Effects of various sugar feeding choices on survival and tolerance of honey bee workers to low temperatures

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Abstract

Beekeepers usually supply their colonies with alternatives to nectar (i.e., sugar feeding) during dearth periods of the year, especially cold times of winter. The objective of the study was to determine the best substances to feed bees to enhance the tolerance and survival of honey bees (Apis mellifera L.) to low temperatures. Seven feeding choices were compared under laboratory conditions. These feeding choices were: sugar syrup, liquid honey, creamed honey, honey candy, sugar candy, honey jelly, and honey/sugarcane juice jelly. The results showed that the number of bees attracted to each feeding choice was influenced significantly by feeding type. Worker bees were attracted to all feeding choices honey/juice jelly. The results showed that the number of bees was enhanced when bees were fed on creamed honey, sugar syrup or honey candy. The mean time at which 50% of bees were able to survive ranged from 3 days (unfed bees) to 15.8 days (honey candy group). The survival rate of worker bees was highest when they fed on honey candy, creamed honey or sugar candy. In light of this study, creamed honey or honey candy can be considered the best feeding choices for bee colonies during winter to enhance their survival and tolerance to low temperatures.

Introduction

Honey bee (Apis mellifera L.) colonies often have a large number of workers reaching 15,000 to 60,000 bees (Southwick & Heldmaier, 1987). Such large numbers of bees require a large amount of food to sustain their survival and development. Sufficient nutrition can boost the development of healthy bee colonies (Brodenschneider & Craitsheim, 2010). Nectar, mainly as a source of carbohydrates, is needed in large amounts by honey bee colonies. Nectar is very important to honey bee workers as a source of energy especially during winter where worker bees engage in strategies to keep the brood temperature within a suitable range from 33 to 36°C for colony survival, including warming of the brood caps superficially, or internally by heating bees (Kleinhenz et al., 2003). During winter, high colony weight loss was reported by Seeley & Visscher (1985) as an indication of high honey consumption during this critical period of the year. Low temperature during winter has been considered as a cause of honey bee colony losses in some Arabian countries (Al-Ghamdi et al., 2016). High colony losses have been reported in regions with low mean temperatures in the USA (van Engelsdorp et al., 2008). Colony losses generally occur during winter. Such losses have been estimated to be 19% in Belgium during 2009/08 (Nguyen et al., 2010) and 22.5% in the USA during 2011/12 (Spleen et al., 2013). Starvation has been considered as one reason behind colony losses from 6.0% to 17.8% during winter in Austria (Brodenschneider et al., 2010). The winter colony losses in Germany were reported to be 3.8% to 15.2% in 2004/05 and 2005/06 (Genersch et al., 2010). Bee colonies suffer greatly from shortage of nectar sources as well as unsuitable conditions for foraging during winter. Thus, beekeepers need to supply colonies with alternatives to nectar. It is commonly known that the best alternative to nectar is pure sucrose (Barker, 1977), but, searching for more cost-effective alternatives with adequate nutritional value to honey bees is needed.

Some feeding, such as heated or old honey is less suitable to bees while acid-hydrolysed carbohydrates can be toxic to bees (Bailey, 1966). There was no reported advantage of feeding high fructose corn syrup or honey over sucrose syrup. Grape syrup, when fed to bees, shortened survival and caused dysentery (Barker & Lehner, 1978). Under field conditions, brood development was increased in colonies fed on honey (Andelković et al., 2011). The
problems of liquid honey are in its ability to spread disease among colonies and high viscosity. Abd El Hamid & Abou-Shaara (2016) presented a simple method to produce creamed honey using powdered glucose and a refrigerator. These authors found produced creamed honey was attractive to honey bees and it was consumed within 24 hours. Using creamed honey (especially that made from low marketable honey choices) could be considered as a natural alternative to commercial sugars. Sugarcane juice is relatively inexpensive if compared with table sugar (sucrose). According to Carrillo et al. (2015), sugar syrup was better than sugarcane juice as energy source for beeswax production. The main problem with juice with rapid fermentation due to its high water content. Using juice in a solid form may solve this problem and make it more effective.

Winter bees can better survive cold conditions if provided with a suitable energy source (i.e., sugar feeding). The objective of the study is to test different feeding choices to enhance the ability of bees to survive under cold conditions (i.e., to boost their wintering ability). Two forms of feeding choices were tested; liquid and solid. The effects of feeding choices on the survival and tolerance of honey bees to cold conditions were tested.

