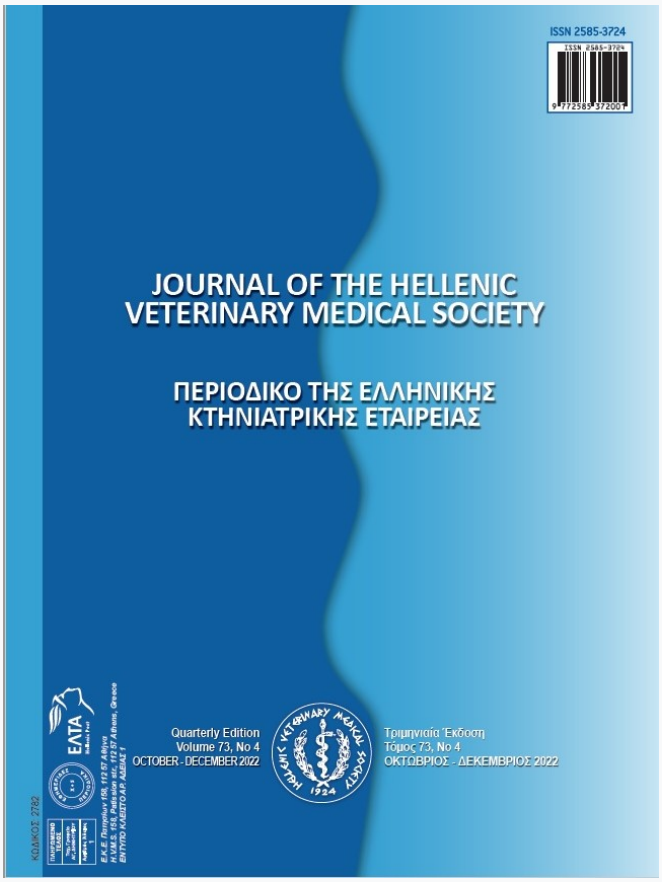
Journal of the Hellenic Veterinary Medical Society

Vol 73, No 4 (2022)



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doi: 10.12681/jhvms.27794

To cite this article:

Karlıdağ, S., Köseman, A., Akyol, A., Saatçıoğlu, G., Şeker, İ, Uyumlu, A., Yılmaztekin, M., & Erdoğan, S. (2023). The effects of different industrial sugars on royal jelly production. *Journal of the Hellenic Veterinary Medical Society*, *73*(4), 4689–4696. https://doi.org/10.12681/jhvms.27794

J HELLENIC VET MED SOC 2022, 73 (4): 4689-4696
ΠΕΚΕ 2022, 73 (4): 4689-4696

Research article
Ερευνητικό άρθρο

The effects of different industrial sugars on royal jelly production

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ABSTRACT: In this study, the larval acceptance rate and the royal jelly yield in honeybee (*Apis mellifera* L.) colonies supplemented with different industrial sugars at different locations were determined. For this purpose, feeding groups (1. Sucrose group, 2. Glucose group, 3. Bee feed syrup group, 4. Control group) and locations (1. Battalgazi, 2. Doğanşehir) were formed. In queenless colonies that produce royal jelly, in order to sustain 5-15 day-old young feeder worker bees, two sealed frames with brood from support colonies were added. The royal jelly yield was harvested seven times. Based on the location, the feeding groups, and the location x feeding groups interaction, 12600 larvae were grafted, 9054 larvae were accepted, and the larval acceptance rate was determined as 71.86%. Based on the location, feeding groups and the location x feeding group interaction, the yield per cell was calculated as 213.15 ± 11.53 mg/cell, the yield per colony as 6.88 ± 0.38 g/app., and the total yield per colony as 34.40 ± 1.91 g/colony. In the study, no statistically significant difference was determined between feeding with sucrose, bee feed and the supplementary feeding with glucose. On the other hand, it was determined that the location where the royal jelly was produced affected both the larval acceptance and the royal jelly yield.

