

## The Feeding Effect of Invert Sugar and Sucrose Solutions on The Quality of Produced Honey and The Performance of Honey Bee Colonies Overwintering\*

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### Abstract

Sugar nutrition is essential for managing and maintaining healthy honey bee colonies, especially during nectar scarcity. This study aimed to evaluate the use of invert sugar as a nectar substitute, comparing its impact on the quality of stored honey and colony performance during overwintering to that of a traditional sucrose solution commonly used by beekeepers. Two separate field experiments were carried out. The first experiment took place in the spring under both isolated and free-flying conditions to assess the quality of honey caused by artificial feeding. Sealed honey was collected and analyzed to determine the percentage of sucrose, glucose, fructose and HMF. Also, the pollen density in honey samples was evaluated as another detector for honey quality. The second experiment was conducted during the winter and nectar scarcity to evaluate the effects of the two supplemental sugar feedings on the overwintering performance of honey bee colonies. We measured the sealed brood and the bee bread areas to indicate the colony's growth and development. The results showed that colonies fed sucrose solutions produced honey that fulfilled standard specifications regardless of whether they were placed under an insulator or free-flying colonies, with sugar percentages and HMF levels that comply with standard specifications and no significant difference in sugar content with honey collected from colonies provided with invert sugar. In contrast, colonies provided with invert sugar had higher HMF values in the produced honey when it was the only sugar source under isolation. However, when the colonies were fed invert sugar during the winter, they became more active in collecting pollen, which increased brood rearing and made them more ready for the beginning of the active season. Because it increases pollen-collecting activity, Invert sugar can be considered as a suitable sugar supplement to honey. It may be more successful than sucrose during periods of overwintering and nectar scarcity, resulting in more brood being raised and better preparation is made before the active season. However, care should be taken because overuse at times when natural nectar sources are available could increase the HMF level in the produced honey, causing low-quality honey production.

**Keywords:** Carbohydrate, Nutrition, Nectar scarcity, Honey quality, Winter losses

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## 1. Introduction

Sugar nutrition is essential for managing and maintaining healthy honey bee colonies, especially during nectar scarcity. Supplemental sugar feeding helps to maintain bee populations by providing an essential supply of carbohydrates for energy generation and overall colony survival, particularly during the winter, which helps to maintain the hive's temperature and perform necessary activities such as brood rearing and foraging (Haydak, 1970; Brodschneider and Crailsheim, 2010; Pudasaini et al., 2020). While sugar supplements are often helpful, balancing them with natural foraging opportunities is vital to prevent any negative effects on honey quality (Kamal et al., 2019; Abdella et al., 2024).

Invert sugar, which consists of glucose and fructose, has long been used as a supplemental diet for honey bee colonies (Standifer, 1980). Bees can metabolize invert sugar better than sucrose, which beekeepers commonly use. The simpler digestion process reduces the energy bees require to convert the sugar into usable forms, resulting in a more sustainable energy source (Barker and Lehner, 1974; Frizzera et al., 2020). However, there are many concerns about the effects of invert sugar. Specifically, inappropriate preparation or storage of the solution can result in the formation of 5-hydroxymethylfurfural (HMF) (Bogdanov et al., 2004).

Honey's different flavors and nutritional benefits are responsible for its rising popularity (Yüzbaşıoğlu, 2022). The local flora, beekeeping practices, and environmental factors impact honey's composition and quality (Anklam, 1998; Kekeçoğlu, 2007; Guler et al., 2014). It is noteworthy that beekeeping practices might affect the sugar structure of honey (Cavdar et al., 2013; Majtan et al., 2021). Most people agree that honey is a functional food with many health advantages. However, feeding different syrups and sweeteners to bees during the beekeeping process can lead to adulteration, which can cause severe problems for consumers and beekeepers and may have a negative impact on honey's nutritional content and health advantages (Cordella et al., 2005; Elflein and Raezke, 2008; Guler et al., 2014).