Materials and methods

Sugar feeding choices

In this study, seven choices of feeding (suitable sources for winter feeding) were tested under laboratory conditions. Feeding choices included the following. I) Sugar syrup: was made by mixing table sugar with water (2:1, w/w). II) Liquid cotton honey: cotton honey is available to colonies during autumn. This type of honey was selected because it has low price, a source beekeepers can utilize to feed colonies. III) Creamed cotton honey: it was produced by mixing 2.4% (w/w) powdered glucose to liquid cotton honey and stored on 4°C until full crystallization following Abd El Hamid & Abou-Shaara (2016), except honey was not heated. IV) Cotton honey jelly: it was produced by adding 10 g of gelatin dissolved in 100 mL of water to 200 g liquid honey. The mixture was heated for 1 minute in water bath. Pectin was not used as solidifying material because toxicity to honey bees (Barker, 1977). V) Honey/sugarcane juice jelly (abbreviated as honey/juice jelly) was produced by adding 5 g of gelatin to a mixture of 100 mL sugarcane juice and 100 g of cotton honey in water bath for 1 minute. Feeding choices 4 and 5 were produced based on some preliminary experiments, but the mentioned amounts of gelatin, honey and juice were only found to be suitable for producing honey or honey/juice jelly. Also, preliminary studies showed that bees are able to absorb food from these choices of jelly. VI) Honey candy: was prepared by saturating honey with powdered sugar in a water bath. VII) Sugar candy: water was saturated with powdered sugar ratio (4 sugar : 1 water, w/w) in water bath.

Sampling and experimental cages

Hybrids of Carniolan honey bee colonies from an apiary located at Damanhour City, Egypt were used. Honey bee workers of approximately foraging age (older than 21 days) were collected from the lateral combs of the colonies in small well ventilated plastic jars. The bees were starved at room temperature for 12 hours prior to each experiment (Wang et al., 2016). Then, the bees were placed in a freezer for 4 minutes to immobilize them prior to distribution into experimental cages. The number of worker bees varied according to the experiment. The experiments were performed during spring 2016. The experimental cages were constructed using petri dishes and small plastic sheets (Figure 1). The cage diameter was 8.5 cm by 5 cm deep. The bottoms of cages were provided with 4 tightly fixed Eppendorf tubes to be filled with water when necessary. According to Simpson (1964), dry sugar is diluted by bees more than concentrated syrup or honey and generally food containing less than 50% of sugar is not diluted by bees. Therefore, each cage was provided with a water source.

Preference of honey bees to tested feeding choices (Experiment 1)

Four cages were used in this experiment. In each cage, small amounts (0.5 grams) of all feeding choices were placed in small plastic plates. Ten bees were placed in each cage. The number of bees attracted to each feeding choice was counted after 3, 6, 9, 12, 15 minutes and after 1, 4, 8, and 12 hours after placing the bees into the cages. The number of bees attracted to each feeding choice was then compared to detect preference of honey bees to specific sugar feeding type.

Tolerance to low temperature (Experiment 2)

Per each feeding type, three cages equipped with 5 g of feeding type and 1.5 ml of water were used in addition to three cages that were provided only with water. Ten bees were placed in each cage (3 cages/30 bees per treatment, total of 240 bees). The bees were allowed to feed on the feeding for 12 hours then the cages were placed in a refrigerator at 6±1°C. The number of surviving bees (bees exhibiting movement) in each cage was recorded each minute. Then, number of surviving bees per each minute was compared. After all the bees in the cages of each treatment were motionless, the cages were placed at room temperature (at 19±1°C). The time at

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Figure 1. Description of cage parts used in the experiments.
which the bees started to recover (showed whole-body movement) was recorded as well as the number of recovered bees after 10 and 15 minutes. The feeding type that caused highest survival rate was then identified. The consumption rate \((5 - \text{consumed amount} / 5\times100)\) of each feeding type was also calculated.

Survival of honey bees (Experiment 3)

Three cages equipped with 10 g of feeding and supplied with 1.5 mL of water were used in this experiment per each feeding type beside three cages supplied only with water. 15 workers were placed in each cage (45 workers per treatment, total of 360 bees). Each cage was also provided with a piece of wax comb \((4\times3\ \text{cm}, \ L\times W)\). This wax piece was added to allow bees to stand on it. The cages were left at room temperature (at about 20±1°C) during the experiment. The cages were inspected daily and the number of surviving bees was recorded until the death of all bees. The number of surviving bees was compared among feeding choices to identify the best feeding choice with respect to enhancing survival ability of bees in cold air temperature conditions. The consumption rate \((10 - \text{consumed amount} / 10\times100)\) of each feeding type was also calculated.