Keywords: Royal jelly, Bee feed, Honeybee (*Apis mellifera* L.), Feeding, Sugar

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Date of initial submission: 11-08-2021
Date of acceptance: 29-05-2022

INTRODUCTION

Royal jelly is a homogeneous, creamy, milky white, secretion substance with an acidic taste and sharp odor secreted by 5-15 day-old worker bees from their hypopharyngeal and mandibular glands after partially digesting honey and pollen (Wongchai and Ratanavalachai 2002; Nie et al. 2017; Yang et al. 2017). This secretion is used to feed worker bee larvae until they are three days old, drone larvae until they are three days old, and the queen during the larvae and the adult stage (Crailsheim, 1990; Fujita et al., 2012; Nie et al., 2017; Yang et al., 2017; Al-Kahtani and Taha 2020).

Royal jelly consists of a mixture of 60-70% water, 9-18% protein, 3-8% lipid, 3-13% fructose, 4-8% glucose, 0.5-2.0% sucrose, 0.8-3% ash and 1.4-2.01% 10-hydroxy-2-decenoic acid (10-HDA) (Sabatini et al. 2009; Yang et al. 2017). Apart from these, remnants of larvae, pieces of beeswax, and pollen from plants visited by worker bees are also present among the contents of royal jelly (Krell, 1996; Renner et al., 2003). The composition of royal jelly slightly differs depending on the season and the geographical origin (Zheng et al., 2010; Yang et al., 2017).

The sugar content of royal jelly has a stimulating effect on feeder bees and promotes the production of more royal jelly. Therefore, feeding royal jelly producing colonies with sugar syrup increases the yield significantly (Köseoğlu et al., 2013). In a study conducted on this subject, the effect of feeding on the larval acceptance rate in colonies and the royal jelly yield per colony was significant, and the highest yield was obtained from colonies fed with a 60% glucose syrup (Sharaf El-Din et al., 2010). Chen et al. (2002) reported that if it is not possible to move the colonies, supplementary feeding with pollen and sugar syrup should be given.

Many factors such as the race of the bee, the strength of production colonies and whether they are queenless or not, ecological conditions, whether production colonies are given supplementary food or not, the number of queen cells given to one production colony, the age of the larvae grafted, the harvest interval and the cell type affect the royal jelly yield of colonies (Fuhai et al., 1993; Kutluca et al., 1998; Zeedan, 2002; Muli et al., 2005).

In studies conducted, it has been reported that queenless colonies are more attracted to grafted cells than queenright colonies (Taber, 1991; Laidlaw and Harry 1992) and that the larval acceptance rate is higher in queenless colonies (Öder, 1989; Johansson and Johansson, 1994). In a study conducted in this context, it was found that more royal jelly was collected from queenless colonies (7.54 g / colony) than queenright colonies (5.67 g / colony) (Ibrahim, 2002) and that the larval acceptance rates were 77% and 70% respectively, for single and double grafting (Emsen et al., 2003).

In another study conducted, the larval acceptance rate and the royal jelly yield per cell and colony were calculated to be higher in colonies harvested every 72 hours than those harvested every 48 or 24 hours (Sharaf El-Din et al., 2010). Furthermore, in a study conducted by Silici (2009), it was reported that 148-281 mg royal jelly was collected from each queen cell after the transfer of 8-24 hour-old larvae 72 hours after the transfer, and in the study by Ergün (2010), it was reported that an average of 200 mg royal jelly was collected from one cell if harvested 60-72 hours after transfer and that an average of 20-50 g was collected every 2-3 days from one colony.

In a study conducted, royal jelly was harvested after 24, 48, 72, and 96 hours after grafting of 1-day larval age queens to investigate changes in macro and trace elements associated with harvesting time. They were significantly affected by harvest time, and the highest yield was obtained 72 hours after grafting (Al-Kahtani and Taha 2020).