Bee feeding is an essential technique beekeeper use to support their colonies and enhance their defenses. The additives may include honey, pollen, sugar syrup, candy or pollen paste, and substitutes. This is especially important when honey bees cannot gather enough natural resources or beekeeping scenarios, like queen-rearing, merging colonies, or improving pollination ability (Goodwin, 1997; Nisbet et al., 2018). During the blooming season, beekeepers may feed their colonies artificial sugars such as sugar syrup, high-fructose corn syrup, glucose, and invert sugar to increase honey production, which is a standard method of adulteration (Li et al., 2012; Guler et al., 2014; Geslin et al., 2017; Kanelis et al., 2022). As a result, artificially feeding honey-producing bee colonies during the blooming season is prohibited and deemed unacceptable aside from its floral or geographic location (Bertelli et al., 2010; Siddiqui et al., 2017; Cotte et al., 2004).

Its carbohydrate composition might be helpful when evaluating honey's quality and spotting adulteration. Many studies now offer many methods for estimating honey's sugar profile to assess its overall quality (Guler et al., 2014; Cavdar et al., 2013; Consonni et al., 2013; She et al., 2019; Kanelis et al., 2022). Techniques like gas chromatography with mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC) are frequently used (Ruiz-Matute et al., 2007; Cordella et al., 2005). Another method for evaluating the quality of honey samples was to analyze the pollen spectrum and pollen number quantity (Von der Ohe, 1994; Kerkvliet et al., 1995; Hermosín et al., 2003). It is also valuable for determining honey's geographical and botanical origin (Chefrour et al., 2009).

Most current research focuses on laboratory experiments rather than field feeding trials. In this study, two field experiments were conducted. The first experiment was conducted during the spring under isolation and free-flying conditions to examine the quality of honey produced after artificial feeding. The second one was carried out under apiary conditions during the winter season to investigate the impact of supplementary sugar feeding on honey bee colonies' performance.

## 2. Materials and Methods

The experiments were conducted in the apiary of the Plant Protection Department (27° 11' 04" N 31° 09' 45 " E), Faculty of Agriculture, Assiut University, Assiut, Egypt. Local hybrid Carniolan honey bee (*Apis mellifera* L.) colonies were used.

## 2.1 Sugar solutions used

Two types of sugar solutions were examined. The first type was a sucrose solution prepared at a ratio of 2:1 (W/V) sucrose to water, heated to 50 °C to dissolve the sugar (Nicolson et al., 2013). The second one was invert sugar obtained from the local market, which was analyzed using HPLC to determine its composition, as shown in *Table 1*.

## 2.2. Experimental design

### 2.2.1. First experiment: Quality of honey produced after feeding with artificial sugar

This experiment was conducted over six weeks (May- June 2023) using twelve colonies to study the quality of the honey produced. The colonies were separated into two groups. The first group consisted of six colonies of equal strength free from stored honey. All six colonies were placed inside an isolator (3.5\*6.5\*4 m) covered with a muslin mesh (16\*16 mesh); three of them were given sucrose solution (IS), while the other three received invert sugar (II). All hives were painted in different colors to help the honey bee workers' orient. The second group were free-flying colonies. Three colonies were provided with sucrose (FS), and the other three received invert sugar (FI).

After six weeks, honey samples were collected from the sealed honey frames of each colony to determine the sugar and 5-hydroxy-methyl-furfuraldehyde (HMF) content and analyzed using HPLC at the Agricultural Research Centre unit, Beekeeping Research Department, as described by Aljohar et al. (2018) and Alghamdi et al. (2020).

The pollen density in honey samples was evaluated. A modified version of the analysis method recommended by the International Commission of Botany (Louveaux et al., 1978) was performed. Ten grams of each honey sample were mixed with 10 ml of hot (40°C) distilled water, and the mixture was centrifuged for 20 minutes at 4500 rpm. The sediment was spread on a slide, fixed in fuchsin-glycerin gel, and examined using a 100-fold magnification ocular micrometer (SP1 10/0.25) microscope, Reichard Diavar for pollen analysis (Nair, 1960). The total number of pollen grains was counted in ten microscope fields, and the average was taken to calculate the number on the slide. As an indicator of pollen density, the number of pollen grains on the slide was calculated by counting the number of pollen grains in the microscopic field and calculating the area on which the sediment was spread. The area of the microscopic field was calculated using a calibrated graduated microscope lens.