Statistical analysis

All the experiments were of complete random design (CRD). The independent factors for the first and second experiment were feeding choices and experimental time while, for the third experiment, only feeding choices was considered as an independent factor. Means and standard errors (S.E.) were calculated for each studied parameter. Then, one way or two-way ANOVA was performed, and means were then compared using Duncan’s multiple range test\(_{0.05}\). Pearson correlation coefficient at 5% level of significance was also calculated to assess correlations between survival period and percent of consumed food. The SAS (Version 9.1.3, SAS Institute, NC, 2004) was used in the statistical analysis.

Results

Preference of honey bees to specific feeding choice

The number of bees attracted to each feeding type was influenced significantly by feeding choices \((P=0.0001<0.05, F=8.88)\) as well as experimental time \((P=0.0001<0.05, F=8.88)\). Also the interaction between feeding type and experimental time was significant \((P=0.0001<0.05, F=8.88)\) as shown by the Two-factor ANOVA. After three minutes, bees attracted to all feeding choices but the highest number of bees were at honey jelly, honey/juice jelly, and creamed honey with no significant differences \((P>0.05)\) as shown in Figure 2. The number of bees decreased at all feeding choices but the highest number of bees were at honey jelly, honey/juice jelly, and creamed honey with no significant differences \((P>0.05)\) as shown in Figure 2. The number of bees decreased at all feeding choices by time and stayed without feeding at 12 and 15 minutes (Figure 3). After one hour, the high number of bees attracted to sugar candy was significantly different \((P<0.05)\) than all the other feeding choices with % of 30, 37.5, 15, and 12.5 of the total (40 bees) at 1, 4, 8 and 12 hours, respectively. At 12 hours, bees attracted only to sugar

Figure 2. Mean number of bees per cage (= number of bees/4) per feeding type during the experiment. (A: Liquid honey, B: Honey jelly, C: Sugar candy, D: Honey/juice jelly, E: Creamed honey, F: Honey candy, G: Sugar syrup).

Figure 3. Preference experiment. A) Distribution of feeding plates within the cage, B) Bees during feeding, and C) Bees during non-feeding.
candy and creamed honey. In the course of the experiment period, sugar candy differed significantly than the other feeding choices and had the highest mean with 1.25±0.30 bees.

**Tolerance to low temperature**

As indicated in Table 1, during the first 5 minutes at 6±1°C, bees of each feeding choice showed 100% survival ability (apparent moving within cages), without significant differences among them. During the next 5 minutes, only bees fed on sugar syrup and honey/juice jelly showed 100% survival ability followed by workers fed on liquid honey, creamed honey, honey candy and sugar candy, respectively. These feeding choices differed significantly than workers fed on honey jelly and unfed bees. After 10 to 15 minutes, the highest survival was for workers fed on creamed honey and sugar syrup followed by liquid honey without significant differences among them. These feeding choices differed significantly than the other feeding choices. The lowest survival ability was recorded for unfed bees. During the last four minutes, no significant differences were found among surviving bees fed on honey candy, creamed honey, sugar syrup, honey/juice jelly, and sugar syrup while bees of all the other treatments stayed motionless. The correlation between survival ability and exposure time to low temperature was negative (r = −0.84, P < 0.0001).

As shown in Figure 4, it is apparent that bees were able to survive up to 19 minutes only when fed sugar syrup, creamed honey, and honey candy. Bees survived up to 18 minutes on sugar candy and honey/juice jelly, and up to 12, 15, and 16 minutes on no food, honey jelly, and liquid honey, respectively. From these experiments it is concluded that creamed honey, sugar syrup, and honey candy are the best feeding alternatives to enhance bee survival ability to cold air temperature conditions followed by honey/juice jelly and sugar candy.

The bees fed on all feeding choices were able to recover (whole body moving ability) within 2.05 minutes (Table 2). No significant differences were found between feeding choices with regard to recovery time but bees fed on sugar syrup showed slightly faster recovery (1.50 minutes). Unfed bees were slower in their recovery than fed individuals. After 10 and 15 minutes, no significant differences were found among bees fed on different feeding choices. Unfed bees had significantly less number of recovered bees than fed ones. The consumption % ranged from 2.66 to 11.33.

