Journal of Apicultural Research

ISSN: 0021-8839 (Print) 2078-6913 (Online) Journal homepage: www.tandfonline.com/journals/tjar20

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To cite this article: Eslam M. Omar, Mostafa Abdella, Salah H. Rateb, Mohammed M. Khodairy & Abdulraouf M. Amro (02 Oct 2025): Impact of some sugar types on hemocytic response, wax glands development, and performance of honey bee, Journal of Apicultural Research, DOI: 10.1080/00218839.2025.2564524

To link to this article: https://doi.org/10.1080/00218839.2025.2564524

Published online: 02 Oct 2025.

JOURNAL OF APICULTURAL RESEARCH
https://doi.org/10.1080/00218839.2025.2564524

ORIGINAL RESEARCH ARTICLE

Impact of some sugar types on hemocytic response, wax glands development, and performance of honey bee

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ABSTRACT

Sugar solutions are a valuable tool in the beekeeper's arsenal. Understanding the nutritional value and diverse effects of sugar solutions on individual and colony populations allows beekeepers to ensure the successful wintering of honey bee colonies. The efficacy of feeding three types of sugar solutions (sucrose, glucose + fructose, invert sugar) compared to honey as a control on honey bees' physiological and morphological parameters was studied during nursing. The results showed that the feeding type influenced consumption, digestibility, wax gland development, and hemocytic parameters. While honey topped the charts in all parameters, sucrose also reflected improvement of wax gland development (oenocytes area) and significantly increased hemolymph plasmatocytes as an index for bee immunity, making it a compelling alternative among the tested solutions. The glucose and fructose solution mixture exhibited neither significant bee attraction nor positive impacts on the fourth wax mirror area, wax gland development, or bee immune response. Invert sugar exhibited the lowest consumption and bee attraction among the solutions, yet it demonstrated a noteworthy effect on the fourth wax mirror expansion. The highest spindle hemocyte percentage of bees fed Invert sugar suggested they experienced stress. In this study, honey, followed by sucrose, is the best feeding choice to prepare bee colonies for winter to promote honey bee immunocompetence. While invert sugar potentially offers several economic advantages. Its limited influence on key colony parameters, such as wax production and overwintering success, requires further study.

ARTICLE HISTORY
Received 17 February 2025
Accepted 9 June 2025

KEYWORDS
Honey bee nutrition; wax production; hemocytes; wax mirrors; inverted sugar; honey alternatives

Introduction

Several investigations pointed out honey bee starvation as the primary cause of the disastrous phenomenon of colony losses, either due to the lack of pollen and carbohydrate sources. Nectar availability is relevant to certain climatic circumstances (Neov et al., 2019; Polatto et al., 2014; Takkis et al., 2015). Thus, bees may experience food shortages during floral scarcity or seasonal dearth. Recent studies show that bee starvation may cause physiological effects. Lack of food limits fat body formation and inhibits the production of vitellogenin, a critical storage protein for immunity and lifespan (Kunat-Budzyńska et al., 2025; Wang et al., 2016), and insufficient food has been associated with shortened lifespan and colony overwintering success (Güneşdoğdu et al., 2024). Accordingly, carbohydrate-rich supplementary food should be supplied for colonies to develop their biological activities (Brodschneider & Crailsheim, 2010), improve honey bee survival, immunity (Abou-Shaara et al., 2023), tolerance to harsh conditions (Abou-Seif et al., 1993), and may reduce wintering losses (Castaños et al., 2023). Various forms of carbohydrates such as sucrose (disaccharide) (Barker & Lehner, 1973), invert sugar (IS) (Taylor et al., 2019), high fructose corn syrup (Quinlan et al., 2023; Severson & Erickson, 1984), rice syrup (Li et al., 2020), and starch syrups (Szczęsna et al., 2021) are considered good alternatives for nectar.

The most prevalent sugars in nectar are glucose, fructose, sucrose, maltose, galactose, raffinose, and mannose (Waddington, 1985). Before bees can process making the honey, they must first convert the disaccharides into monosaccharides and undergo enzymatic transformation (Barker, 1977) because only monosaccharides can pass to the bee's hemolymph and be utilized by cells (Crailsheim, 1988). The yield mixture of invertase action is known as an IS, composed of an equal ratio of D-glucose and D-fructose (Lombard et al., 2014).