### 2.2.2. Second experiment: Effect of sucrose and invert sugar feeding on bee bread and sealed brood areas

The experiment was conducted from early winter to early spring (December 2023 to March 2024) during the nectar scarcity season. Eight colonies were headed by sister queens and had equal strength with eight frames. All hives were monitored for *Varroa* infection using a sticky board (Dietemann et al., 2013). Colonies were divided into two groups, with four replicates for each treatment. The first group had a sucrose solution, considered a control group, which reflects the normal feeding practice used by beekeepers in the region throughout the winter months, while the second group had invert sugar.

A graduated cup was used to introduce a certain amount of each solution into the hives (four colonies were fed with sucrose, and the other four were fed invert sugar). The amount of solution consumed was calculated as the difference between the solution at the beginning of feeding and the amount left after 48 hours of being introduced into the colony. This procedure was repeated every seven days for each treatment, and the total consumption of each solution during the experimental period was calculated.

The sealed brood and bee bread area per colony (in<sup>2</sup>/colony) was measured every 12 days using a square-inch frame. The different areas of each colony were calculated and added together (Jeffree, 1958).

## 2.3. Statistical Analysis

Data was analyzed using GraphPad Prism 8.00 for Windows (www.graphpad.com). Parametric tests were performed after checking the normality of the data using the Shapiro-Wilk test. In the first experiment, a one-way analysis of variation (ANOVA) with a balanced, fixed-effect, independent design was followed by Tukey's post-hoc test, which was performed to compare the characteristics of honey produced. The second experiment used a student T-test to compare the effects of invert sugar and sucrose solution consumption, colonies' bee bread, and brood area. The effect of each treatment on colonies before and after winter was assessed using a paired T-test. The significance level of a test was set as 0.05.

### 3. Results

#### 3.1. First experiment: Quality of honey produced after feeding with artificial Sugar

Various characteristics were measured to investigate the quality of the resulting honey. After analysis, there were insignificant changes across the four treatments regarding the percentage of sugar types (fructose, glucose, and sucrose). All honey samples contained more fructose than glucose, which is typical for natural honey. Honey of free-flying colonies (FS and FI) showed slightly lower levels of fructose and glucose than honey collected from isolated colonies (IS and II), as shown in *Table 1*. In addition, sucrose was found in all the tested honey samples with approximately the same percentage, in the range of 2.02~2.55%. The HMF content was found to be below the permissible limit. However, the highest amount was recorded in II honey (9.79 mg/kg).

The moisture content and pH of the honey yield from free-flying colonies were higher than those inside the isolation ( $p < 0.05$ , *Table 1*). The pH of free-flying colony samples (FS and FI) was 4.0 and 4.1, respectively, while isolated colonies (IS and II) had a lower pH of 3.7 and 3.6. Pollen density was higher in the honey of free-flying colonies, especially FI ( $40796 \pm 5182$  PG/g), which had significantly more pollen than the isolated colonies ( $8548 \pm 2062$  PG/g in IS and  $9534 \pm 1524$  PG/g in II).

#### 3.2. Second experiment: Effect of sucrose and invert sugar feeding on bee bread and sealed brood areas

During the experiment, there was no difference in total consumption (ml/colony) between invert sugar and sucrose solution (*Table 2*). To measure the increase of colony growth in the two tested honey bee colony groups, bee bread and sealed brood areas were measured from the beginning to the end of the experiment (*Table 2* and *Figure 1a and b*). Statistical analysis (T-test) showed significant differences between bee bread and sealed brood areas during experimental periods due to the sugar solution feeding ( $p < 0.05$ ). In December 2023, bee bread and sealed brood areas were found to be low in all experimental colonies, with insignificant differences between the two groups ( $p > 0.05$ ). However, by March 2024, the averages of bee bread and sealed brood areas in the two groups increased significantly. Invert sugar significantly increased bee bread ( $T=11.04$ ,  $p=0.0016$  \*\*) and sealed brood areas ( $T=12.98$ ,  $p=0.0010$  \*\*\*) at the end of the experiment. Colonies supplied with invert sugar had a greater growth rate, on average, of bee bread and sealed brood areas than colonies supplied with sucrose solution, as shown in *Table 2*.