![Figure 4](image-url). Decreasing the percent of surviving bees during time for different feeding choices after exposition to low temperature. (A: Liquid honey, B: Honey jelly, C: Sugar candy, D: Honey/juice jelly, E: Sugar syrup, F: Creamed honey, G: Honey candy, H: Bees without feeding).

Table 1. Means of surviving bees ± standard error for each feeding choice at 6±1°C per each five minutes during the experiment (3 cages and 10 bees per cage for each feeding choice).

| Time* (minutes) | Liquid honey | Honey jelly | Sugar candy | Feeding choices | Honey/juice jelly | Creamed honey | Honey candy | Sugar syrup | Without feeding |
|-----------------|--------------|-------------|-------------|-----------------|-------------------|---------------|-------------|-------------|-----------------|
| 0 to 5          | 10.0±0.0ab   | 10.0±0.0ab  | 10.0±0.0a   | 10.0±0.0a       | 10.0±0.0a         | 10.0±0.0a     | 10.0±0.0a   | 10.0±0.0a   | 10.0±0.00a       |
| 6 to 10         | 9.8±0.1a     | 7.4±0.8a    | 9.0±0.4a    | 10.0±0.0a       | 9.7±0.1a          | 9.2±0.4a      | 10.0±0.0a   | 9.2±0.2a    | 8.0±0.2a         |
| 11 to 15        | 4.4±0.3ab    | 0.6±0.2ab   | 2.1±0.5ab   | 2.9±0.2bc       | 5.9±0.9c          | 3.4±0.5bc     | 5.3±0.9a    | 0.1±0.1c    | 0.1±0.1c         |
| 16 to 19        | 0.0±0.0b     | 0.0±0.0b    | 0.4±0.2ab   | 0.5±0.2ab       | 1.0±0.3b          | 1.1±0.4b      | 0.7±0.2ab   | 0.0±0.0b    | 0.0±0.0b         |

*Means followed by the same letter in the same row are not significantly different according to Duncan’s multiple range test a.

Table 2. The time at which bees started to recovery, means of recovered bees (showed moving ability) at 19±1°C after 10 and 15 minutes, and the consumption % of each feeding choice at the end of the experiment. Values are ± standard error.

| Feeding choice | Start to recovery (minutes) | After 10 minutes | After 15 minutes | Consumption % |
|----------------|-----------------------------|------------------|------------------|---------------|
| Liquid honey   | 1.68±0.26ab                | 8.33±1.20a       | 8.66±1.33a       | 4.66±0.66ac   |
| Honey jelly    | 1.55±0.33b                 | 6.00±0.57a       | 7.33±0.33a       | 2.66±0.66a    |
| Sugar candy    | 1.71±0.24ab                | 9.66±0.33a       | 9.66±0.33a       | 9.33±0.66ab   |
| Honey candy    | 1.75±0.40ab                | 8.00±1.52a       | 8.00±1.52a       | 6.66±1.33bc   |
| Honey/juice jelly | 2.05±0.27ab            | 9.33±0.33a       | 9.33±0.33a       | 4.00±1.154ad |
| Creamed honey  | 1.99±0.20ab                | 8.66±1.33a       | 8.66±1.33a       | 8.66±1.33ab   |
| Sugar syrup    | 1.50±0.27b                 | 6.33±2.02a       | 7.66±1.45a       | 11.33±1.76a   |
| Without feeding | 2.58±0.01b                | 0.66±0.06a       | 1.66±0.33b       | -             |

*Means followed by the same letter in the same column are not significantly different according to Duncan’s multiple range test a.
Table 3 it is evident that bees consumed significantly more amounts of sugar syrup and liquid honey than the other feeding choices. Honey candy was least preferred. Feeding choices in descending order according to consumed % are sugar syrup, liquid honey, honey candy, honey/juice jelly, creamed honey, sugar candy, and finally honey jelly. During the experiment, bees were observed to absorb nutrients from honey jelly without cutting it into smaller fragments (Figure 6A). In the case of honey/juice jelly, bees were able to absorb nutrients and to remove and store fragments of this feeding choice in combs (Figure 6B and C).