Over the last decade, IS syrup has gained favor among Egyptian beekeepers over regular sugar

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syrup for bee feeding. This preference stems from two key reasons. The first one, IS syrup, is generally lower cost. Secondly, it crystallizes less, making it more accessible to bees, especially during colder months. This, in turn, allows beekeepers to stimulate hive activity and effectively provide supplemental feeding whenever necessary. Ceksteryte and Racys (2006) documented that feeding inverted sugar syrup to winter bees prevents exhaustion, as almost all sucrose was previously converted to glucose and fructose before storage. However, Vranić et al. (2017) mentioned that inverted sugar syrup produced a very high content of 5-hydroxymethyl furfural (HMF) about 25.80 times compared to authentic honey because of the heating of sugars and the addition of citric acid to the inversion of sucrose, given concerns about HMF's potential toxicity to bees, which may contribute to increased winter mortality, as raised by numerous authors (Gregorc et al., 2019; Přidal et al., 2023; Vranić et al., 2017; Zirbes et al., 2013). In addition, (Mirjanic et al., 2013) concluded that bees fed with an invert syrup produced through acid hydrolysis had damage to the epithelial layer of their mesenteron in comparison with sucrose feeds.

Hoarded nectar of honey bees is kept in a comb made of wax produced by the wax glands of adult workers, located in the abdomen, at the anterior part of sterna IV to VII (Cassier & Lensky, 1995). Therefore, studying wax gland development is critical for understanding honey bee colony growth since wax makes comb frames for storing food and brood. Efficient wax production indicates colony health and productivity, whereas reduced gland activity may suggest stress, insufficient nutrition, or disease (Seeley, 2009; Winston, 1991). Monitoring wax gland growth provides knowledge of colony behaviors and helps improve bee health. A wax mirror consists of a system of microtubules that transport wax precursors from fat body cells and oenocytes to the surface of the cuticle, where they solidify and crystallise to become wax scales (Cassier & Lensky, 1995). Oenocytes also play an essential part in wax formation by producing fatty acids and hydrocarbons, which are then transferred to the wax glands for secretion (Cavalcante et al., 2000). This process is critical for constructing and maintaining comb structures. Disruption in oenocyte activity might impair wax formation, hence affecting colony development. Previous research has linked protein to wax gland growth (Abou-Shaara, 2024; Al-Ghamdi et al., 2021; Shawer & Mousa, 2016); or prebiotic and probiotic feed supplementation (Bugarova et al., 2021; Pătruică et al., 2012), but carbohydrate's impact is unknown.

Numerous investigations have been conducted to determine the optimal and cheaper options of carbohydrate feed that provide enough nutritional value for honey bees, especially after international sugar prices increase (Abou-Shaara et al., 2023; Sammataro & Weiss, 2013). Specific parameters are usually used to judge the efficacy of specific carbohydrates for feeding honey bee colonies. These parameters include colony performance (Abou-Shaara et al., 2023), survival (Abdella et al., 2024; Al-Ghamdi et al., 2021; Škerl & Gregorc, 2014), and some criteria such as wax production (Carrillo et al., 2015) and hemolymph parameters (Abou-Shaara et al., 2023; Amro et al., 2016). However, the role of carbohydrate feeding types on honey bees' histological, physiological, and hematological parameters remains obscure. To further contribute, the present study has been established to understand the real impact of sucrose syrup, honey, fructose, glucose, and inverted sugar syrup on honey bee workers' consumption, survivability, digestibility, wax gland, and wax mirror development, and hematological response.

Materials and methods

The experiments were conducted in the Economic Entomology Laboratory, Department of Plant Protection, Faculty of Agriculture, Assiut University, Assiut, Egypt, in May 2023, during the active season of honey bee colonies.

Bees used

One colony of local hybrid, Carniolan honey bee workers (*Apis mellifera carnica polamannl* × *A. m. lamarkii cockerell)* without any detected diseases was selected. Three sealed brood combs were chosen and incubated at 34.5 °C and 65% relative humidity (Williams et al., 2013) to obtain newly emerged honey bee workers (0–24 h old). The next day, newly emerged bees were collected from each brood comb, combined, and randomly divided into groups for the tested treatments.

Cages design

Wooden cages (15 × 15 × 5.5 cm) were used, closed with a movable glass panel on one side (to remove bees), and the other side was covered with a grid (to allow air ventilation). A wax comb was attached to the side of each cage to simulate the natural conditions experienced by honey bees. A 5 ml syringe is used to introduce the tested sugar solutions through a hole in the top of the cages. Sixteen cages were divided into four groups (four cages each), with 100 worker bees in each cage. Throughout the experiment, all cages were kept in a dark incubator at

Table 1. Chemical parameters and sugar percentage of clover honey, sugar solutions, and invert sugar used to feed bees.

	Clover honey	Diluted honey (HS)	Invert sugar (IS)	SS	G + F
Fructose %	38.65	19.33	34.19	–	25
Glucose %	34.21	17.11	33.72	–	25
Sucrose %	2.55	1.28	2.09	50	–
Moisture %	20	60	29.1	50	50
Other components %	4.59	2.29	0.9	–	–
HMF	6.72 ppm	3.36 ppm	9.41 ppm	–	–
pH	4	4.1	4.3	7.53	7.03

34.5 °C and 65% relative humidity (Brodschneider et al., 2022; Williams et al., 2013).