**Table 1. Chemical parameters and quantitative pollen analysis of honey samples**

	Honey samples				Invert sugar
	FS	FI	IS	II	
Fructose %	38.58 a $\pm$ 1.17	39.86 a $\pm$ 1.42	38.92 a $\pm$ 1.78	40.19 a $\pm$ 1.30	34.19
Glucose %	34.12 a $\pm$ 0.91	33.81 a $\pm$ 1.89	34.26 a $\pm$ 1.68	34.66 a $\pm$ 1.11	33.72
Sucrose %	2.56 a $\pm$ 0.56	2.38 a $\pm$ 0.83	2.52 a $\pm$ 0.68	2.02 a $\pm$ 0.38	2.09
Moisture %	20.00 a $\pm$ 0.8	19.00 ab $\pm$ 0.7	18.00 b $\pm$ 0.5	18.00 b $\pm$ 0.6	29.1
Other components %	4.74 $\pm$ 2.2	4.95 $\pm$ 1.73	6.30 $\pm$ 2.79	5.13 $\pm$ 2.66	0.9
HMF (ppm)	6.27 b $\pm$ 0.34	6.91 b $\pm$ 0.26	6.72 b $\pm$ 0.37	9.79 a $\pm$ 0.43	9.41
Ph	4.00 a $\pm$ 0.13	4.10 ab $\pm$ 0.12	3.70 bc $\pm$ 0.13	3.60 c $\pm$ 0.14	4.3
Pollen grain number (PG/ g of honey)	8894 b $\pm$ 2164	40796 a $\pm$ 5182	8548 b $\pm$ 2062	9534 b $\pm$ 1524	---

One-way ANOVA was used, followed by Tukey's post-hoc test. Means in the same row followed by the same letter do not differ significantly at the 5% level of probability, where FS: Free-flying colonies fed Sucrose, FI: Free-flying colonies fed Invert Sugar, IS: Isolated colonies fed Sucrose and II: Isolated colonies fed Invert Sugar.

