The effect of different levels of protein and fat on dietary preference and survival of western honey bees workers, *Apis mellifera* L.

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Abstract

This study was conducted at the bee laboratory in the Faculty of Agriculture at the University of Kufa to investigate the possibility of using artificial feeding with protein and fat as an alternative to pollen. The study also aimed to examine the preference of nurse honey bees for artificial feeding mixtures and their consumption compared to a sugar solution (Sucrose 1 M), and finally to determine the effect of feeding mixtures on the lifespan of nurse bee workers. The results showed that the highest consumption rate of nurse honey bees was in the control treatment (Sucrose 1 M) at 72.57 microliter /bee /day, while the lowest consumption rate was 27.70 microliter /bee /day in the treatment (15:1 P: F). The consumption rates in the treatments (0:1 P:F), (1:1 P: F), (5:1 P: F), and (10:1 P: F) were 64.80, 47.24, 44.70, and 32.42 microliter /bee /day, respectively. The results also revealed that the highest survival rate was in the control treatment (Sucrose 1 M) at 96.25%, while the treatment (0:1 P: F) had the lowest survival rate at 67.50%. The survival rates in the treatments (1:1 P: F), (5:1 P: F), (10:1 P: F), and (15:1 P: F) were 81.25%, 73.75%, 83.75%, and 86.25%, respectively.

Keywords: Protein, Fats, Artificial feeding, dietary preference, Survival of honey bees.

Introduction

The honey bees, *Apis mellifera* L., are considered one of the important social insects and its classification comes at the top of the scale of these insects in terms of economic importance [1]. Bee are always active throughout the year despite climate changes and temperature variations, so it has become an important element in its economic field. With its activity, it has a close and strong relationship with man, as it has been observed in recent years the growing interest in breeding and caring for honey bees in most countries of the world, due to the great importance that bees have in various fields of life, including agricultural, therapeutic, nutritional, economic and cosmetic [2]. A lot of research has been conducted that included all aspects of bees, such as the effect of diet on protein concentration, hypopharyngeal gland development, and virus load in honey bees *A. mellifera* L. [3]. Besides because bees are one of the most important pollinators, as they completely depend on the flowering products of plants for nutrition, in addition to the ease of breeding them in large numbers in hives that can be moved from one area to another. Therefore, they represent 80% of insects pollinating plants, which play an important role in increasing plant production [4].

Food is considered one of the main factors for strengthening the colony of bees because it provides the basic needs for bee growth, development and reproduction [5, 6]. Stayed there substitutes or supplements [7, 8] in any period of the year in which food is scarce, as long as bee swarming has been prevented for any reason, whether due to unfavorable weather conditions, scarcity, lack of pollen, scarcity of nectar sources, and insufficient food in the hive [9]. Studies show that bee colonies require more than 6.8 kg of honey and more than 19.9 kg of pollen during periods of the year, especially during the spring season. The density of bees decreases, and here the bee-keeper must intervene by providing alternatives to honey and pollen and using artificial feeding with alternatives to natural bee food and its supplements [10].

Bees, like any living organism, need carbohydrates, proteins, fats, minerals, and water for growth, development, and reproduction, and they obtain them through collecting water, nectar, and pollen [11]. The nectar collected by bees from the floral nectar glands is a source of sugar, while pollen is a source of proteins, fats, vitamins, minerals and other nutritional requirements [12]. The honey bee sect consumes some of what it collects from them to sustain its various activities and store more than it needs in the hexagonal springs to benefit from it at the time of need and during the period in which the bees' external nutrition sources are reduced or absent in the winter and summer months [13], which leads to the sect's consumption of its food stocks and thus Egg laying decreases and bee density decreases, forcing beekeepers to conduct artificial feeding with alternatives and supplements to honey and pollen grains, and by external or internal feeding methods determined by the strength of the colonies and their number in the apiary [11]. Pollen is essential for the glandular development of young worker bees [14]. Studies have confirmed that pollen quality affects the activation of ovaries [15, 16] and the development of hypopharyngeal glands (HPGs) in worker honey bees [17, 18]. Pollen meals significantly affect the development of honey bees' hypopharyngeal glands and ovaries [19, 20]. Some studies confirmed that the use of the degree of development of the hypopharyngeal glands and the development of the ovary is very important for evaluating pollen quality [21, 22]. Hypopharyngeal gland development deficiency may occur if the workers do not receive adequate pollen nutrition at the beginning of the puberty stage [23]. At the same time, pollen grains rich in protein can promote the development of ovaries of workers in queen less colonies, that is (Laying workers) [24]. Low-protein pollen also stunts the development of ovaries [25].