In a study, cell acceptance rates were highest in the 24-h-old larvae (74.5%) and least in 48- and 60-h-old larvae (35%) (Muli et al., 2005). Chen et al. (2002) stated that the weight of royal jelly in each queen cell is about 650 mg and harvested about 30 g/colony royal jelly every three days. Salem et al. (2021) conducted that the mean royal jelly production attained 353, 398.33 and 296 mg/ cup when grafting 1 day old Carniolan larva and 290.67, 358 and 246.33 mg/ cup for 2 days old grafted Carniolan hybrid larva and when harvesting process was performed at 2, 3 and 4 days post grafting, respectively.

Korkmaz and Öztürk (2010) stated that 6.46 - 10.56 g of royal jelly could be obtained from one colony in one transfer. In addition to this information, Silici (2009) reported that as the number of larvae transferred to the colonies increased, the amount of royal jelly per cell decreased, but that the total amount of royal jelly would increase. In the study on this subject, Jianke (2000) stated that 69-100 g of royal jelly is collected from 125-170 queen cell cells formed 72

hours after grafting.

Due to its potential benefits, royal jelly is widely used in the food industry, pharmaceutical and cosmetic industries (Sabatini et al., 2009). China produces about 90% (~ 3500 tons) of the royal jelly in the world (Cao et al., 2016; Lee et al., 2017; Nie et al., 2017). On the other hand, despite its high beekeeping potential, Turkey is not fully benefiting from the extensive royal jelly market. For Turkey to improve its share in this market, it is necessary to conduct studies towards increasing the royal jelly production and yield, which are low under natural conditions.

This study aims to determine the effect of supplementary feeding of honeybee colonies at different locations with different industrial sugars on the larval acceptance rate and royal jelly yield. It is anticipated that the data obtained as a result of the study will contribute to performing higher value-added beekeeping.

Figure 1. Study locations

MATERIAL AND METHODS

Material

Forming the research groups: In the study, two different locations were determined to establish the effect of different florae on the royal jelly yield. Figure 1. While deciding these locations, the sites being far from each other and having different flora were considered. The location groups formed were as follows:

1.The Malatya province Battalgazi group (38 ° 13 ′ 27 ″ N 38 ° 27 ′ 34 ″ E): This is spread over a wide area and is rich in flora where many plants are present and grown in cultured and wild forms. In agricultural areas, vegetables (tomato, pepper, cabbage, etc.), fruit gardens (apricot, cherry, walnut, apple, etc.), vineyards and nurseries are among important agricultural products.

2.The Malatya province Doğanşehir group: Doğanşehir is 74 km away from the Malatya city center (350 34 ‘390 03’ N 38 ° 45 ‘39 ° 08’ E). Beside apples, apricots and cherries, agricultural products such as beans, lentils and tobacco are grown in the region. On the other hand, herbaceous plants such as bromus, milk vetch, randal grass, thyme, thorns, poppies, spurge and festuca, wild almond, hawthorn, aleppo oak, juniper, poplar and willow communities and plants such as wild rose, silverberry, and blackberry grow.

To determine the effect of different industrial foods (crystallized granulated sugar (sucrose) syrup, pasteurized bee feed syrup and glucose syrup) on royal jelly yield, feeding groups and a control group to compare these groups were formed. Beekeepers feed colonies with industrial carbohydrate sources to increase the amount of royal jelly and achieve cell acceptance rates of higher than 95%. In this study conducted with this objective, sugar sucrose, which is commonly used to feed colonies by beekeepers, pasteurized bee feed syrup produced to feed colonies, and glucose, which is also widely used by beekeepers to feed colonies and is present in human foods, have been preferred for feeding the colonies being studied.

The supplemented groups formed:

1. The sucrose group,
2. The bee feed group,
3. The glucose group,
4. The control study group.

The study had a 2 (location groups) x 4 (feeding groups) factorial design. In the study, five colonies were used for each of the feeding groups. Thus, a total of 40 queenless starter colonies, 20 in each location group, were used in the production of royal jelly.

Supply of larvae: In each location, a colony with

young queen bees and a good brood colony was selected to provide the larvae to be grafted. For grafting, 24-hour-old larvae were used.

