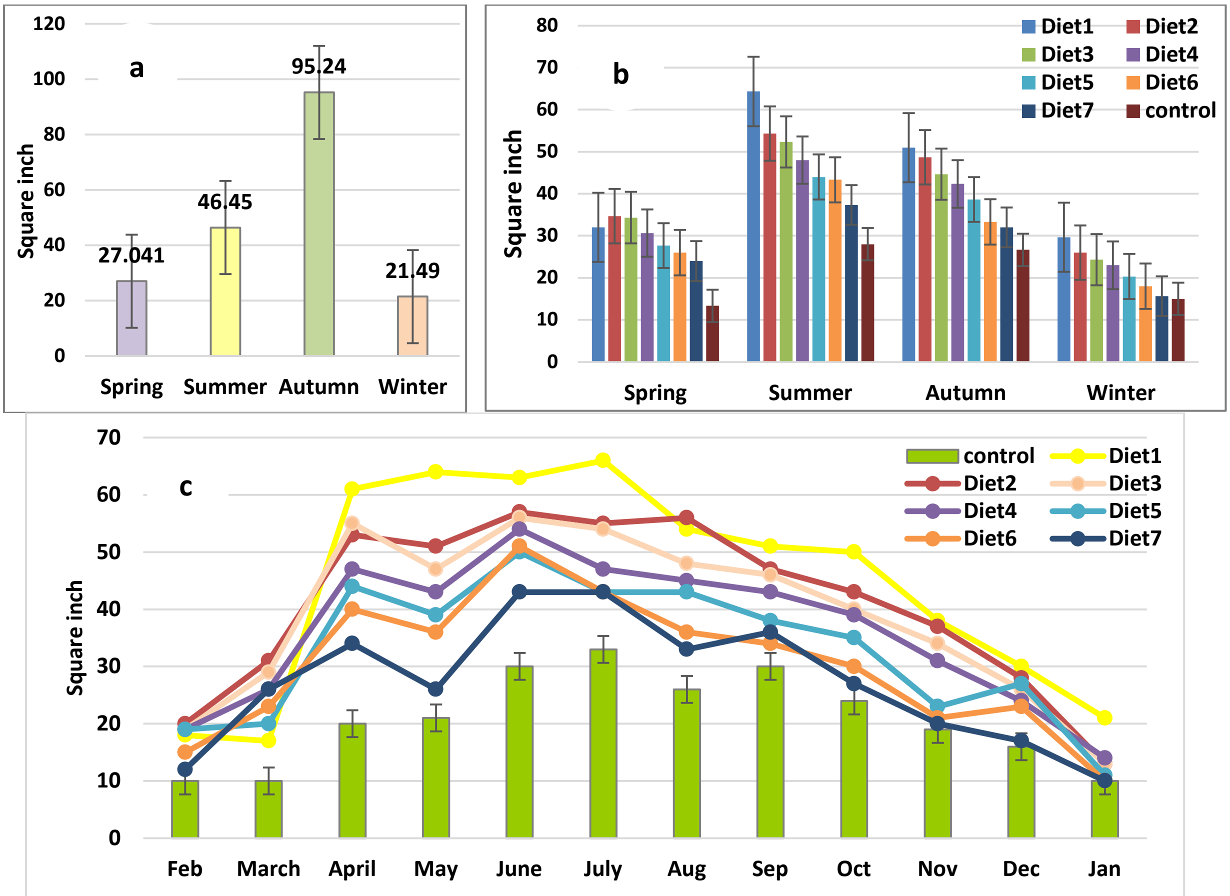


Figure2. Changes in drones sealed brood area as affectedby different pollen substitutes during the first year 2021-2022, **a)** mean seasonal changes during the experimental period; **b)** seasonal changes per colony; **c)** monthly changes per colony.