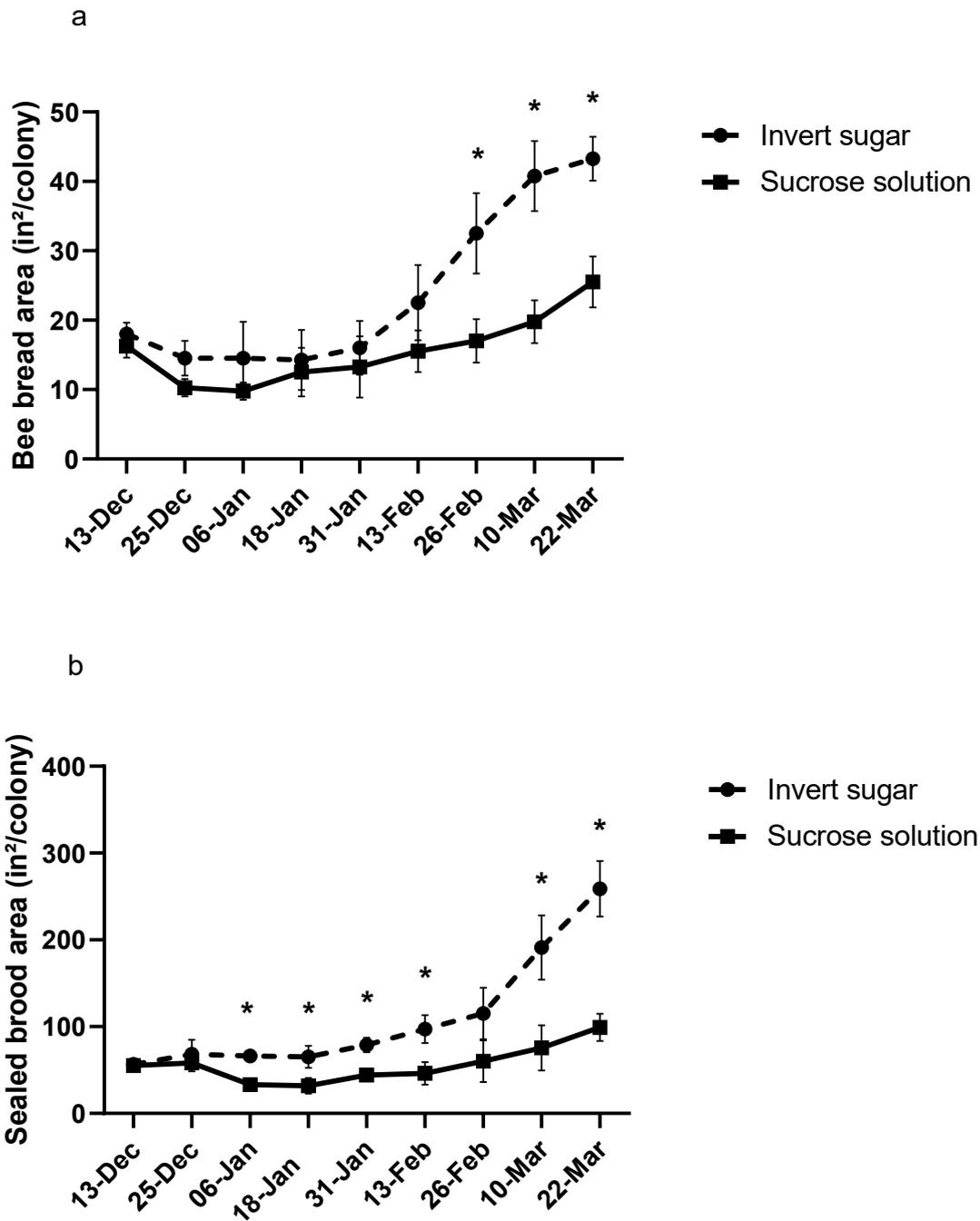


Figure 1. Shows (a) bee bread areas and (b) sealed brood areas in honey bee colonies during the winter to early spring period (n = 4 colonies). (\*) indicates a significant difference between treatments (Student T-test,  $P < 0.05$ ).

**Table 2. Solution consumption, sealed brood, and bee bread areas of honeybee colonies fed different sugar solutions at the experiment's beginning (December 2023) and end (March 2024).**

Sugar solution	Total solution consumption (ml/colony)	Sealed brood area (mean ± SD) (in <sup>2</sup> /colony)				Bee bread area (mean ± SD) (in <sup>2</sup> /colony)			
		Dec-22	Mar-23	T-test (P value)	Development (%)	Dec-22	Mar-23	T-test (P value)	Development (%)
<b>Invert sugar</b>	2682.5 a ± 157.8	56.5 a ± 7.1	258.8 a ± 31.9	12.98 (***)	362.7 ± 71.3	18 a ± 1.6	43.3 a ± 3.2	11.04 (**)	142.71 ± 37.5
<b>Sucrose</b>	2651.3 a ± 106.2	55 a ± 4.1	99.3 b ± 15.8	6.172 (**)	80.4 ± 25.5	16.3 a ± 1.7	25.5 b ± 3.7	6.441 (**)	56.9 ± 16.2
<b>T-test (P value)</b>	0.0121 (ns)	0.368 (ns)	8.945 (***)			1.481 (ns)	7.259 (***)		

A paired t-test was used to compare the effects of each sugar solution on the bee bread and sealed brood areas from December 2023 to March 2024.  $p > 0.05 = ns$  (non-significant),  $p < 0.05 = *$ ,  $p < 0.01 = **$ ,  $p < 0.001 = ***$  (highly significant) for within-group comparisons (paired t-test). Different superscript letters (a, b) within the same column indicate significant differences between treatments at the same time point (unpaired t-test,  $p < 0.05$ ). Areas were measured according to Jeffrey, 1958. SD = standard deviation.