The supply and preparation of nutrients:

a) To prepare the sucrose syrup (1 part sucrose:1 part water), crystal granulated sugar used as human food and marketed in 50 kg sacks,

b) the glucose syrup (1 part glucose:1 part water) commercial fresh glucose syrup produced within the last year and sold in stainless metal packaging (Brix 82, DE value (dextrose equivalent) 37, Dextrose 14, Maltose 12),

c)the bee feed syrup pasteurized bee feed syrup supplied by a manufacturer (sucrose; 30-36%, glucose; 27-30%, fructose; 37-40%, dry matter; 72% ± 2) were used.

Methods

Preparation of the colonies: The maintenance of the colonies purchased from the market in the spring was carried out in the apiary located on the İnönü University Campus**.** The colonies purchased were divided into research groups in the spring, as described above. The spring feeding of the research groups was performed with the nutritional source of their group. During the royal jelly production period, nutritional sources were added to the feeders as they were depleted. The colonies were transported to their appropriate locations on the 14th and 15th of June, and royal jelly production took place between June 18 and July 3. Due to the dry weather flow, the period of nectar flow was short and royal jelly could be harvested seven times. In the queenless colonies that produce royal jelly, to sustain 5-15-day old young feeder worker bees, two sealed frames with brood from support colonies were added.

The preparation of the queen cells and grafting: Molds made of hard wood with the length of 10 cm and a diameter of 9 mm were used to make the cells. Of these molds, 15 were mounted on a wooden bar at 2.5 cm intervals, and the 10 mm edges of the frames connected to the mandril prepared were dipped into melted bee wax 3-4 times. The cells obtained were fixed to the wooden bars (15 cells/bar). Each treatment colony was given a cell grafted with 45 larvae (3 bars) (Laidlaw, 1979).

For grafting, royal jelly diluted at a ratio of 1:1 was placed at the bottom of the cells. Then 24 hour-old larvae obtained from the comb cells grafting needle were transferred on to the diluted royal jelly. During the transfer, a cold light source was used to ensure that the breeder larvae in the comb chamber could be obtained easily and without damage, and care was taken not to damage the larvae.

The preparation of queenless starter colonies: In the study, royal jelly was produced in a total of 40 queenless starter colonies at both locations, and 20 colonies were used as supporting colonies. Queenless starter colonies were arranged as colonies with abundant nurse worker (young) bees and food having no larvae requiring care. For this purpose, the queen was removed from 40 of the two-story colonies of equivalent strength, and bees were shaken off into the brood chamber and squeezed into a single story, and queenless starter colonies were formed. While eight frames containing honey-pollen and sealed combs with brood near hatching were left in the colony, the others were removed from the combs in the brood chamber. After 4-5 days, the queenless starter colonies were checked, and the queen cells made were destroyed. The procedure of destroying queen cells was performed twice with a one-week interval. The frames with grafted larvae were given to the queenless starter colonies, and 48 hours later, these frames with larvae fed with royal jelly were removed from the queenless starter colonies, and their royal jelly was collected. During the production season, larval grafting, and the royal jelly collection were repeated every 48 hours, and these colonies were supplemented with two frames of sealed brood. No honey was harvested from the colonies. However, since the starter colonies being fed continuously had no larvae to feed, honey was stored on the combs in the colony. After each frame grafted was given to the colonies of the treatment groups, they were fed ad libidum with the food of their group.

Royal jelly harvest: The larvae in the queen cells in the graft frame removed from the queenless starter colonies were obtained using a fine tip forceps, and then the royal jelly deposited in each cell was harvested using a royal jelly harvest spoon. The royal jelly harvested every 48 hours was weighed with a 0,01 g precision scale, and the amounts were recorded and stored in a deep freezer at -18 ° C for evaluation later.

Statistical analysis

To determine the effect of location (1: Battalgazi, 2: Doğanşehir) and feeding (1: sucrose, 2: bee feed, 3: glucose, 4: control) on royal jelly yield and the lar-

val acceptance rate, a (2x4) factorial study design was used and the definitive statistics of the features evaluated were calculated.