When studying the behavior of bees towards foodstuffs that they visit naturally or are offered to them artificially, it has been observed that there is a nutritional preference by workers towards some foodstuffs over others [26, 27] and that there are substances that increase the number of workers attracted to food sources, unlike other materials. The fatty substances such as sterols and free fatty acids found in pollen have a role in attracting bees [28, 29], and the importance of fats has been proven in vitro against some viruses such as deformed wing virus (DWV) [30]. Adding anise, chamomile, and artificial honey fragrance to dry pollen substitutes increased the bees' attractiveness to them [31]. Also, the lack of attraction of bees to pollen-free supplements is due to the lack of attractants [32]. Benzan, which is extracted from pollen, attracts 90% of bees compared to alternatives not treated with this substance; in addition to pollen and flower color that have a role in attracting bees, either as a function of acidity (PH), it has little to no role in attracting bees [33]. Likewise, bees were consuming more sugar solution (glucose), then date paste (molasses), then black honey (date molasses) when placed in Petri dishes in front of honey bee hives [34], as 217 workers were attracted to glucose, 129 workers to black honey, and three workers to molasses 15 minutes after serving food [35]. The nurse honey bee workers preferred a 25% date molasses solution in the laboratory compared to the control (1M glucose solution) [26].

The research aimed to study the effect of artificial mixtures of the different food combinations of proteins and fats on western honey bee workers' food preference and life.

Materials and Methods

The research was carried out in the bee laboratory of the Faculty of Agriculture / University of Kufa, from 11/10/2022 - 23/10/2022, The study included conducting an experiment consisting of 24 beekeeping boxes of hybrid Carniolian bees *A. mellifera*, to the effect of Artificial mixtures on the nutritional preference and lifestyle of western honey bee workers.

Caged Honey Bees

Two parcels of local bees, *A. mellifera*, were selected with a bee density of 5 frames covered with bees. It was taken into account that the colonies are similar in strength, The queen is one year old (according to the record of the beekeeper from whom the parcels were purchased). The bees were fed with proteins and fats and six per cent of feeding mixtures were artificial, including the control treatment (Table 1). Each treatment included four boxes or replicates using laboratory breeding boxes and laboratory feeding tubes (Eppendorf tubes). The boxes containing bees were kept in a laboratory insect incubator at a temperature of 33 m o and a humidity of 66% [27].

Table (1): Tested artificial feeding mixtures and their components

No.	Treatments	Artificial feed ingredients	
		Protein	Fat
1	0 : 1 P : F	0 %	0.6 %
2	1 : 1 P : F	0.6 %	0.6 %
3	5 : 1 P : F	3 %	0.6 %
4	10 : 1 P : F	6 %	0.6 %
5	15 : 1 P : F	9 %	0.6 %
6	Control	Sugar solution (1M)	

Treatments

A sugar solution (Sucrose 1 M) was prepared by adding 342.3 g of sucrose after dissolving it in distilled water to make the sugar solution's volume (1 liter) , and shaking well to ensure that the sugar was completely dissolved. It was kept in a sealed glass container in the laboratory refrigerator for later use, as a control treatment or to dissolve the protein to-fat ratios (P: F). Milk protein (casein) and soybean fat (lecithin) were used in the current study as the basis for protein and fat materials, respectively. The materials were prepared as shown in table (1). The treatment was prepared (15:1 P: F) by weighing 9 gm of protein and 0.6 gm of fat in a sensitive balance (Sartorius) and Johnson's materials, then completing the volume to (100 ml) with sugar solution, and placing it in a water bath at a temperature of 80 °C to dissolve From this percentage, the remaining percentages are present.