Tested sugar solutions

The tested feeding solutions were prepared daily to ensure they were not fermented, as follows:

1. Clover honey solution (HS) mixed with water (1:1, w/v). Egyptian clover honey is the predominant honey available at low prices and can be used by beekeepers to feed honey bee colonies.
2. Sucrose solution (SS): was made by mixing table sugar with water (1:1, w/v).
3. Glucose and fructose (G + F): Water was saturated with an equal amount of glucose and fructose powder ratio (1 glucose: 1 fructose: 2 water, w/v).
4. IS: fresh commercial solution obtained from the local market.

To measure the sugar and 5-hydroxy-methyl-furfuraldehyde (HMF) content of clover honey and IS, HPLC analysis was performed at the Agricultural Research Centre unit, Beekeeping Research Department, as reported by Aljohar et al. (2018) and Alghamdi et al. (2020). Also, a pH meter was used to determine the acidity of all examined solutions (Table 1). All treatments were provided with a protein-rich diet of pollen mixed with 10% water to form a homogeneous dough. The pollen dough was frozen and thawed at room temperature before being given to the cages daily. The protein source was provided to all cages for 12 days, as worker bees consume less protein at this age (Crailsheim et al., 1992; Omar et al., 2017).

Consumption rate

From the beginning of the experiment, all sugar solution consumption was measured daily using the syringe scale. Pollen consumption was measured by weighing the dough before and after 24 h of introducing it to cages using a sensitive electronic balance. Solutions and pollen dough consumption were calculated for precisely 24 h per bee. Dead bees were removed from the cages and considered to have no consumption that day.

Digestibility

To measure the honey bee's ability to digest the food provided, on day 12, three workers from each replicate were randomly selected ($n = 12$), and the hindgut was dissected, weighed, and compared to total protein consumption (Slansky & Scriber, 1985; DeGrandi-Hoffman et al., 2016 and Omar et al., 2022; Abdella et al., 2024).

Wax gland parameters

Areas of wax oenocytes

Three 16-day-old bees were chosen randomly from each cage, with 12 bees from each treatment. After anaesthetization, the worker bee was fixed ventrally on a wax plate. Dissection was performed using scissors and forceps. Two superficial cuts were made along the lateral side of its abdomen exoskeleton. A binocular dissecting microscope (Olympus SZ 40) was utilized to make a more precise distinction. To obtain a clear view of wax oenocytes, according to Svečnjak et al. (2019) and improved by the authors, the wax glands were extracted and then transferred to a mixture of ether and xylan in a 1:1 ratio for three days to clear the tissue in an embedding agent to dissolve the lipid layer around the oenocytes. Using a light microscope (Olympus –CH20i BIMU), the oenocyte areas were measured by Image J software according to Schneider et al. (2012) and Mohamed et al. (2023).

The fourth wax mirror area

The morphology of the fourth wax mirror area was also assessed to predict the development of bee workers. The bees used in a previous experiment and wax gland dissection were also used to measure the wax mirror areas. The fourth wax mirror was removed following the established wax gland dissection protocol. The area of wax mirrors was also measured using a binocular dissecting microscope (Olympus SZ 40) and the Image J software program.

Hematological studies

Differential hemocytes count

In this experiment, 9 and 16-day-old worker bees were used. 12 workers from each group (3 from each replicate) were randomly chosen for the differential

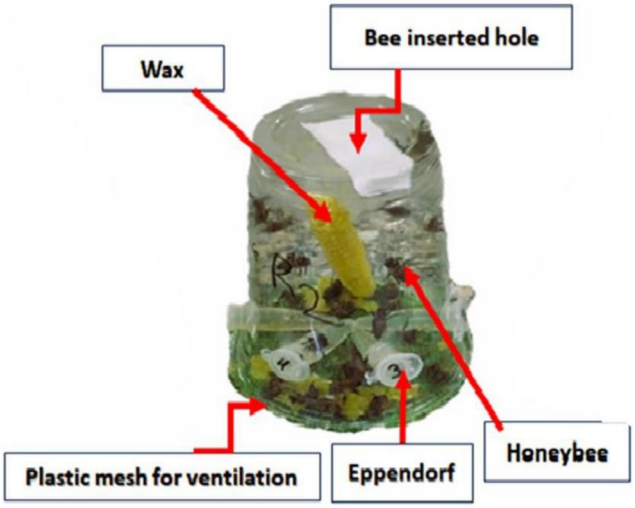

Figure 1. Description of the cage parts used in the experiments.

hemocytes count (DHC). A drop of hemolymph (~5 µl) was collected from the head of each adult worker according to the method of Borsuk et al. (2017). The drop was then placed on a clean slide and spread with the edge of another one. The swabs were immediately dried at room temperature and soaked in ethyl alcohol for 5 min. The slides were then air-dried, stained with Romanowski-Giemsa stain diluted (1 Romanowski-Giemsa stain: 9 distilled water) for 15 min, air-dried, and then washed in distilled water for 5 min before being air-dried (Omar & Amro, 2023).