The Larva Acceptance Rate (%): In each application, the larval acceptance rate was determined by dividing the number of larvae accepted by the number of larvae grafted in the feeder colonies of different research groups.

Royal Jelly Yield Per Cell (mg/cell): The royal jelly yield per cell was found by dividing the amount of royal jelly obtained from the cells in the research colonies by the number of cells accepted.

Royal Jelly Yield Per Application (g/application): The amount of royal jelly harvested from colonies after each application was assessed as yield per application.

The Total Royal Jelly Yield Per Colony (g/colony): The amounts of royal jelly collected from the colonies throughout the production period were added, and the total royal jelly yield per colony was calculated.

The effects of location and nutritional factors and the interactions between these on the royal jelly yield per cell (mg/cell), royal jelly yield per application (g/app.) and the total royal jelly yield per colony (g/col.) were analyzed following the mathematical model below using the General Linear Model (GLM) procedure.

As the model:

$Y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + e_{ijk}$

In the model:

Y_{ijk} = observational value of the feature being evaluated

μ = population average

a_i = The effect of location (I=1: Battalgazi, 2: Doğanşehir)

b_j = The effect of feeding (j=1: sucrose 2: bee feed, 3: glucose, 4: control)

$(ab)_{ij}$ = The effect of the interaction between location x feeding

e_{ijk} = The random error term is N (O, σ^2).

The Duncan Multiple Range Test (DMRT) was used post hoc in the multiple group comparisons of the factors that were identified as significant (Akgül 2005; Özdamar 2003), and for the larval acceptance rates, the Arc sin $\sqrt{p}\sqrt{p}$ transformation was applied; the analyses were then performed (Yıldız and Bircan, 1991). The descriptive values and analysis results pertaining to the larval acceptance rate with no transformation applied have been presented in the findings section. All statistical analyses were performed using the SPSS version 11.5 (SPSS, 2005).

RESULTS AND DISCUSSION

Larval acceptance rate: The larvae transfer results of honeybee colonies supplemented with different industrial sugars at different locations have been presented in Table 1.

In the study, the number and rate of larval accepted at Doğanşehir (4758 and 75.52%) were found to be higher than that of Battalgazi (4296 and 68.19%). In the feeding group, the control group had a lower larval acceptance rate (65.49%) than the others. In the location x feeding group interaction, the lowest larval acceptance rate occurred in the Battalgazi control group (62.03%) and the highest in the Doğanşehir sucrose group (81.71%). In the study, although 6300 larvae were grafted for each location in the evaluation based on the location, a 31.81% loss occurred in Battalgazi, resulting in a larval acceptance rate of 68.19%. At Doğanşehir, the number of larval accepted was 7.33% higher than that of Battalgazi. According to the location x feeding group interaction, similarly, a higher larval acceptance rate occurred at Doğanşehir in all feeding groups (Table 1). This difference that occurred despite the same number of grafts shows that the conditions that facilitated the larval acceptance were more favorable at Doğanşehir.

The differences between the larval acceptance rates according to the location were calculated to be statistically significant ($P<0.01$), and a higher larval acceptance rate occurred at Doğanşehir. Feeding also had a statistically significant effect on the larval acceptance rate ($p<0.01$). The differences between the larval acceptance rates of the feeding groups, the sucrose, bee feed and the glucose groups were not found to be significant ($P>0.05$). Despite this, it was determined that the differences between the control and sucrose, bee feed, and glucose groups of the feeding group were significant ($P<0.01$) (Table 1).

Table 1. The results of larvae transfer in honeybee colonies supplemented with various industrial sugars at different locations.