Effects of P: F diets on some biological aspects

Dietary preference

The sealed brood frames were placed two days before emergence in the incubator at a temperature of 33 °C and a humidity of 66%. Newly emerged bees were collected at a rate of 20 bees per replicate, and four replicates were used for one treatment. The newly emerged bees were placed in a laboratory breeding box, dimensions 20 × 6 × 11 cm, made of Perspex plastic. Each box contains 6 holes in which Eppendorf feeding tubes are placed. It has a capacity of 2 ml and is equipped with four openings of diameter (3 mm) to access the feeding, where 4 tubes were filled with artificial feeding mixtures, and 2 tubes were filled with distilled water (Figure 1). The boxes containing the honey bees used in the experiment were placed in the lab incubator at a temperature of 33 °C because it is the optimum grade for bees. The experiment was repeated for 14 days, and the readings were taken daily by weighing the tubes containing artificial feeding mixtures before feeding the bees and after feeding the bees to find out what food was consumed by the nurse honey bee workers, as follows, according to the method of Stabler *et al*. [36].

1 -Total consumption: where the volume of the consumed solution is calculated as a whole, including proteins, fats, and sugars as a single mixture.

2 -Protein consumption: where only the volume of protein consumed is calculated by multiplying the total volume by the percentage of protein per 1 ml.

3 -Fat consumption: only the volume of fat consumed is calculated by multiplying the total volume by the percentage of fat per 1 ml.

4- Carbohydrate consumption: only the volume of carbohydrates consumed is calculated by multiplying the total volume by the percentage of sugar per 1 ml.

Figure (1): A plastic lab breeding box in which honey bees were reared and fed

Survival of honey bee workers

The effect of artificial feeding mixtures on the survival of honey bees workers was studied by recording bees survival and counting and removing dead bees daily.

Statistical Analysis

Statistical analysis of dietary preferences data was achieved using one-way ANOVA by Minitab ®19.1 software (Minitab, LCC, USA). Comparisons were made using the Tukey method and 95% confidence with a significance at $p \leq 0.05$. Survival data was analyzed with Graph Pad Prism 7.0 Software (Inc., La Jolla, USA) using chi-square according to data normality, verified by Log-rank (Mantel-Cox) test and the significance was set at $P < 0{\cdot}05$.

Results and Discussion

Dietary preference of artificial feed mixtures consumed by nurse worker bees

The results showed in (Figure 2) that the preferences of nurse honey bees for the artificial feeding mixtures provided to them varied, as it was noticed that there are significant differences between the treatments ($P = 0.0020$, $F_{5.23} = 5.81$). It was noticed that the highest consumption rate in the control treatment (Sucrose 1 M) amounted to 72.57 microliter /bee /day. In contrast, the lowest consumption rate was 27.70 microliter /bee /day in the very rich protein treatment (15:1 P: F), The results also showed that the food preference in the second degree was for the completely protein-free treatment (0:1 P: F), as the average consumption of nurse honey bees was 64.80 microliter /bee /day, then the treatment (1:1 P: F), which amounted to 47.24 microliter /bee /day, and finally the treatments (5:1 P: F) and (10:1 P: F), which amounted to 44.70 and 32.42 microliter /bee /day, respectively.

