Kafkas Univ Vet Fak Derg
25 (6): 849-855, 2019
DOI: 10.9775/kvfd.2019.21998

Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org
Online Submission: http://submit.vetdergikafkas.org

Research Article

The Effect of the Supplemental Feeding of Queen Rearing Colonies on the Reproductive Characteristics of Queen Bees *(Apis mellifera* L.) Reared from Egg and Different old of Larvae

Mahir Murat CENGİZ [1,a] Kemal YAZICI [2,b] Servet ARSLAN [3,c]

[1] Ataturk University, Erzurum Vocational School, Department of Plant and Animal Production, Yakutiye, TR-25240 Erzurum - TURKEY
[2] Ardahan University, Posof Vocational School, Department of Plant and Animal Production, Posof, TR-75800 Ardahan - TURKEY
[3] Akdeniz University, Vocational School of Technical Sciences, Department of Organic Agriculture, TR-07000 Antalya - TURKEY
[a] ORCID ID: 0000-0002-9844-4229; [b] ORCID ID:0000-0002-1369-5994; [c] ORCID ID: 000-0003-3892-8130

Article ID: KVFD-2019-21998 Received: 07.02.2019 Accepted: 20.06.2019 Published Online: 21.06.2019

How to Cite This Article

Cengiz Mm, Yazıcı K, Arslan : The effect of the supplemental feeding of queen rearing colonies on the reproductive characteristics of queen bees *(Apis mellifera* L.) reared from egg and different old of larvae. *Kafkas Univ Vet Fak Derg,* 25 (6): 849-855, 2019. DOI: 10.9775/kvfd.2019.21998

Abstract

This study was carried out to determine the effect of supplemental feeding on the reproductive characteristics of queen bees reared from different stages of brood. Queen were reared from one and two-day-old larvae grafted by Doolittle method and were reared from the eggs were transferred by Karl Jenter set and given to the starter colonies prepared as queenless. The starter colonies are arranged as follows: B1, one-day-old larva were grafted; B2, two-day-old larvae were grafted; B3, two-day-old eggs were transfered; and F1, four grams of vitamin a, protein, and minaral mixture were added to the sugar syrup at a ratio of one to one (w/w) per day; F2, no supplemental feeding and the bees benefited only from natural resources. In general, supplemental feeding of starter colonies increased the acceptance rate of larvae and eggs. Colonies fed with a supplemental diet had a higher acceptance rate (82.35%) compared to unfed colonies (62.74%). The highest queen emergence weight (205.75±1.46 mg) was obtained from the two-day-old egg transfer. In the fed group, the average emergence weight of the queen bee was found to be 195.01±2.03 mg, while this value was determined as 186.30±2.09 mg in the group that was not fed. Supplemental feeding of the colonies increased the spermathecae diameter of the queens from 0.98±0.025 mm to 1.09±0.025 mm, while the number of spermatozoa in the spermathecae increased from 4.26±0.679 million to 4.54±0.648 million.

Keywords: *Egg transfering, Honeybee, Larva grafting, Queen rearing, Reproductive features, Supplemental feding*

Yetiştirme Kolonilerinde Ek Besleme Yapmanın Yumurta ve Farklı Yaştaki Larvalardan Yetiştirilen Ana Arıların *(Apis mellifera* L.) Üreme Özellikleri Üzerine Etkisi