Generally, rearing drones requires a large amount of nutrients, so they are only reared in large numbers by colonies with abundant food resources **(Boes 2010)**, The accumulation of pollen in the colonies helps produce more active males and early initiation of flight **(Rueppell*et al.*, 2006)** and they do not require pollen feeding during their sexual maturity **(Stürup*et al.*, 2013).**Sealed brood area increased more in colonies fed on patties than in colonies fed sugar syrup only (control group).Similar results by **Lamontagne-Drolet*et al.*(2019)**and **Islam *et al.* (2020)**indicated an increase in sealed brood area after consumption of different pollen substitutes by honeybees. Also, **Abd El-Wahab *et al.* (2016)** revealed an increase in the sealed brood area in the bee colonies that were provided with the supplements compared to the un-supplemented colonies.Moreover, **Sihag and Gupta (2013)** reported that despite the lack of effectiveness of supplementary diets in stimulating different activities in honeybee colonies, they area good stimulator of population density compared to those fed with sugar syrup only.

3.2. Drone's body weight

3.2.1. The first year 2020-2021

Data graphically illustrated by**Fig. (3 a & b)** showed the changes in body weight of both larvae and adultsof dronesas affected by nutritional composition.Regarding the larvae weight, data in **Fig. (3a)** indicated that the lowest mean of body weight of larvae (0.117– 0.118 g/larva) was observed when the colonies which fed on lactic acid patty and control group which fed on sugar syrup only and. Also, data showed that the highest mean of larvae body weight in colonies which fed withGlycerol patty by mean (0.128g/larva) and followed by colonies which fed with germinated chickpea patty by mean 0.126g /larva. On the other hand, the feeding bees with germinated chickpeapatty the highest mean of adult drone'sbody weight (0.092 g/drone) and followed by colonies which fed with glycerol patty with a mean value of (0.091 g/drone). Whilethe lowest drone's body weight (0.077g/drone) was recorded in control treatment which fed on sugar syrup only **(Fig. 3b)**. This might be due to the highly antimicrobial activity against bee pathogens which protect colony from pathogens, and this led to good colony members included drones **(Lopes *et al*., 2016).**

3.2.2. The second year 2021-2022

Results graphically illustrated by**Fig. (3a)**showed that the lowest mean of body weight of larvae (0.110 – 0.116 g/larva) was observed when the colonies fed on control group which fed on sugar syrup only and lactic acid patty. Also, data indicated that the highest mean of larvae body weight in colonies which fed with chickpea patty by mean (0.128g/larva) and followed by colonies fed with glycerol patty by mean 0.127g /larva. Regarding the weight of adult drones, results illustrated by **Fig. (3b)** provided that feeding bees with germinated chickpea patty the highest mean of drone's body weight (0.091 g/drone) and followed by colonies which fed with glycerol patty with a mean value of (0.089 g/drone). In addition, while lowest drones body weight (0.061 g/drone) in control treatment which fed on sugar syrup only.

Although several researchers were agreed with the current study that the drone body mass in both the larvae and adult groups corresponded to the average drone mass reported in publications and ranged between 0.201 and 0.290 g **(Gençer and Firatli 2005; Mazeed and Mohanny 2010; Gençer and Kahya 2011)**, others disagreed and found that the drone larvae reach 0.262–0.419 g, and the weight of freshly emerged drones was found to be 0.277–0.290 g**(Duay*et al*., 2003).**

Generally, the honeybee drone's body weight was higher in the second year (2021/2022) than in the first year (2020/2021), this trend was true on both larvae and adult drones.Due to their mass, drone larvae require significantly more food with a more diverse protein composition than worker bee larvae, they reach their high final body weight after a longer period of development during which spermatogenesis and organ formation takes place **(Hrassnigg and Crailsheim, 2005)**. Pollen deficiency or lack of necessary nutrients can limit colony growth. It may therefore be beneficial to feed pollen supplements or substitutes**(Czekońska*et al*., 2015)**. This feeding is particularly useful for stimulating brood rearing, especially drone brood to take advantage of honey flow and spring pollination,and important for queens rearing and mating **(Gençer and Kahya, 2011).**Finally, the feeding of drone larvae can affect their body mass, their ability to empty their copulatory and ejaculatory apparatus, or their sperm count and viability**(Czekońska*et al*., 2015).**