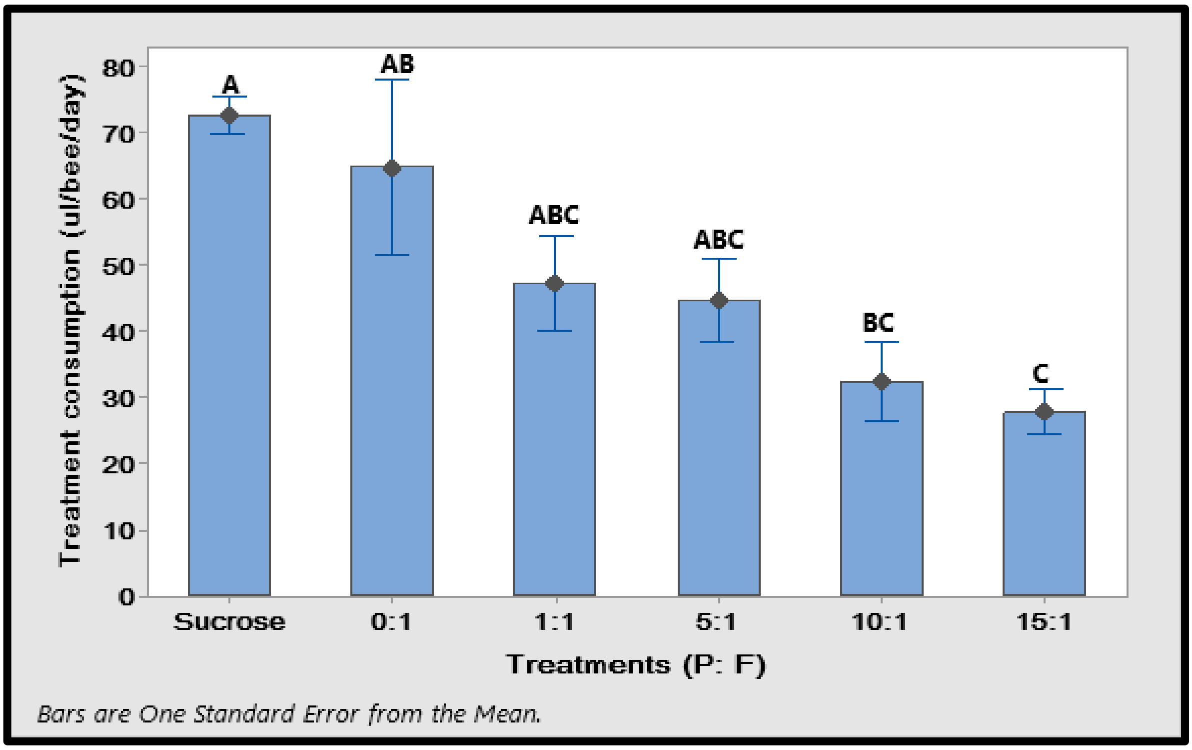

Figure (2): Average daily consumption of artificial feeding mixtures at different concentrations for nurse honey bees workers *A. mellifera* under lab conditions, N = 4 with an average of 20 bees per replicate. Columns with the same letter do not differ significantly at the probability level (P = 0.05) (One way ANOVA, Tukey test)

It is clear from the above results that increasing the protein concentration in the artificial feeding mixture-led to a decrease in the dietary preference for the food consumed by nurse honey bee workers (Figure 2). At the same time, increasing the protein concentration in the artificial feeding mixtures positively affected the lives of nurse honey bee workers (Figure 6).

The bees' intake of more protein in mixtures containing proteins, fats, and sugars results from the bees' need to reach the target concentration of the body's need for fats, for example, or carbohydrates. Conversely, the need for protein may lead to the consumption of additional quantities of fats to reach what is recently called " Intake Target" (IT) [36, 37]. This feeding behavior indicates that the worker bees may overeat protein to meet the need for fat and overeat fat to meet the need for protein. Concerning food preference, there are several factors due to which bees are attracted to foodstuffs, including the concentration of protein or sugary substances in the food and their containment of attractive substances, in addition to the density of these substances and the ease of obtaining those [38]. A study also revealed that bees selectively collect pollen from flowers based on their protein-to-fat ratio (P: F) [39].

The results of the current study agreed with the findings of Stabler *et al*., [36], that honey bees *A. mellifera* regulated the intake of protein and fats at a rate ranging between (1:1 P: F) and (5:1 P: F). Also bees nurse honey bees *A. mellifera* ate more protein when fed a low-fat diet and an equal-fat diet than a high-fat one.

Dietary preference for protein consumed in artificial feeding mixes

The results showed in (Figure 3) that the preferences of nurse honey bees for the protein in the artificial feeding mixtures provided to them varied, as we notice significant differences between the treatments ($P = 0.0000$, $F_{5.23} = 34.95$). We note that the highest rate of protein consumption in the very rich protein treatment (15:1 P: F) was 13.91 microliter /bee /day, while the lowest consumption rate was 1.428 microliter /bee /day in the treatment (1:1 P: F), and the results also showed that the food preference in the second rank for protein was for the treatment (10:1 P: F), as the average consumption of nurse honey bees was 10.46 microliter /bee /day, then the treatment (5:1 P: F), which reached 6.80 microliter /bee /day.