The prepared slides were examined under a light Microscope (Olympus—CH20i BIMU) with an objective lens (40 magnification). Five types of hemocytes were identified and classified using Ribeiro and Brehélin (2006) key as prohemocytes (PR), plasmatocytes (PL), granulocytes (GR), spindle cells (SP), and spherocytes (SPH). Each type of hemocyte in each prepared smear was counted in ten microscopic fields, and the percentage of each type was calculated for each diet.

Total soluble solid percentage

The total soluble solid percentage (TSS %) was determined in 9 and 16-day-old worker bees ($n = 10$) by refraction index using a hand refractometer (Euromex Brix, USA), according to method 22.024 of the AOAC (Association of Official Analytical Chemists, 1984).

Preference for sugar solutions

This experiment was designed to determine honey bees' preferences for different sugar solutions. Plastic cups were used as experimental cages, with three cups as replicates. Each cup was modified by creating four holes on the sides to insert 1.5 ml Eppendorf tubes containing the various sugar solutions, with three holes at the end to allow the bees to suck the solution from the inside (Figure 1). A wax comb was placed in each cup to simulate a natural environment. The cup nozzle was covered with plastic mesh to allow for ventilation. For each replicate, 15 forager bees were collected from the entrance of the hives and introduced into the cups through a hole in the base of the cup. Before the experiment, the bees were starved for two hours to ensure they would actively consume the sugar solutions provided upon introduction. Bee attraction to each sugar solution was assessed based on the number of bees visiting each Eppendorf tube. The number of bees in each solution was recorded at three-time intervals: 5, 30, and 60 min after the experiment started.

Statistical analysis

The Costate software (Version 6.303, CoHort, USA, 1998–2004) and GraphPad Prism 8.00 for Windows were used to perform a normal distribution analysis (Shapiro-Wilk) and an analysis of variance (ANOVA) procedure on all gathered data. Means were compared using Duncan's multiple range tests at $p \leq 0.05$ (Duncan, 1955).

Results

Food consumption

The total solution consumption (µl/bee/18 days) significantly varied among the four tested sugar solutions ($p < 0.001$, Figure 2a). The highest consumption was observed for the HS group (682.1 ± 15.5 µl/bee/18 days), followed by SS (440.7 ± 36.5 µl/bee/18 days), then G + F (328.5 ± 24.2 µl/bee/18 days), and the lowest for IS (272.1 ± 21.2 µl/bee/18 days).

The type of sugar solution provided significantly influenced the bee pollen intake ($p = 0.0002$, Figure 2b). Among the different treatments, the highest pollen

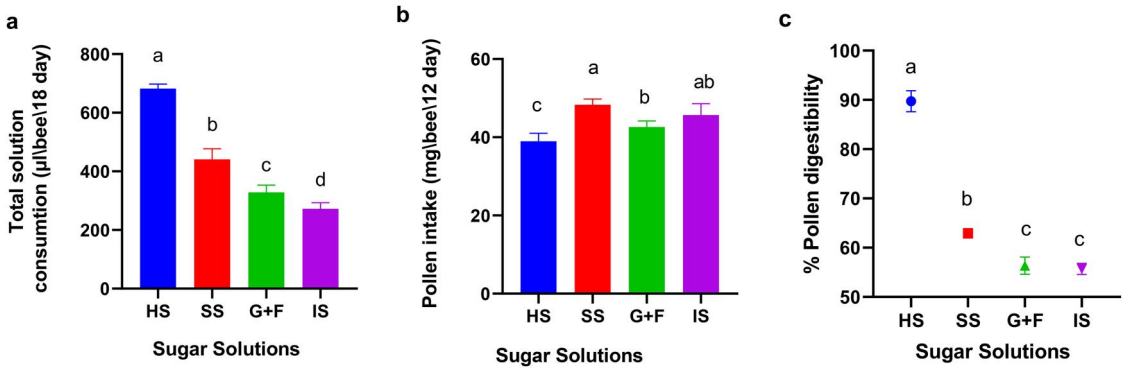

Figure 2. (a) Total consumption of carbohydrate solutions (µl/bee/18 days); (b) Protein intake (mg/bee/12 days); (c) digestibility % by honey bee workers feeding with HS (clover honey), SS (Sucrose solution), G + F (Glucose and fructose), and IS (invert sugar) for the experiment period. Letters within solutions indicate statistically significant differences ($p < 0.0001$).