#### 4. Discussion

The two current experiments were conducted to investigate the effect of sugar feeding on the potential impact on honey quality and colony performance overwintering. In the first experiment, the honey-production conditions affected specific physicochemical characteristics of honey, such as whether the colonies were fed sucrose or invert sugar and whether they were isolated or free-flying. The only monosaccharides in honey are glucose and fructose, which, when mixed in various ways, make up the di- and trisaccharide components of floral honey. According to surveys of floral honey composition, the three largest components are fructose, glucose, and water, with averages of 38.2, 31.3, and 17.2% (Doner, 1977). In the current study, isolation conditions helped produce honey with less moisture content. The average amount of monosaccharides ranged between 72.86 and 74.85%, meeting the Egyptian standards ES: 355-1/2005 and Codex Alimentarius Commission on Sugar (2001). It is also considered a suitable range compared to many other honeys, which can contain up to 80% sugar (Juan-Borrás et al., 2014; Rodríguez-Flores et al., 2019). The fact that honey contains more fructose than glucose is one of its primary qualities. In honey, there are around 40% fructose and 30% glucose, although these numbers can vary based on several factors, including nectar source, temperature, and length of storage (White and Doner, 1980). Sucrose was also present in all samples, but all were less than 5%. Except for honey from a few plants, the minimal amount is ideally no more than 5% (ES: 355-1/2005; Codex Alimentarius Commission on Sugar, 2001).

Beekeepers typically offer artificial sugar feeds to honeybee colonies to ensure effective overwintering and during periods of nectar scarcity. There are various types of nectar substitutes. Traditionally, sugar beets or cane have been applied successfully. Invert sugar syrups, which contain simple sugars derived from starch or sucrose, have recently gained popularity (Přidal et al., 2023; Rana and Singh, 2024). In the present investigation, the sugar solution feeding under free-flying (sucrose vs. invert sugar solutions) or isolation conditions did not affect the honey's essential sugar content (fructose, glucose, and sucrose), as these values were similar throughout all samples. Our results are consistent with those obtained by Farag and Rag (2020) and Přidal et al. (2023). However, when colonies depended on sugar solutions only, moisture percentage, pH, and pollen density were affected in the isolation condition. Moisture content and pH levels of honey slightly decreased under the isolator compared to the free-flying colony samples. This result suggested that isolation conditions might provide a more controlled environment where foragers are prevented from bringing nectar from any other source. As reported, botanical sources affect honey's physical and chemical characteristics (El Sohaimy et al., 2015; Adalina et al., 2024). According to Lieux (1972) and Chefrou et al. (2009), the total quantity of pollen grains can distinguish between different honey varieties. The high pollen count in free-flying samples reflected additional floral sources accessed by the free-flying colonies. This was demonstrated in FI colonies with a significant content of pollen grains (*Table 1*).

The high HMF level observed in treatment group II was exceptionally elevated, primarily because invert sugar syrup was the sole carbohydrate source provided to the hives maintained inside the insulator. Notably, this syrup already had a high initial concentration of HMF (as shown in *Table 1*), which likely contributed to the overall increase in HMF levels detected in the resulting honey. In contrast, free-flying hives that had access to natural forage (nectar from flowering plants) exhibited lower HMF levels, suggesting that dilution or replacement of the invert syrup with nectar from natural sources helped mitigate HMF accumulation. This highlights the buffering effect of natural nectar sources in maintaining honey quality. From a chemical standpoint, HMF is a known degradation product formed during the breakdown of fructose, particularly under acidic and high-temperature conditions. During the industrial or domestic preparation of invert sugar, sucrose is hydrolyzed into its monosaccharide components - glucose and fructose - typically in an acidic environment. When this process is conducted at elevated temperatures (common in syrup production), it can significantly enhance the formation of HMF. Fructose is more reactive than glucose in this context and is especially prone to acid-catalyzed dehydration, leading to the formation of HMF (Kowalski et al., 2013; Saklani and Kumar, 2021). Thus, feeding bees with pre-processed invert syrups, particularly those stored under suboptimal conditions (e.g., heat exposure), may pose a risk of HMF contamination in honey, which is not only a marker of honey deterioration but also a potential toxicant to honeybees at high concentrations.