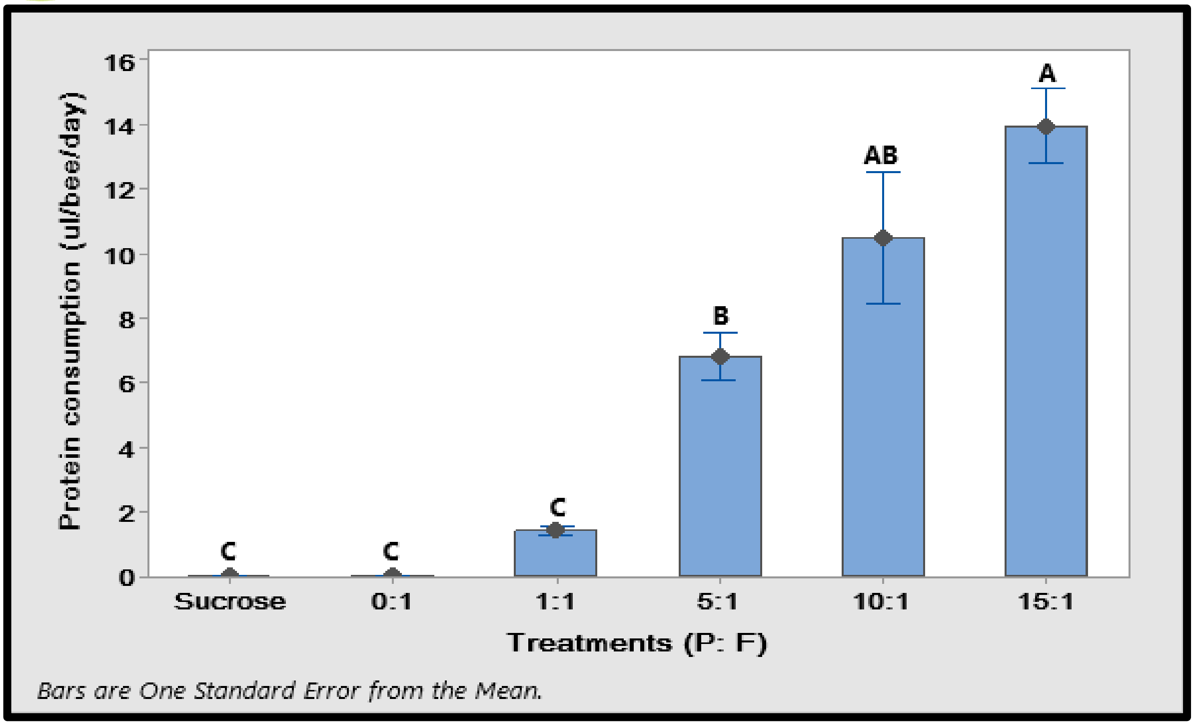

Figure (3): Average daily consumption for protein in artificial feeding mixtures at different concentrations for nurse honey bees workers *A. mellifera* under lab conditions, N = 4 with an average of 20 bees per replicate. Columns with the same letter do not differ significantly at the probability level (P = 0.05) (One way ANOVA, Tukey test)

There is debate among researchers about whether honey bees can distinguish between diets according to their quality. For example, Hendriksma & Shafir [40], found that honey bees could distinguish between similar diets depending on their amino acid components. On the other hand, researchers Corby-Harris *et al*., [41] revealed that nurse honey bees either cannot or do not evaluate the nutritional value of pollen, and thus, researchers suggest that nurse honey bees cannot tell foraging honey bees about the quality of the pollen. The vaccine they collect. On the other hand, some studies revealed that bees selectively collect pollen from flowers based on the ratio of protein to fat (P: F) [39]. Stockhoff [42] also mentioned that the early stage larvae of honey bees preferred the high-protein food mixture. The results of the current study agreed with what Stabler *et al*., [36] concluded, as nurse honey bees *A. mellifera* ate more protein when fed on two diets low in fat and equal in fat than they ate in the high-fat diet, this feeding behavior indicates the worker bees may overeat protein to reach their need for fat. The same researcher reported that honey bees consumed less protein in artificial diets containing a higher fat concentration, indicating that honey bees discontinued the diet after reaching or exceeding the fat intake target (IT).

It is clear from the above results that the increase in protein consumption positively affected the life of nurse honey bee workers (Figure 6).

Dietary preference for fat consumed in artificial feeding mixtures

The results showed in (Figure 4) that the preferences of nurse honey bees for the fat in the artificial feeding mixtures provided to them varied, as we notice significant differences between the treatments (P = 0.0000, $F_{5.23} = 13.98$). We note that the highest consumption rate of fat was in the completely protein-free treatment (0:1 P: F), which amounted to 1.735 microliter /bee /day, while the lowest consumption rate was 0.9783 microliter /bee /day in the (15:1 P: F), which is very rich in protein. The results also showed that the dietary preference for fat was in the second place for the treatment of (1:1 P: F), as the average consumption of nurse honey bees was 1.5 microliter /bee /day, then the two treatments (5:1 P: F) and (10:1 P: F), which amounted to 1.435 and 1.1 microliter /bee /day, respectively.