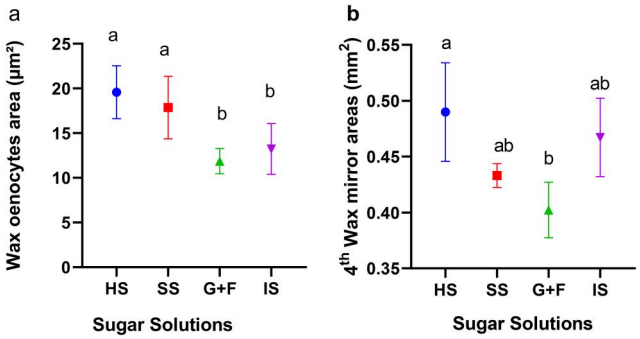

Figure 3. (a) Wax oenocyte areas (μm^2) and (b) 4th wax mirror area (mm^2) influenced by carbohydrate types: HS (honey solution), SS (Sucrose solution), G + F (Glucose and fructose), and IS (invert sugar). Letters within solutions indicate statistically significant differences ($p < 0.05$).

intake was observed in the SS group (48.33 ± 1.4 mg/bee/12 days), significantly greater than HS (38.98 ± 2.1 mg/bee/12 days). The G + F group exhibited intermediate pollen consumption (42.65 ± 1.5 mg/bee/12 days), significantly higher than HS but lower than SS. Interestingly, IS (45.66 ± 2.9 mg/bee/12 days) did not differ significantly from SS but was also categorized with G + F.

Pollen digestibility also differed significantly between sugar solution treatments (Figure 2c). The HS group had the highest pollen digestibility (89.72 ± 2.1%) compared to all other groups ($p < 0.0001$). The SS group had an intermediate digestibility (62.92 ± 0.96%), significantly lower than HS but higher than G + F and IS, with similar digestibility values (56.34 ± 1.8% and 55.69 ± 1.1%, respectively). Mortality rates ranged from 10.75% to 14.75% across treatments, with no statistically significant differences detected ($p > 0.05$).

Morphological parameters

The HS and SS groups showed the highest wax oenocyte areas (19.58 ± 2.6 and 17.86 ± 3.5 μm^2, respectively), with no significant difference ($p > 0.05$, Figure 3a). The G + F and IS groups had significantly reduced oenocyte areas (11.87 ± 1.4 and 13.23 ± 2.9 μm^2, respectively), showing no significant difference but a significant decrease compared to HS and SS. This suggests that HS and SS were more effective diets for wax production than IS or a mixture of G + F.

The analysis of the 4th wax mirror areas (mm^2) across the different sugar solutions indicated substantial differences in effect. As shown in Figure 3b, the HS group had the largest mean wax mirror area (0.49 ± 0.04 mm^2), which was significantly different from the G + F group (0.40 ± 0.02 mm^2, $p < 0.05$), which exhibited the smallest wax mirror area among all treatments. The SS and IS treatments showed intermediate values (0.43 ± 0.01 and 0.47 ± 0.04 mm^2, respectively), with no statistically significant difference from either HS or G + F.

Hematological studies

DHC

Five hemocyte types were identified in the hemolymph of 9- and 16-day-old worker bees across all tested sugar solutions: PR, PL, GR, SP, and SPH. Table 2 demonstrates a significant variation in hemocyte composition across different sugar diets (HS, SS, G + F, and IS). The largest hemocyte type in all treatments was (PL), followed by (GR) and (SPH), while (PR) had the lowest percentage in all groups.

Table 2. Effect of tested carbohydrate solutions on the relative abundance of 9and 16-day-old worker's hemocytes type.

	Hemocyte types (% ± SD)									
	PR		PL		GR		SP		SPH	
Solutions	9 d	16 d	9 d	16 d	9 d	16 d	9 d	16 d	9 d	16 d
HS	1.81 ± 0.34 ab	0.70 ± 0.17 a	51.92 ± 2.45 b	57.97 ± 2.71 a	34.97 ± 2.13 a	39.13 ± 3.01 a	0.79 ± 0.16 d	0.41 ± 0.13 d	10.48 ± 1.26 b	1.78 ± 0.35 ab
SS	1.47 ± 0.28 bc	0.73 ± 0.34 a	51.12 ± 4.36 b	55.80 ± 2.79 ab	32.46 ± 4.33 a	38.97 ± 2.90 a	1.65 ± 0.28 c	2.33 ± 0.33 c	13.27 ± 1.58 a	2.15 ± 0.45 a
G + F	1.92 ± 0.51 a	0.65 ± 0.26 a	58.79 ± 3.46 a	54.03 ± 5.58 b	25.59 ± 2.99 b	38.00 ± 5.14 a	3.41 ± 0.47 b	5.76 ± 1.61 b	10.27 ± 1.62 b	1.55 ± 0.68 b
IS	1.12 ± 0.69 c	0.39 ± 0.14 b	58.13 ± 2.20 a	53.65 ± 3.09 b	23.84 ± 2.47 b	36.57 ± 3.80 a	8.50 ± 1.73 a	7.99 ± 2.19 a	8.39 ± 2.34 c	1.90 ± 0.56 ab
F	6.56	5.00	4.19	2.88	35.75	1.64	170.87	73.28	15.88	2.70
p ANOVA	0.0009***	0.0045**	<0.0001***	<0.028*	<0.0001***	0.0194 ns	<0.0001***	<0.001***	<0.0001***	0.057 ns