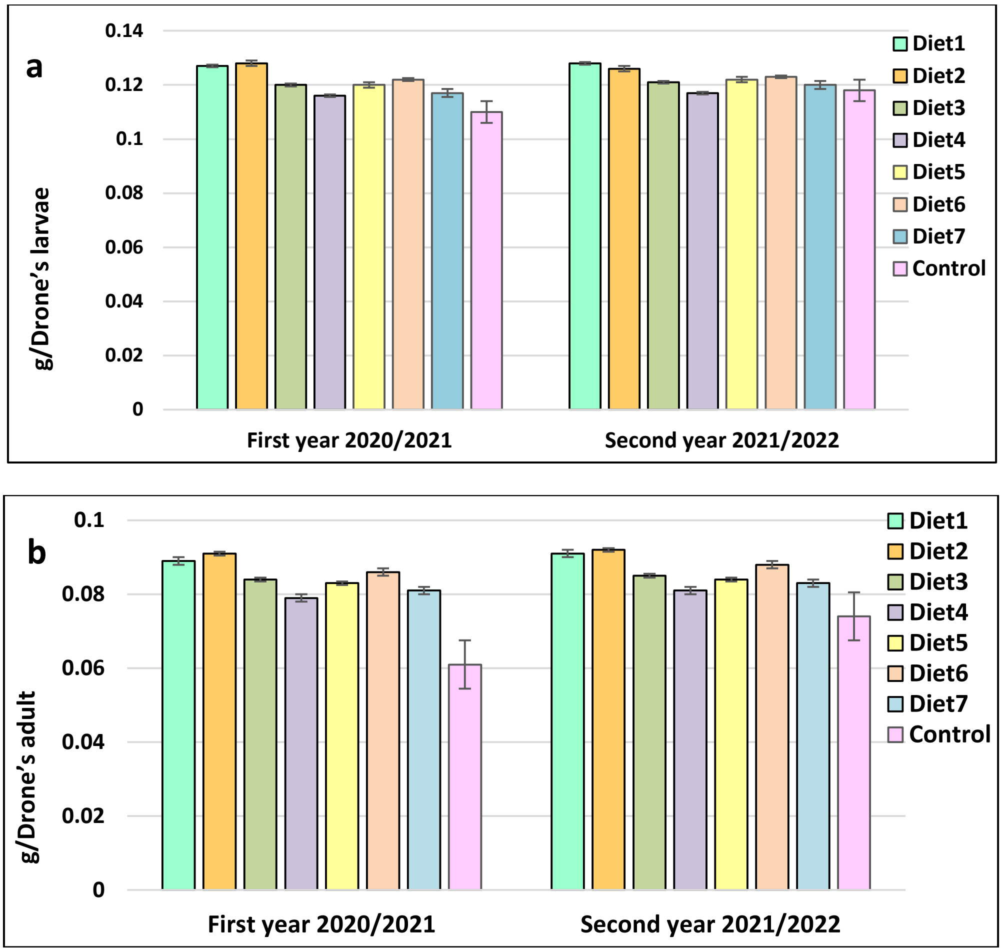


Figure3. Changes in drone'sbody weightas affectedby different pollen substitutes during both years2020/2021 and 2021/2022, **a)** larvae; **b)** adults.

3.3. Drone's fertility

Results in**Table (1)** showed that the drones fed on protein diet produce largersemen volume (ml) than not received protein diets. Data showed that the highest semen volume in colonies fed on Glycerol patty, followed by colonies fed on germinated chickpea patty with means (1.28 and 1.27mL) while the lowest semen volume recorded in control group which fed on sugar syrup only with mean (1.07mL).Moreover, data indicated that the highest sperm count in Diet 1 followed by Diet 2 with means values (2.77 and 2.66mL). On the other side, it was observed that sperm count/dronewas higher in the group received Diet 1followed by that received Diet 2 with means 3.10 and 3.08, respectively. While the lowest number recorded in group fed on Diet 7 and control group with means 3.01 and 2.99, respectively.