Öz

Bu çalışma ek beslenmenin yumurta ve farklı yaşlardaki larvalardan yetiştirilen ana arıların üreme özellikleri üzerine etkisini belirlemek amacıyla yapılmıştır. Bir ve iki günlük yaştaki larvalar Doolitle yöntemiyle ve yumurtadan ana arı üretimi ise jenter seti yardımıyla transfer edilerek ana arısız olarak hazırlanan başlatıcı kolonilere verilmiştir. Başlatma kolonileri aşağıdaki şekilde düzenlenmiştir: B1, bir günlük larva transferi; B2, iki günlük larva transferi; B3, iki günlük yumurta transferi; F1, günlük bir litre bire bir oranında (bir suya bir şeker) şeker şurubuna dört gr vitamin, protein ve minarel karışımı ilave edilmiştir. F2, ek besleme yapılmayarak arıların sadece doğal kaynaklardan yararlanması sağlanmıştır. Genel olarak, başlatma kolonilerine yapılan ek beslemeler larva ve yumurta kabul oranını arttırmıştır. Tamamlayıcı diyetle beslenen koloniler (%82.35), beslenmemiş kolonilere (%62.74) kıyasla daha yüksek kabul oranına sahip olmuşlardır. Yetiştirme grupları içinde en yüksek ana arı çıkış ağırlığı (205.75±1.46 mg) iki günlük yumurta transferinden elde edilmiştir. Besleme yapılan grupta ortalama ana arı çıkış ağırlığı 195.01±2.03 mg olarak bulunurken, bu değer ilave beslenme yapılmayan grupta 186.30±2.09 mg olarak belirlenmiştir. Kolonilere ek besleme yapmak ana arıların spermatheca çapında 0.98±0.025 mm'den 1.09±0.025 mm'ye bir artış sağlarken, sparmatheka içindeki sperm sayısında ise 4.26±0.679 milyondan 4.54±0.648 milyona bir artış sağlamıştır.

Anahtar sözcükler: *Yumurta transferi, Larva aşılaması, Balarısı, Ana arı yetiştirme, Ek besleme, Üreme özellikleri*

İletişim (Correspondence)
+90 442 2313723 GSM: +90 542 6919437
mcengiz@atauni.edu.tr

INTRODUCTION

Usually a honey bee colony has one queen, a few hundred drones and thousands of worker bees. However, due to its anatomical, physiological and behavioral characteristics and its functions within the hive, queen is the most important individual in a colony [1,2]. It is possible to identify colony performance with the performance of the queen bee [3].

Having quality queen bees can help to inctease the performance of honey bee colonies. However, the quality of a queen varies depending on many physical properties, such as emergence weight, diameter of spermatheca and sperm number stored in the spermatheca [4-6]. Physical characteristics that affect the quality of the queen are influenced by various factors such as rearing season, genotype, feeding, age and number of transferred larvae [6-8]. Many investigators have reported a negative relationship between larval age and quality of the queen [9-11].

Recently, supplemental feeding and dietary formulations of queen breeding colonies have become a common approach in beekeeping. Pollen, nectar, syrup and vitamins are important nutritional components for queen rearing colonies [12,13]. In addition, it has been reported that providing additional nutrients to the rearing colonies significantly increases the quality of the transferred larvae and hence the quality of the queen bees [14,15].

The need for large quantity and high-quality queen bee breeders in commercial queen breeding has led to different searches for methods to increase queen quality [7]. Doolittle [16] was the first person to instill worker larvae into artificial queen cells, and, since then, many studies have been conducted on the factors affecting the larval acceptance rate. Some researchers reported that the supplemental feeding of queen rearing colonies positively affected the larval acceptance rate [17,18]. Another researcher reported that the acceptance rate of egg transfer was lower than the transfer of larvae, but it was reported that the queen bees from the egg transfer had higher weights compared to the queen bees that reared from the larvae transfer [19,20].

Queen bee emergence weight can be used as a quality factor in the evaluation of queen bees because high emergence queen bees have a larger spermatheca diameter, a higher ovariol number, and a higher number of spermatozoa [4,21]. On the other hand, it is stated that queen body size or emergence weight can be used as a reliable index for determining the quality of the queen bee [22].

This study was carried out to determine effect of supplemental feeding on reproductive characteristics of queen bees reared from different stages of brood such as heights of sealed queen cell, emergence weight, spermatheca diameter and number of spermatozoa in spermathecae.