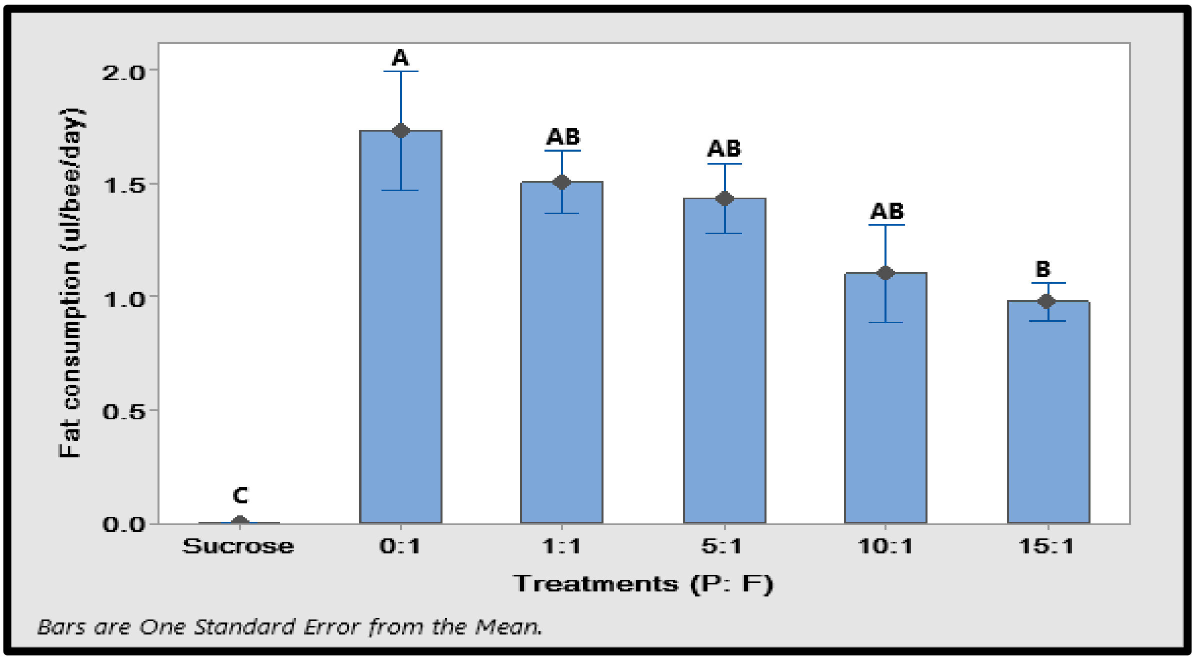

Figure (4): Average daily consumption for fat in artificial feeding mixtures at different concentrations for nurse honey bees workers *A. mellifera* under lab conditions, N = 4 with an average of 20 bees per replicate. Columns with the same letter do not differ significantly at the probability level (P = 0.05) (One way ANOVA, Tukey test)

It conclude from the above results that increasing the protein concentration in the artificial feeding mixtures led to decreased in fat consumption by nurse honey bee workers (Figure 4).

Fat is one of the most important biochemical components of an animal's diet, playing a major role in growth, survival and reproduction [46]. Besides its primary function as an energy source, fats have many functions such as preventing dehydration and communicating with insects of the same species via pheromones [47]. Stockhoff [42] stated that the preference for fats for larvae depends on the age of the larvae, as the last instar larvae of the *Lymantria dispar* insect preferred a diet high in fats. The author attributed this preference to the fact that dietary fats are a richer energy source than dietary proteins, and therefore , fat storage may become more important at older larval ages due to the future energy needs of the pupal and adult stages.

Al-Esawy [27] stated that honey bees, *A. mellifera*, preferred foods with high fat content, which caused greater growth of the hypopharyngeal glands and fewer pathogenic viruses such as Deformed Wing Virus in honey bees (DWV) [30].