Factors	n	Grafted larvae (pieces)	Accepted larvae (pieces)	Larval acceptance rate (%)
Location				
Battalgazi (1)	20	6300	4296	68.19 ± 1.20^{A}
Doğanşehir (2)	20	6300	4758	75.52 ± 0.97^{B}
General	40	12600	9054	71.86±0.80
Nutrition Groups				
Sucrose (1)	10	3150	2349	74.57 ± 1.86^{x}
Bee food (2)	10	3150	2327	73.87 ± 1.41^{x}
Glucose (3)	10	3150	2315	73.49 ± 1.32^{x}
Control (4)	10	3150	2063	65.49 ± 1.32^{y}
General	40	12600	9054	71.86±0.80
Location x Nutrition Groups				
1x1	5	1575	1062	67.43 ± 2.97^{c}
1x2	5	1575	1152	73.14 ± 2.28^{bc}
1x3	5	1575	1105	70.15 ± 1.78^{c}
1x4	5	1575	977	62.03 ± 2.12^{d}
2x1	5	1575	1287	81.71 ± 1.49^{a}
2x2	5	1575	1175	74.60 ± 1.69^{bc}
2x3	5	1575	1210	76.82 ± 1.82^{ab}
2x4	5	1575	1086	68.95 ± 2.13^{c}
Total	40	12600	9054	71.86±0.80
Location			**	
Nutrition Groups			**	
Location x Nutrition Groups			**	

**:P<0.01

A, B: The differences between averages marked with different letters in the same column are significant (P<0.05)

x, y: The differences between averages marked with different letters in the same column are significant (P<0.05)

a,b,c,d: The differences between averages marked with different letters in the same column are significant (P<0.05)

The effect of the location X feeding group interaction on the larval acceptance rate was also found to be statistically significant (P<0.01) (Table 1).

In the study, the larval acceptance rates of the sucrose, bee feed and the glucose groups of the feeding groups were similar. However, based on the location x feeding group interaction, the statistically significant highest larval acceptance at Doğanşehir occurred in the group fed with sucrose syrup. At Battalgazi, the larval acceptance was higher in the group fed with bee feed than the others (Table 1).

In the study, it was observed that the control group in the feeding group had a lower larval acceptance rate than those fed with sucrose, bee feed and glucose. This indicated that supplementary feeding based on sugar had a positive effect on larval acceptance regardless of its nature, whereas no supplementary feeding results in lower larval acceptance. According to the principle that the weight of vitality is of more priority than the weight of yield while feeding, bees should be fed adequately for high larval acceptance. A lower larval acceptance occurs under unfavorable conditions that prevent bees from reaching nutrients available or lead to insufficient nutrients in the flora, especially such as excessive rainfall or drought. Indeed, due to the fact that the season was dry during the study, the plants dried up rapidly, causing bees to reach less nectar and pollen. The insufficiency of food in the region where the study was conducted resulted in lower larval acceptance in the control groups that were not given supplementary food. Jianke et al. (2003) reported that the amount of feeding should be determined according to the nectar in nature, honey stored in the beehive with bees and the number of frames with brood and that in general, 50-100 ml of food should be calculated per frame with bees. And also the age of the larvae should be as equal as possible, and no more than 24 h old. The larvae should be healthy and not separated from the brood food during transfer (Chen et al., 2002).

In the study we conducted, taking into account the

time, effort and cost advantage demonstrated in literature reports, it was preferred to perform a single grafting in the colonies. Emsen et al. (2003) reported that they achieved a 77% larval acceptance rate with single grafts in the study they conducted. As observed, a higher larval acceptance occurs with single grafting compared to double grafting. Despite this, in our study, the larval acceptance rates achieved from the locations and their general average were lower than these reports in the literature. In another study conducted that gave 45 cells per colony and harvested royal jelly every 48 hours (Kutluca et al., 1996), the average larval acceptance rate (81.98%) was higher than the larval acceptance rate achieved in this study.

Royal jelly yield: The findings of the royal jelly yield per cell and colony and the total yield per colony of the colonies supplemented with different industrial sugars at different locations have been presented in Table 2.