Tested sugar solutions: HS (honey solution), SS (Sucrose solution), G + F (Glucose and fructose), and IS (invert sugar syrup). Means in the same column followed by the same letter do not differ significantly at the 5% level of probability (PR, prohaemocytes; PL, plasmatocytes; GR, granular cells; SP, spindle cells; SPH, spherulocytes). Values are mean ± SD = standard division.

PL amounts were significantly lower in bees fed SS, G + F, and IS than those fed HS ($p < 0.05$). The SS, G + F, and IS treatments showed a significant decrease in GR compared to HS, confirming that these diets affect specific hemocyte populations. In contrast, HS (SP) increased significantly with SS, G + F, and IS treatments. The bees fed IS showed the most significant increase at both ages ($p < 0.0001$), followed by those fed G + F and SS, suggesting that artificial sugar solutions have a greater impact on spindle development. Notably, SS-fed bees showed hemocyte levels more like HS-fed bees, especially concerning PL and GR levels. This suggests that the benefits of natural honey in maintaining certain hemocyte distributions may be partly matched by sucrose.

Overall, the results show that the composition of the sugar solution affects hemocyte differentiation, with IS and G + F causing an increase in more significant SP percentages. At the same time, HS maintains higher PL and GR amounts. These results demonstrate that dietary sugar sources affect worker bees' immune-related hemolymph cell composition.

Total soluble solid percentage (TSS %)

The TSS% in 9-day-old worker bees did not differ significantly among the studied sugar solutions ($p > 0.05$, Figure 4). However, in 16-day-old worker bees, the type of sugar solution had a significant impact on TSS%. The highest TSS% was seen in bees-fed SS (14.51%), followed by G + F (12.59%) and IS (11.44%). In contrast, HS had the lowest TSS% (10.48%). Bees fed SS showed significantly higher TSS% than HS- and IS-fed bees ($p < 0.05$), with G + F-fed bees showing intermediate values. The results suggest that the sugar supply had an important impact on hemolymph TSS%, especially in older worker bees.

Sugar solution preference

The experiment assessed honey bees' preferences for the different sugar solutions examined. Figure 5 represents the mean percentage of bees attracted to each solution across three experimental replicates at three different times: 5-, 30-, and 60-min. HS was the preferred carbohydrate source, attracting the highest percentage of bees (36.74 ± 7.5%) and showing a significant difference from other sugar solutions ($p < 0.05$). SS had a moderate attraction rate of 27.18 ± 2.6%, which was not significantly different from HS ($p > 0.05$) but was still higher than the other tested solutions. G + F attracted about 22.87 ± 1.9% of bees, significantly lower than HS and SS ($p < 0.05$), while IS had the lowest attraction, with only 13.22 ± 7.2% of bees choosing it.

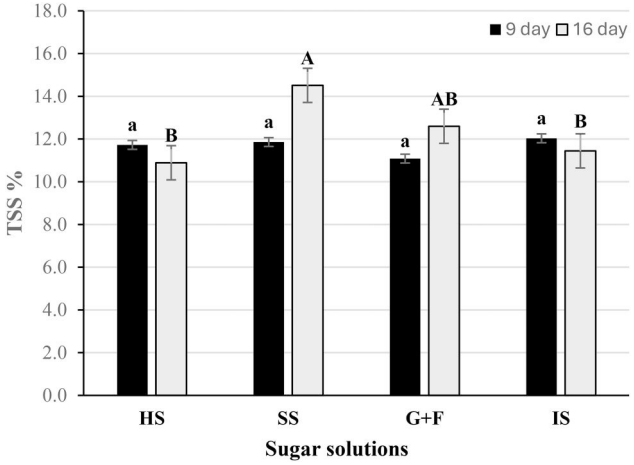

Figure 4. Effect of different carbohydrate solutions (HS = Honey Solution, SS = Sucrose Solution, G + F = Glucose + Fructose Solution, and IS = Inverted Sugar) on honey bee workers' total soluble solids percentage (TSS%). Statistical differences were analyzed using a one-way ANOVA for each age group separately. Means denoted by different small letters indicate significant differences among 9-day-old workers, while capital letters indicate significant differences among 16-day-old workers ($p < 0.05$).