Dietary preference for carbohydrates consumed in artificial feeding mixe

The results showed in (Figure 5) that the preferences of nurse honey bees for carbohydrates in the artificial feeding mixtures provided to them varied, it was noticed a significant differences between the treatments (P = 0.0000, $F_{5.23}$ = 8.04). There was the highest rate of consumption of carbohydrates in the control treatment (Sucrose 1 M) amounted to 143.06 microliter /bee /day, while the lowest consumption rate was 61.06 microliter /bee /day in the treatment (15:1 P: F), which is very rich in protein. And the completely protein-free treatment (0:1 P: F) gave the second best consumption rate of 108.3 microliter /bee /day, then the treatments (1:1 P: F), (5:1 P: F) and (10:1 P: F) as it reached 93.98; 89.58 and 68.9 microliter /bee /day, respectively.

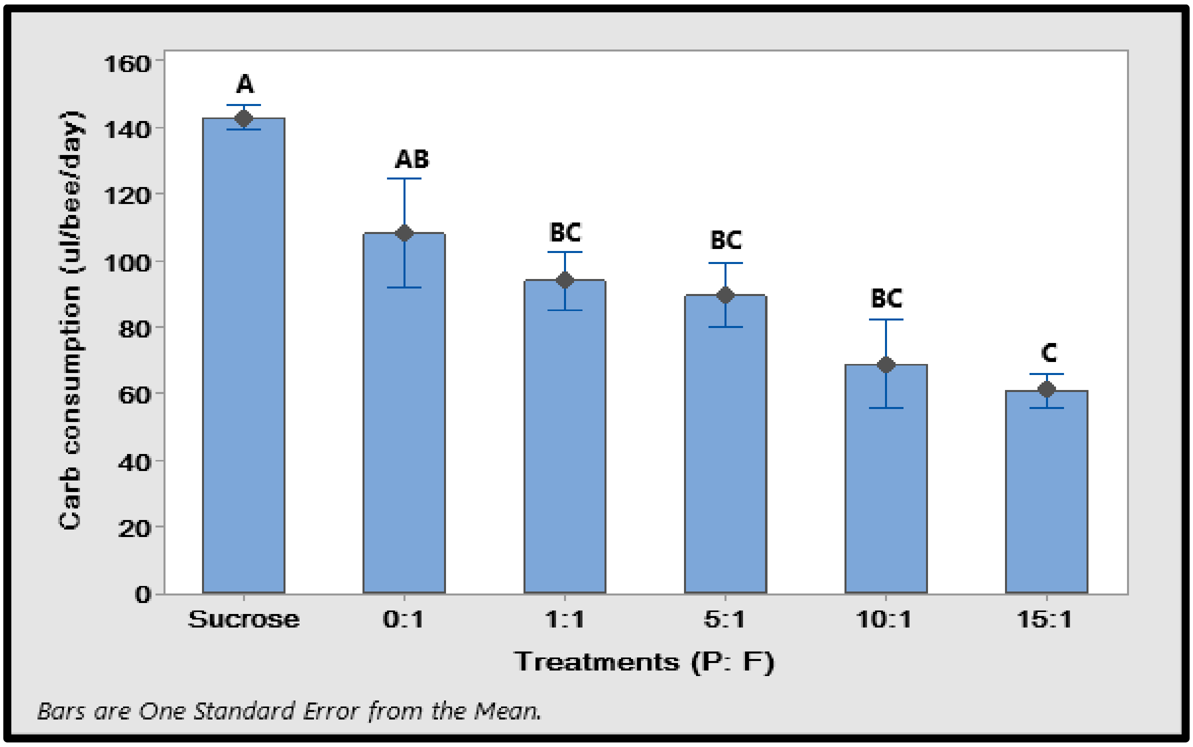

Figure (5): Average daily consumption for carbohydrate in artificial feeding mixtures at different concentrations for nurse honey bees workers *A. mellifera* under lab conditions, N = 4 with an average of 20 bees per replicate. Columns with the same letter do not differ significantly at the probability level (P = 0.05) (One way ANOVA, Tukey test)

It is evident from the above results that increasing the concentration of proteins in the artificial feeding mixtures led to a decrease in the consumption of carbohydrates by nurse honey bee workers (Figure 5).

There are several factors due to which bees are attracted to foodstuffs, including the concentration of protein or sugary substances in the food and its containment of attractive substances, as well as the density of these substances and the ease of obtaining those [38]. Some studies mentioned that the food provided to honey bees is a sugar solution and it is considered a well-known and preferred food for bees after natural honey. Honey helps lay eggs and stimulates brood productivity [48]. The results of the current study agreed with the fact that the bees consumed an amount of carbohydrates in all the artificial feeding mixtures, less than the diet that contained sucrose only [27] (Figure 5).