MATERIAL and METHODS

The research was conducted between June and August of 2017 in the beekeeping and research center of Ardahan University in Turkey. In the study, the Caucasian honey bee *(Apis mellifera caucasica)* colonies were used. The six sisters queen bees, which were reared in the previous year was used as starter colonies. Six colonies, each with ten frames, were reduced to five frames, and then the starter colonies were formed equalized according to adult bees, brood area, honey and pollen frame.

The starter colonies were arranged as follows: B1, one-day-old larva transfer; B2, two-day-old larvae transfer; B3, two-day-old egg transfer; F1, four grams/kg of a vitamin, protein, and mineral mixture were added to the sugar syrup at a ratio of one to one (w/w) per day (vitamin A, 6.000.000 I.U; vitamin D3, 1.200.000 I.U; vitamin E, 1000 mg; vitamin B12, 24 mg; vitamin C, 5000 mg; biotin, 10 mg; folic acid, 100 mg; choline, 3000 mg; inositol, 3000 mg; carotene, 100 mg; methionine, 3000 mg; lysine, 6000 mg; threonine, 3000 mg; tryptophan, 3000 mg; manganese, 300 mg; iron, 300 mg; zinc, 300 mg; copper, 100 mg; iodine, 50 mg; magnesium 10.000 mg; potassium, 20.000 mg; and phosphorus 3000 mg); F2, no supplemental feeding and the bees provided benefited only from natural resources. Eggs and larvae transfers to each group were given to six starter colonies. 34 larvae/eggs were transferred to each of these colonies. One and two-day-old larvae were transferred by the Doolittle method and queens reared from the egg were transferred by Karl Jenter set and given to the starter colonies prepared as queenless [19,20,22]. The starter colonies were fed two days before the transfers and feeding was continued until the queen cells were sealed [14]. In this case, eight days (eight liters of syrup) to the B1 starter colony, seven days (seven liters of syrup) to the B2 starter colony, and 10 days (10 liters of syrup) were given to the B3 starter colony.

The acceptance rate was determined by counting the larvae/eggs which were accepted two days after the larvae/egg transfer. Larvae/egg acceptance rates were calculated as a percentage with the following formula:

$$\text{Acceptance Rate} = \frac{\text{(Accepted Larvae/Egg)}}{\text{(Total Grafted Larvae/Egg)}} \times 100$$

On the tenth day, queen cells were measured with using calipers and then caged, and emergence time was provided. The queens were weighed in a sensitive scale (mg), and the emergence weights were determined. The selected queens were introduced to the combs that covered with worker in mating nucs and mated. Twenty days after mating, the spermatheca of ten laying queens were removed in each group, the trachea on the spermatheca was cleaned, the sperm sac was taken on a lamella slide, and the diameters of spermatheca were measured with