In the current study, royal jelly was produced in queenless starter colonies. Taber (1991) and Laidlaw and Harry (1992) reported that queenless colonies were more attracted to cells grafted than queenright colonies, and Öder (1989) and Johansson and Johansson (1994) reported that the larval acceptance rate was higher in queenless colonies. In this context, in the study conducted by Ibrahim (2002), it was reported that 7.54 g/colony royal jelly was collected in queenless colonies and 5.67 g/colony was collected in queenright colonies. In the study we conducted in queenless colonies, 7.59 g/colony royal milk was obtained at the Battalgazi location, 6.17 g/colony at Doğanşehir, and an average of 6.88 g/colony at the Battalgazi and Doğanşehir locations (Table 2). Although the yield obtained from queenless colonies at Battalgazi was a bit higher than the yield obtained from queenless colonies by Ibrahim (2002), Doğanşehir, and the average of both locations were lower than the general yield. However, the value obtained from

Table 2. The royal jelly yield of honeybee colonies supplemented with different industrial sugars at different locations.

Factors	n	Yield per cell (mg / cell) $\bar{x} \pm S_{\bar{x}}$	Yield per application (g/app.) $\bar{x} \pm S_{\bar{x}}$	n	Total yield per colony (gr / col.) $\bar{x} \pm S_{\bar{x}}$
Location					
Battalgazi (1)	28	245.78±13.81	7.59±0.51	20	37.96 ±2.56
Doğanşehir (2)	28	180.52±16.50	6.17±0.55	20	30.83±2.73
General	56	213.15±11.53	6.88±0.38	40	34.40±1.91
Nutrition Groups					
Sucrose (1)	14	215.20±19.66	7.19±0.76	10	35.94 ± 3.79^{x}
Bee food (2)	14	223.62±22.89	7.48±0.83	10	37.41 ± 4.14^{x}
Glucose (3)	14	218.96±20.62	7.15±0.64	10	35.73 ± 3.18^{x}
Control (4)	14	194.82±29.63	5.70±0.82	10	28.50 ± 4.08^{y}
General	56	213.15±11.53	6.88±0.38	40	34.40±1.91
Location x Nutrition Groups					
1 x 1	7	250.33±17.52	7.61 ± 0.92^{ab}	5	38.04 ± 4.62^{AB}
1 x 2	7	268.59±29.59	8.87 ± 1.17^{a}	5	44.33 ± 5.85^{A}
1 x 3	7	248.78±26.30	7.75 ± 0.78^{ab}	5	38.77 ± 3.89^{AB}
1 x 4	7	215.43±36.24	6.14 ± 1.12^{ab}	5	30.69 ± 5.59^{AB}
2 x 1	7	180.07±30.93	6.77 ± 1.26^{ab}	5	33.85 ± 6.28^{AB}
2 x 2	7	178.65±26.85	6.10 ± 0.98^{ab}	5	30.49 ± 4.90^{AB}
2 x 3	7	189.14±29.22	6.54 ± 1.01^{ab}	5	32.69 ± 5.06^{AB}
2 x 4	7	174.21±48.49	5.26 ± 1.25^{b}	5	26.30 ± 6.28^{B}
Total	56	213.15±11.53	6.88±0.38	40	34.40±1.91
Location		*	-		-
Nutrition Groups		-	-		*
Location x Nutrition Groups		-	*		*

-:P>0.05, *:P<0.05

a,b: The differences between averages marked with different letters in the same column are significant (P<0.05)

A, B: The differences between averages marked with different letters in the same column are significant (P<0.05)

x, y: The differences between averages marked with different letters in the same column are significant (P<0.05)

the Battalgazi and Doğanşehir locations, and the general averages of both locations were higher than the value obtained by Ibrahim (2002) from queenright colonies.