The effect of artificial feeding mixtures on the survival of honey bee workers

The results showed in (Figure 6) that there is a significant effect (Long-rank (Matel-Cox) test, $\chi^2 = 25.36$, P = 0.0001) of laboratory artificial feeding mixtures on the survival rate of nurse honey bee workers. The control treatment (Sucrose 1 M) achieved the highest survival rate of 96.25% , followed by a treatment (15:1 P:F) with a rate of 86.25% , then the treatment (10:1 P:F) with a rate of 83.75% , which are considered very rich in protein, in while the (0:1 P:F) treatment gave the lowest survival rate of 67.50% , the two treatments (1:1 P:F) and (5:1 P:F) recorded survival rates of 81.25 and 73.75% respectively.

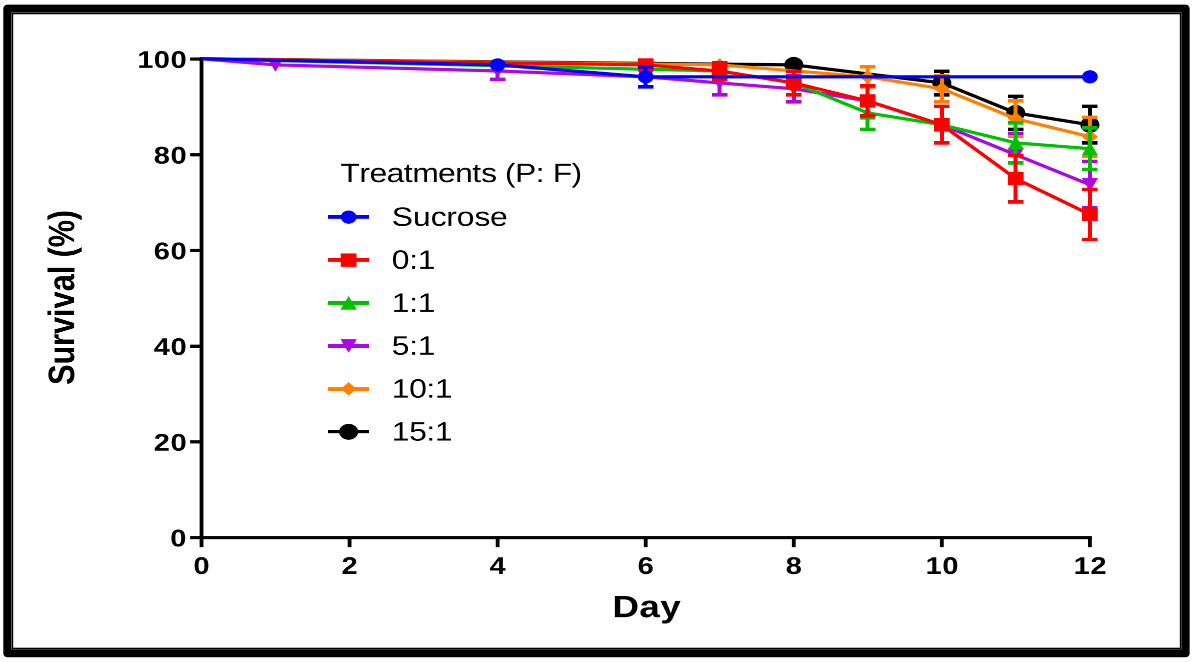

Figure (6): The cumulative survival rate of nurse honey bee workers, *A. mellifera* after 12 days of feeding with artificial lab. feeding mixtures, N = 4, with 20 bees per replicate

It conclude from the above results that increasing the protein concentration in the artificial feeding mixture had a significant positive effect on the survival of nurse honey bee workers, despite the lack of acceptance by the bees, as was previously indicated (Figure 1), the results also show that increased protein consumption had a positive impact on the survival of nurse honey bee workers (Figure 6).

The results of the current study agreed that eating a diet rich in protein improves the survival of African honey bees *A. mellifera scutellate*. Also, bees have very strong requirements for carbohydrates and carbohydrate deficiency leads to a high risk of death (Figure 6) [14]. However, it did not agree with some studies that proved that consuming excessive amounts of protein leads to the risk of death [38, 43, 44 and 45] (Fig. 6).

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