In this concern, **Schlüns *et al*. (2003)** found that supplementing colonies during the spring significantly increase the semen production of drones. In line with our study which showed an increase in semen quantity in colonies fed pollen substitutes, sucrose syrup, or both in the spring; meaning that undernutrition of honeybee larvae can affect the health and development of adult beesa low-protein diet during development has been shown to adversely affect some reproductive parameters, including lower semen volume and fewer sperm per ejaculate at maturity in several mammals. **(Brodschneider and Crailsheim 2010).**Also, **Goins and Schneider (2012)**

Additionally,**Czekońska *et al*. (2015)** found that honeybee colonies do not abandon drone brood rearing immediately when access to pollen is restricted. Worker bees breed a brood that produces lower-mass drones, ejaculates less frequently, and produces smaller amounts of semen. However, the LP colonies did not raise brood with poorer semen quality as judged by sperm concentration and the number and viability of sperm in the ejaculate. This could be due to malnutrition in drone larvae caused by faster cell sealing.With a different vision, **Ayoub *et al*. (2021)** addressed the effect of nutrition on male fertility through some questions, the most important was show an average of many flights per day. Do drones work during mating season? Queen breeders could use this information as a basis for measures to increase flight numbers, such as alternative feeding of bee colonies during the drone flight season.

Table 1. Semen volume, sperm count per ml and per one drone of *A. mellifera* drones in various experimental groups.

		Diet 1	Diet2	Diet3	Diet4	Diet5	Diet6	Diet7	control
Semen volume (ml)		1.28	1.27	1.26	1.24	1.23	1.23	1.09	1.07
Sperm count	**per mL ($\times10^9$)**	2.77	2.66	2.55	2.48	2.45	2.40	2.31	2.28
	per one drone ($\times10^6$)	3.10	3.08	3.70	3.05	3.04	3.03	3.01	2.99

4. Conclusion

Our investigation focuses onhoneybee drone's activitiesas affected by feeding with protein diets, these activities included (broad rearing, body weight and fertility). This study concluded that all applied diets (pollen substitutes patties) were better than sugar syrup only in colony feeding and thus reflexed on drone's activities. Regarding the competent nutrient composition of honeybee drones in the present study, the patties contained glycerol was the best one for all drone's activities because it was distributing quickly and uniformly in the colony and can provide a good dispersion when used as a carrier substance.

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تحديد بعض التأثيرات للمعاملات الغذائية المختلفة على ذكور نحل العسل.

تقى فتحي مشعل، رضا السيد عمر، متولي مصطفى خطاب والحسيني السيد نوار
قسم وقاية النبات، كلية الزراعة، جامعة بنها، مشتهر، القليوبية 13736، مصر.

من المعروف أن الوظيفة الأساسية لذكور نحل العسل هي التكاثر فقط. في الدراسة الحالية، تمت دراسة مواصفات ذكور النحل التي تتأثر بالتغذية ببدائل حبوب اللقاح خلال عامين متتاليين 2021/2020 و2022/2021. لقد درسنا تأثير سبع بدائل لحبوب اللقاح على تربية الحضنة ووزن الجسم (اليرقات والحشرات الكاملة) وكذلك الخصوبة (حجم السائل المنوي وعدد الحيوانات المنوية) لذكور نحل العسل. تم ملاحظة التغير في مواصفات حضنة الذكور وكان اعلاها في الخريف والصيف خلال عامي الدراسة. بينما خلال شهر سبتمبر كل طوائف الذكور أكثر نشاطًا في استهلاك جميع البدائل المستخدمة مقارنة بالأشهر الأخرى في كلا العامين. في النهاية، فيما يتعلق بخصوبة الذكور، كان البديل المحتوي على الجلسرين هو الأفضل في كل معايير الخصوبة، وكان حجم السائل المنوي 1.28 ملم/ذكر ، وكان عدد الحيوانات المنوية / ملم2.77×10^9، وعدد الحيوانات المنوية / ذكر 3.10×10^6مما يؤكد فرضيتنا بأن التغذية الاصطناعية ببدائل حبوب اللقاح يؤثر على أنشطة الذكور.