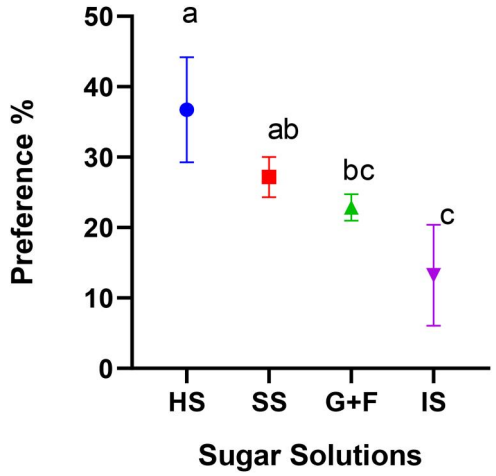

Figure 5. Preference percentages of carbohydrate types in the free choice set are HS (Honey solution), SS (Sucrose solution), G + F (Glucose and fructose), and IS (invert sugar). Letters within solutions indicate statistically significant differences ($p < 0.05$).

Discussion

In this study, the solution intake test highlights that honey intake significantly overshadowed the other solutions. On average, they consumed HS twice as much as SS and three times as much as IS or G + F. This interpretation is consistent with HS being a more absorbable energy source than glucose, fructose, or a mixture of G + F. According to Barker and Lehner (1973), bees prefer HSs to sucrose or mixed sugars containing 13 different sugars. On the other hand, Hough and Phadnis (1976) reported that sucrose was the most attractive of all sugar solutions. This is also demonstrated by the bees' preference for honey and sucrose in a multiple-choice experiment. Honey contains sugars (mainly glucose and fructose) and small amounts of minerals, amino acids, and bioactive compounds that may attract bees (Bogdanov et al., 2008; Gheldof et al., 2002). This study found that IS and a mixture of glucose and fructose were the least preferred carbohydrate solutions. This may be related to reduced digestibility of fructose-based syrups, as reported by recent studies showing negative impacts on gut microbiota and bee digestibility efficiency (Abdella et al., 2024; Quinlan et al., 2023), making these solutions less consumed than naturally balanced nectar sources. The higher consumption of HS compared to other solutions may be attributed to its lower sugar concentration after dilution. Bees regulate their intake to meet energetic demands and thus may consume larger volumes of less concentrated solutions (Barker & Lehner, 1974). This also explains the reduced consumption of IS by bees. The variation in consumption may also be due to the scent of the sugar solutions, which influences the bees' response and consumption (Pel et al., 2023). Another factor that may influence bees' consumption is the acidity of sugar solutions (Waller, 1972). HS, with a pH of 4.1, was the most consumed. However, the IS, which has a similar pH (4.3), recorded the lowest consumption, likely due to HMF, which negatively affects bee acceptance and health (Frizzera et al., 2020). These findings suggest that the type and concentration of sugar solution influence consumption behavior, with HS being the most preferred and IS the least preferred. This trend may reflect differences in the solutions' palatability or metabolic efficiency.

When one identical protein source was provided in all bee cages in this study, bees showed significant differences in their protein intake, and the highest intake was in cages provided with SS. This may be because SS significantly affected the enzymatic secretion compared to IS, as reported in (Brodschneider & Crailsheim, 2010; Lichtenberg-Kraag, 2014). In response to differences in sugar types, bees adjust their enzymatic secretion accordingly. For sucrose,

they release a greater proportion of carbohydrate metabolizing enzymes from the hypopharyngeal glands (de Brito Sanchez, 2011), ensuring efficient digestion of sucrose into its constituents glucose and fructose. The higher protein intake by bees in sucrose cages could be a response to compensate for the greater use of protein resources during enzyme production in their hypopharyngeal glands. The lowest intake was recorded in cages provided with honey. Bees' reserved honey may self-regulate their intake because honey contains lower free protein levels and bioactive compounds. However, it is still not a significant protein source, leading to lower total food and protein consumption (Démares et al., 2016).

The effect of honey on protein digestion could influence protein intake since it offers the necessary nutrients from a smaller amount of protein. As shown in Figure 2a, HS had the most positive impact on pollen digestibility, followed by SS. This may be due to the different effects of solutions on the gut microbiota, which plays a significant role in protein digestion (Motta & Moran, 2024; Raymann & Moran, 2018). HS and SS stimulate the development of bacterial populations in the bees' gut. This causes a rise in the digestibility rate, which indicates how effectively bees digest and absorb nutrients from the food they ingest. Feeding with IS or G + F reduced the percentage of digested food (D'alvise et al., 2018; Taylor et al., 2019; Wang et al., 2020).