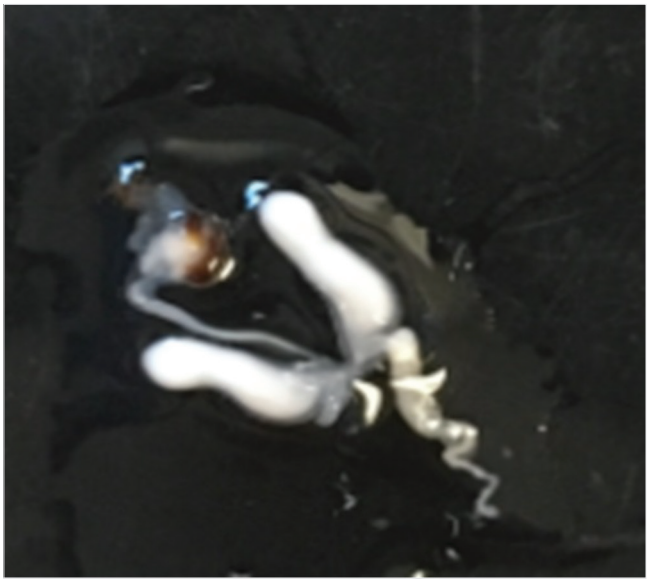

Fig 1. Queen bee reproductive organs

Fig 2. Queen bee and spermathecae

an ocular micrometer at a 4.5x10 magnification microscope [21,23-25]. The spermathecae were then discharged with a fine insect needle and fine forceps in 1 mL of saline solution (0.9%). Tap water was added to make 10 mL total volume. The sample that was taken from this mixture was dropped between the lamella and the lamella slide; then the number of spermatozoa in the square part of the Thoma slide were counted, and the total amount of spermatozoa (million pieces/queen) found in the 10 mL mixture and also in the sperm sac of the queen bee was calculated [23-27].

In the statistical analysis of the data heights of the sealed queen cell, the emergence weight, the diameter of the spermathecae and number of spermatozoa were compared according to one-way analysis of variance[28]. The chi-square test was used for the statistical analysis of cell acceptance rates. The DUNCAN multiple comparison test was used to determine the differences between the groups.

RESULTS

The acceptance rates in one, two-days-old larvae and two-day-old eggs groups were determined 72.06%, 83.82%, and 61.76%, respectively *(Table 1)*. The highest acceptance rate was obtained from two-day old larvae while the lowest acceptance rate was obtained from two-day old egg. The difference between larval acceptance rates was statistically insignificant while the difference between larval and egg transfer rates was statistically significant (P<0.01). The acceptance rate of egg transfer was lower than larva transfers.

In general, giving a feed containing proteins, vitamins, and minerals to the initiating starter colonies increased the larval and egg acceptance rate *(Table 1)*. The rate of acceptance in the fed colonies (82.35%) was found higher than the acceptance rate of non-fed colonies (62.74%). The difference was observed in the acceptance rate of larvae and eggs between the fed and non- the fed groups was found significant (P<0.01).

The results show that the longest sealed queen cell was obtained from two-days-old egg transfer group. The difference between the groups in terms of sealed queen cell was found to be statistically significant (P<0.01). Although the acceptance of egg transfer is low, it is thought to be an important application in terms of increasing the sealed queen cell length. While 29.05±0.24 mm long sealed queen cell was obtained from fed colonies, this value was determined to be 27.03±0.39 mm in non-fed colonies. The difference observed in terms of sealed queen cell between groups was found to be significant (P<0.01).

Table 2 shows emergence weights of queens raised from the one-day-old larvae, two-day-old larvae, and two-day-old eggs. The highest emergence weight (205.75±1.46 mg) was obtained from the two day old egg transfer. The difference observed between the emergence weights of the queens raised from different stages of the brood was significant (P<0.01). In other words, egg transfer was significantly effective for the emergence weight of the queen bee.

In the feeding group, the average (±S.E.) emergence weight of the queens was found to be 195.01±2.03 mg, and this value was determined to be 186.30±2.09 mg in the group which was non-fed. The effect of feeding on the emergence weight of the queen was found to be significant (P<0.05). Feeding the starter colonies influenced the emergence weight of the queen bee.

Table 3 presents the diameter of the spermathecae and

Table 1. *Average larvae/egg acceptance rates of the groups (%)*

Groups	Number of Grafted Larva and EGG	Number of Accepted Larvae and Egg	Grafting Success Rate (%)
Breeding Method (B)			
1 day old larvae (B1)	68	49	72.06[ab]
2 day old larvae (B2)	68	57	83.82[a]
2 day old egg (B3)	68	42	61.76[b]
Feeding Medhod (F)			
Fed colonies (F1)	102	84	82.35[a]
Unfed colonies (F2)	102	64	62.74[b]
B x F Interaction			
B1XF1	34	28	82.35[ab]
B1XF2	34	21	61.76[c]
B2XF1	34	32	94.11[a]
B2XF2	34	25	73.53[b]
B3XF1	34	24	70.58[b]
B3XF2	34	18	52.94[c]

[a,b,c] *Means with no common superscripts differ (P<0.01)*

Table 2. *The average (±S. E.) of sealed queen cell length and emergence weight of queen bees in research groups*