The royal jelly obtained per cell in the study conducted (213.15 mg/cell) (Table 2) was higher than the amount obtained by Kumar (2015) from harvests with 48-hour intervals (148.48 mg/cell), by Kutluca et al. (1996) from harvests from 45 cells with intervals of 48 hours (152.528 ± 8.708 mg/cell), by Ergün (2010) obtained 60-72 hours after the transfer (200 mg/cell) and between the values reported by Silici (2009) (148-281 mg/cell). The total yield per colony in our study (30.40 g/colony) (Table 2) remained between the amount obtained by Ergün (2010) (20-50 g/colony) and was lower than the amount obtained by Jianke (2000) (69-100 g). While Jianke (2000) achieved this yield from 125-170 queen cells, in our study, the yield came from 45 cells. Korkmaz and Öztürk (2010) stated that 6.46-10.56 g of royal jelly could be obtained from one colony in one grafting. Muli et al. (2005) was stated that for the 3-day cycle to have higher royal jelly yields per cup (349.5 mg) than the 2-day cycle (236.3 mg). They reported that harvesting royal jelly on a 2-day cycle could be better if one has a higher number of colonies to compensate for the lower yields per cell cup. Chen et al. (2002) stated that the harvesting interval for royal jelly is either three days (68-72 h) or two days (48 h). The total royal jelly production for the two-day cycle in one month is higher than that of the three-day cycle. Harvest times should be decided according to the manpower available in the apiary.

As the number of larvae grafted increases, the amount of royal jelly per cell decreases, but despite this, an increase in the royal jelly amount is achieved (Silici, 2009). The timing of harvest is also important for the amount of royal jelly obtained. Many studies report that the most yield is achieved from harvests done every 72 hours (Kumar, 2015; Sharaf El-Din et al., 2010). Due to the aging of the growing larvae and the subsequent increase in royal jelly consumption, the amount of royal jelly decreases in harvests made 96 hours after grafting (Kumar, 2015). In another study reported that the average royal jelly per queen cup was highest in 24-h-old larvae (419.5 mg) and least in queen cups grafted with 48- or 60-h-old larvae (181.5 mg). Royal jelly yields from larvae grafted at the age of 24 h were significantly different from those of larvae grafted at the ages of 36, 48 and 60 h (Muli et al., 2005). Salem et al. (2021) reported that the highest royal jelly production was attained when grafting one day old larvae.

In the study conducted, at Battalgazi the yield per cell (245.78 mg/cell), yield per application (7.59 g/app.), and the total yield per colony (37.96 g/col.) was higher than that of Doğanşehir (180.52 mg/cell, 6.17 g/app. and 30.83 g/col.). In the feeding group, the control group achieved a lower value than the others in terms of all three criteria (194.82 mg/cell, 5.70 g/app. and 28.50 g/col.). In the location x feeding group interaction, the lowest value in all three criteria occurred in the Doğanşehir control group (174.21 mg/cell, 5.26 g/app. and 26.30 g/col.) and the highest in the Battalgazi bee feed group (268.59 mg/cell, 8.87 g/app. and 44.33 g/col.) (Table 2). The yield of royal jelly is related to the weight of royal jelly per cell and the number of queen cups in a colony. Deciding on the appropriate number of queen cups according to the size of the colony population is important to make full use of the colony's potential. This range of published values reflects many variables such as bee subspecies, climatic differences, floral resource.

CONCLUSION

In the study, it was determined that supplemental feeding of bees with sucrose, bee feed or glucose had significant positive effects on the larval acceptance rate compared to the control group. However, there was no significant difference between the supplementary feeding methods in terms of their effects on the larval acceptance rate. On the other hand, the locations where the royal jelly is produced affect both the larval acceptance rate and the royal jelly yield. At Doğanşehir, one of the study locations, a higher larva acceptance rate was obtained than Battalgazi. At Doğanşehir, the highest larval acceptance occurred in the group fed with sucrose. The royal jelly yield was higher in Battalgazi. At Battalgazi, the highest royal jelly yield was achieved in the group fed with bee feed. It has been concluded that supplementary feeding with various industrial sugars can be carried out and that such implementations would be beneficial, and that based on the preference of larvae or royal jelly production, determining the locations considering the Battalgazi and Doğanşehir results of this study, will contribute to increasing the royal jelly yield. It has been evaluated that it would be important to make decisions separately in the assessment of the effect of feeding on the contents of royal jelly and the relationship between production cost and yield.

ACKNOWLEDGEMENTS

We would like to thank Scientific Research Project Fund of Inonu University (FCD-2018-1118) for their financial contribution to our study.

CONFLICT OF INTEREST STATEMENT

The authors declare there is no conflict of interest.

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