The present results highlight the role of different types of sugar in the wax oenocyte area. Statistical analysis reveals that HS and SS have the most significant effect on oenocyte areas, reflecting their positive effect on wax gland development compared to other sugar solutions. The results agree with those obtained by Carrillo et al. (2015), who tested sucrose syrup, IS and sugarcane juice. They documented that IS showed minimal wax gland development, while the SS showed the maximum development. The current study showed that HS and SS promote gland growth by increasing the functioning and abundance of hemocytes. PL and PR are likely the essential hemocyte types influencing gland development due to their functions in immune response and cell signaling (Lavine & Strand, 2002). The data presented in Table 2 revealed that honey and sucrose are more beneficial for developing these two types of hemocytes in bees and are more suitable for meeting the physiological needs of bees, including the growth of wax glands.

Several studies have shown that feeding honey bee colonies with sugar syrups can stimulate the development of wax glands and enhance beeswax production. For example, Carrillo et al. (2015) and Pătruică et al. (2012) reported that energetic feeding supports comb construction and gland activation, especially with supplements. These findings suggest that the type and quality of sugar feed directly influence wax output, which aligns with observations from this study. The present study confirmed the positive effect on wax production. It demonstrated that SS and IS could effectively continue this trend, enhancing the wax mirror and scale size and maximizing wax production. Honey bee wax scales, although roughly pentagonal with gentle curves and a slight convexity, mirror the outlines of the wax mirror cuticle as they develop, ensuring a smooth and seamless transition during comb construction. The effect of IS was seen on the wax mirrors, not the glands. This is because IS provides immediate energy and does not require breaking down like sucrose and honey (Taylor et al., 2019). This results in poorer nutrient absorption, and the immediate effects may be seen in areas of high metabolic activity, such as wax mirrors (Winston, 1991). Furthermore, the absence of IS and G + F hydrolysis revealed a poor food digestion rate (Figure 2c), which subsequently appeared in nutrient absorption expressed by TSS% (Figure 4). In this context, the increase in TSS % reflected the active status of insect metabolism (Amro et al., 2016; Omar & Amro, 2023). Dahlman (1969) documented that TSS indicates the amounts of soluble solids in liquid and is dominated by the portion of soluble proteins, amino acids, carbohydrates, and other organic materials in the insect hemolymph.

Negri et al. (2014) found that differences in morphology and relative abundance of certain types of hemocytes within a species can indicate different physiological conditions. Park and Stanley (2006) also confirmed that PL and granular cells play an essential part in the immunity defence mechanism of honey bee workers against pathogens. Previous researchers found that bees exposed to environmental stress (Abou-Shaara et al., 2023) or malnutrition (Omar & Amro, 2023) showed an increase in the relative abundance of SP. The data suggest that natural and supplemental sugars such as HS and SS are more beneficial for developing and maintaining bee hemocytes. This may be due to the additional nutrients in honey or the metabolic pathways of these solutions in which these sugars are involved, which may be more compatible with the physiological needs of bees. The lowest efficacy of G + F and IS may indicate that while these sugars provide energy, they may not support other physiological needs of bees, such as immune function, as effectively as more complex sugars. This may affect bee health, particularly their ability to resist pathogens and maintain homeostasis. These supported the work herein, which showed that SP recorded the highest percentage of worker bees fed on IS and G + F. Meanwhile, the hemolymph of worker bees taken from cages fed on HS and SS recorded the lowest percentages of the same cells (Table 2).

The present study attempts to uncover the effect of alternative sugar sources on honey bee performance, wax production, and DHC as an indicator of the immune response. In conclusion, honey feeding, followed by sucrose, had the best impact on bee performance. These two also positively affected wax gland development and enhanced the immune system. IS and G + F mixture were the least consumed, and bee interaction was among the solutions. Interestingly, they also appeared to negatively affect the bees' wax gland development and immune response, suggesting that they were under stress.

Conclusions

The present study seeks to unveil the impact of alternative sugar sources on honey bee performance, wax production, and immune response. In conclusion, honey, followed by sucrose, was a more readily preferred energy source. Furthermore, these two alternatives positively influenced wax gland development and enhanced the immune system. IS and the G + F mixture received the least consumption and bee interaction among the solutions. Interestingly, they also appeared to negatively impact wax gland development and the bees' immune response, suggesting they experienced stress. Despite potential cost benefits, IS's negligible effects on key colony health aspects, such as wax production and wintering, necessitate further study.

Acknowledgments

The authors thank the beekeeper who manages the Assiut University's Faculty of Agriculture apiary for his assistance. We also appreciate Prof. Dr. Mohamed Omar's support and Prof Dr. Youssef M. Omar's assistance with his Economic Entomology lab.

Ethics approval

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*).

Disclosure statement

The authors declare no competing interests.

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Data availability statement

The datasets generated during and analyzed during the current study are available from the corresponding author upon reasonable request.

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