Groups	N	Sealed Queen Cell Length (mm)	N	Emergence Weight (mg)
		X±Sx		X±Sx
Breeding Method (B)				
1 day old larvae (B1)	49	27.52±0.30[b]	48	191.90±1.46[b]
2 day old larvae (B2)	57	24.20±0.37[c]	56	174.32±1.94[c]
2 day old egg (B3)	42	31.40±0.30[a]	42	205.75±1.46[a]
Feeding Medhod (F)				
Fed colonies (F1)	84	29.05±0.24	84	195.01±2.03[a]
Unfed colonies (F2)	64	27.03±0.39	62	186.30±2.09[b]
B x F Interaction				
B1XF1	28	28.25±0.34[c]	28	195.85±1.68[c]
B1XF2	21	26.80±0.41[d]	20	187.95±2.07[d]
B2XF1	32	25.30±0.48[e]	32	179.85±2.67[e]
B2XF2	25	23.10±0.44[f]	24	168.80±2.28[f]
B3XF1	24	32.60±0.34[a]	24	209.35±2.33[a]
B3XF2	18	30.20±0.32[b]	18	202.15±1.40[b]

[a,b,c,d,e,f] *Means with no common superscripts differ (P<0.01); (P<0.05)*

number of spermatozoa of queens raised from the one day, two-day-old larvae and two-day-old eggs. The spermatheca diameters for the queens raised from the one-day, two-day-old larvae and two-day-old eggs were determined to be 1.04±0.018 mm, 0.83±0.017 mm, and 1.23±0.021 mm, respectively *(Table 3)*. The largest spermatheca diameter was obtained from egg transfer (two-days old), while the lowest spermatheca diameter was obtained from the queens raised from two-days-old larvae. The difference between the breeding groups was statistically significant (P<0.01). The results indicated that the egg transfer method has been increased the spermatheca diameters in honey bee queens. In addition, the ages of grafted larvae have a significant effect on diameter of the spermathecae and number of spermatozoa stored in sperm sac.

When the feeding colonies (1.09±0.025 mm) were compared with non-fed (0.98±0.025 mm), the diameter of spermatheca increased significantly (P<0.05). Supplemental feeding to the colonies increased the spermatheca diameter of the queen bees *(Table 3)*.

The breeding and feeding method influenced the number

Table 3. *The average (±S.E.) spermathecae diameters and numbers of spermatozoa stored in sperm sac in groups*

Groups	Diameter of the Spermathecae (mm)		Number of Spermatozoa (×10⁶)	
	N	X±Sx	N	X±Sx
Breeding Method (B)				
1 day old larvae (B1)	20	1.04±0.018[b]	20	4.44±0.429[b]
2 day old larvae (B2)	20	0.83±0.017[c]	20	3.81±0.451[c]
2 day old egg (B3)	20	1.23±0.021[a]	20	4.95±0.271[a]
Feeding Medhod (F)				
Fed colonies (F1)	30	1.09±0.025[a]	30	4.54±0.648[a]
Unfed colonies (F2)	30	0.98±0.025[b]	30	4.26±0.679[b]
B x F Interaction				
B1XF1	10	1.10±0.019[c]	10	4.56±0.693[c]
B1XF2	10	0.98±0.025[d]	10	4.33±0.371[d]
B2XF1	10	0.88±0.22[e]	10	3.99±0.509[e]
B2XF2	10	0.78±0.23[f]	10	3.62±0.462[f]
B3XF1	10	1.29±0.032[a]	10	5.08±0.204[a]
B3XF2	10	1.18±0.030[b]	10	4.82±0.288[b]

[a,b,c d,e,f] *Means with no common superscripts differ (P<0.01); (P<0.05)*

of sperms in the spermatheca (P<0.01). Queens raised from two-day-old larvae have a significantly smaller number of spermatozoa than the queens raised from one-day-old larva or two-day-old eggs *(Table 3)*. The highest number of spermatozoa were obtained from the queens raised from the two-day-old egg *(Table 3)*. The difference in number of spermatozoa among the breeding groups was significant (P<0.01). When fed colonies were compared with non-fed colonies, it was observed that the queens had more spermatozoa in the spermatheca. Feeding of starter colonies had an important effect to increase the number of sperms in the spermatheca, and this increase was found to be significant (P<0.01).