The second experiment was conducted from the beginning of winter through early spring. During that time, honeybee colonies were maintained under controlled conditions and provided with supplementary carbohydrate

feeding in the form of two sugar solutions: sucrose syrup and invert sugar syrup. Interestingly, the rate of sugar consumption did not show any statistically significant difference between the two feeding groups. Both syrups were consumed at similar rates, a finding that aligns with the results reported by Pridal et al. (2023), who observed comparable consumption behaviour in overwintering colonies regardless of sugar type. However, despite similar consumption patterns, colonies fed invert sugar exhibited superior physiological and developmental performance. Specifically, these colonies showed significantly higher brood production and greater stores of bee bread in the spring and the beginning of the active season. These observations were consistent and measurable under the environmental and climatic conditions of the Assiut region (Table 2). Regarding honey storage, it is interesting to note that the second experiment was conducted during the winter season, when nectar availability is limited and colonies typically do not store honey. As a result, honey yield was not deemed an essential parameter in the current study.

Melnichuk (1964) suggested that invert sugar promotes the growth of overwintering colonies because the invert sugar solution does not require bee enzymes for metabolism, unlike sucrose. The higher digestibility of invert sugar may have increased colony activity (Taylor et al., 2019), enabling bees to collect more pollen when available. This improved protein intake could explain the observed increase in bee bread storage and sealed brood area. Hence, invert sugar feeding has the lowest tendency to physiologically exhaust workers (Barker and Lehner, 1974; Frizzera et al., 2020). However, in subsequent experiments, Ceksteryte and Racys (2006) and Pridal et al. (2023) found that there was no significant difference in the colony strength between those supplemented with invert sugar and those fed sucrose solution, and many other factors affected the colonies' subsequent development in spring. Therefore, confirming that the colonies' physiological exhaustion could be avoided while supplementing with invert sugar was impossible. Another possible explanation for the increased pollen collecting and brood raising in colonies fed invert sugar is that carbohydrates improve protein digestion and processing (Abdella et al., 2024). Efficient carbohydrate metabolism may enhance nurse bee activity by boosting their ability to utilize nutritional proteins from pollen and bee bread, which helps in hypopharyngeal gland development (Omar et al., 2017) and hence promotes brood growth (DeGrandi-Hoffman and Chen, 2015; Brodschneider and Crailsheim, 2010).

In the present study, invert sugar feeding supported the colonies in collecting more pollen, increasing the stored bee bread area and brood development. Protein is thought to have an essential function in the growth and rearing of brood in colonies (Herbert et al., 1977; Brodschneider and Crailsheim, 2010; Omar and Amro, 2023). Overwintering colonies fed invert sugar had a larger stored bee bread area, which appeared to be a significant difference from the seventh week until the end of the experiment. This was also reflected in the sealed brood areas, particularly at the end of the experiment in the early spring, which is considered the most critical period in which the colonies prepare for the activity season (Figure 1). These findings demonstrate that invert sugar syrup is a more efficient supplemental feed for increasing brood production in honeybee colonies, most likely due to its better digestibility and rapid bioavailability compared to sucrose. The higher metabolic efficiency associated with monosaccharides (glucose and fructose) found in invert syrup may aid in energy utilization required for thermoregulation and brood rearing, especially under inadequate foraging settings. Our findings are consistent with those published by Tsvetanov and Balkanska (2025), who also observed improved colony performance and greater brood area in colonies fed invert syrup as a primary carbohydrate source during nectar scarcity.

## 5. Conclusions

Finally, invert sugar is considered a suitable sugar supplement to honey and may be more effective than sucrose during periods of overwintering and nectar scarcity, as it increases pollen-collecting activity. This results in increased brood rearing and better preparation before the active season. However, caution should be taken because excessive use during periods of abundant natural nectar sources may lead to the production of adulterated and poor-quality honey.

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### **Ethical Statement**

To achieve the goals of this study, no approval of the Research Ethics Committee was required, as the experimental work involved an unregulated invertebrate species (*Apis mellifera*).

### **Conflicts of Interest**

We declare that there is no conflict of interest between us as the article authors.

### **Authorship Contribution Statement**

Concept: Omar, E. M.; Design: Omar, E. M.; Data Collection or Processing: Abdella, M.; Statistical Analyses: Omar, E. M.; Literature Search: Abdella, M.; Writing, Review and Editing: Omar, E. M., Rateb, S. H., Khodairy, M. M.

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