### Discussion

**Preference of honey bees to specific feeding choice**

During the first three minutes, bees were attracted to all feeding choices with high preference to honey jelly, honey/juice jelly and creamed honey. This indicates that all feeding choices are desirable to starved bees and can be consumed without difficulty. During the following six and nine minutes, no bees were attracted to honey jelly or sugar syrup which indicates that these two feeding choices are not highly attractive to replete bees. At twelve or fifteen minutes, the bees seemed to be satiated and remained without feeding. After one hour, the majority of bees tended to feed on sugar candy instead of the other feeding choices. This may be explained by the high sweetness of sucrose (sugar candy) comparable to other feeding choices. According to Hough & Phadnis (1976), sucrose has the greatest sweetness over other carbohydrates or even sucrose derivatives. Therefore, it could be expected that sugar candy (a solid form of

### Table 3

|                | Liquid honey | Honey jelly | Sugar candy | Honey/juice jelly | Creamed honey | Honey candy | Sugar syrup | Without feeding |
|----------------|--------------|-------------|-------------|-------------------|---------------|-------------|-------------|----------------|
| **ST0**        | 13.3±2.3ab   | 5.8±1.8c    | 13.3±0.3ab  | 10.6±0.8b         | 14.6±0.8ab    | 15.8±0.1a   | 11.8±1.9ab  | 3.0±0.5c       |
| **ST50**       | 20.6±1.3a    | 10.6±0.3b   | 21.3±2.7a   | 18.3±2.3a         | 17.0±0.5b     | 21.0±1.5b   | 17.6±3.3c   | 4.3±0.3c       |
| Consumption%   | 67.0±3.6ab   | 3.2±0.8c    | 32.3±0.8c   | 43.0±3.4c         | 55.0±3.8c     | 47.3±7.3c   | 71.0±16.7a  | -              |

*Means followed by the same letter in the same row are not significantly different according to Duncan’s multiple range test.*

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**Figure 5.** Decreasing mean % of surviving bees over time for different feeding choices (A: Sugar syrup, B: Liquid honey, C: Creamed honey, D: Honey jelly, E: Honey/juice jelly, F: Honey candy, G: Sugar candy, H: Bees without feed).

**Figure 6.** Worker bee is feeding on honey jelly (A). Worker bee is feeding on honey/juice jelly (B). Workers were able to cut small pieces of the honey/juice jelly and stored them in the wax cells (jelly pieces are pointed out with white arrows) as shown in (C).
(sucrose) was able to provide worker bees with sufficient energy to survive only 13.67 days. The survival period recorded by Abou-Shaara et al. (2012) was less than the survival period of bees fed with honey/juice jelly as an energy source. Also, sucrose syrup in this type of jelly may increase the ability of worker bees to absorb nutrients from it over honey jelly (which has high viscosity and no sugarcane juice). Honey bees used in the experiments were able to cut fragments of honey/juice jelly and stored them in comb. This reflected the ability of bees to utilize this feeding choice over honey jelly. Bees fed on honey jelly were able to survive up to 6.3 days more than unfed bees. Due to the individual variations among tested bees, STₜ₀ did not differ significantly among tested feedings, except honey jelly and unfed bees. It is known that hydroxymethylfurufural (HMF) is toxic substance to honey bees (Brodtschneider & Crailsheim, 2010). This substance is increased by heating honey for longer times (e.g. Sahinler & Gul, 2005). The lowered longevity of worker bees fed on tested jellies cannot be attributed to the high HMF level because the honey was only heated in water bath for only one minute during jelly production. It is not expected that HMF levels were increased substantially to cause adverse impacts on worker bees tested. Providing bee colonies during cold air temperature with honey candy or creamed honey is suggested especially as these feeding choices showed the ability to enhance worker tolerance to cold temperatures conditions is worthy of being mentioned that the nutritional status of the colonies can not only impact the activity of bees but as well as their long-term behavioural development (Schulz et al., 1998).

The form of the feeding type (either liquid or solid) impacted the consumed amounts. It was clear the bees were able to consume significantly more amounts of liquid feedings (i.e. honey or sugar syrup). Consumption of solid feeds was correlated with the ability of worker bees to absorb nutrients. This ability was high for honey candy, honey/juice jelly, creamed honey, sugar candy, and finally honey jelly, respectively. The consumption % was moderately correlated by 60% with survival period until 100% death. However, the high consumption percent did not always indicate longer survival of workers. The utilization ability of feeding type as source of energy appears important as well.

### Conclusions

The tested sugar feeding choices indicated variations in attractiveness of starved bees and to enhance the survival of bees to low air temperatures. Creamed honey, honey jelly or honey/juice jelly had high attractiveness to honey bees during the onset of experiments, but sugar candy attracted bees until the completion of experiments. Creamed honey, sugar syrup, and honey candy showed the highest ability to improve the tolerance of honey bees to cold air temperatures. During cold periods providing bee colonies with honey candy or creamed honey can be considered as suitable energy source to boost the survival of bee colonies.

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