DISCUSSION

In this study, average acceptance rates in one, two-days-old larvae and two-day-old eggs groups were determined 72.06%, 83.82%, and 61.76%, respectively. The larva acceptance rate lower than Okuyan and Akyol [11] findings which indicate that the average acceptance rates from one and two-day-old larvae 81% and 85% respectively. However, the finding that the larval acceptance rate was found to be compatible with Gençer et al.[14] which indicate that the average acceptance rates from one and two-day-old larvae 73.4% and 82.3% respectively. Although the acceptance rate of the two-day-old larvae was high, no statistically significant difference was found between larval transfers. The lowest acceptance rate (61.76%) was obtained from egg transfer. This result is similar to that of Şahinler [19] (64%). The acceptance rates could be affected by rearing season, rearing methods and transfer material. In general, feeding increased larval and egg acceptance rates in all groups. Feeding of the queen rearing colonies with a feed containing protein, vitamins, and minerals can be said to increase the acceptance rate. According to Gençer et al.[14] and Sagili et al.[29], adding pollen or a vitamin mixture to syrup increases the larval acceptance rate.

The average height of queen cell cups obtained from one, two-day-old larvae and two-day-old eggs was 27.52±0.30 mm, 24.20±0.37 mm and 31.40±0.30 mm respectively. The average height of queen cell cups higher than Genç et al.[30] findings which indicate that the average height of queen cell cups obtained from one and two-day-old larvae 25.70±1.4 mm and 23.90±0.3 mm respectively. However, the height of queen cell cups was lower than findings of Cengiz et al.[22] which indicate that average height of queen cell cups from one-day-old larvae was 30.71±0.14 mm. The average heights of queen cell cups obtained in this study are similar to results obtained by Emsen et al.[9] which indicate that the average height of queen cell cups from one and two-day-old larvae 29.98±0.08 and 24.27±0.78 respectively. The findings of these researchers illustrate that height of queen cell cups can have a high variability.

According to Vaziritabar and Esmaeilzade [31], there is a positive correlation between the sealed queen cell and the emergence weights of queen bees. When evaluated in this context, the sealed queen cell obtained from egg scales can be said to be an important application for increasing the emergence of queen bees. A sealed queen cell obtained from the fed groups are longer than from the non-fed groups. In other words, feeding increased the height of the sealed queen cell. According to Njeru et al.[8] and Mahbobi et al.[15] the supplemental feeding has a positive effect on all morphological characteristics of

queen bees. When these results are evaluated, it can be said that adding supplemental feeding to queen rearing colonies increases the sealed queen cell length.

The average reared queen from one, two-day-old larvae and two-day-old eggs weight was 191.90±1.46, 174.32±1.94 and 205.75±1.46 respectively. The queen weights are higher than Gençer et al.[14] findings which indicate that the average of queen weights reared from one and two-day-old larvae 166.6±1.74 mg and 160.8±1.22 mg respectively. However, the weight of queen was lower than findings of Akyol et al.[4] which indicate that classified reared queens into three different group as heavy, medium and light and the average weight of these were 207.63, 193.47, and 175 mg respectively. it was found consistent with the emergence weight reported by Cengiz et al.[22] and Dodoloğlu et al.[32] which indicate that the average of queen weights reared from one-day-old larvae 199.07±7.55 mg and 206.13±3.20 mg respectively.

In this study, the average emergence weight of the queen from the egg transfer was found to be 205.75±1.46 mg; while the average emergence weight reported by Şahinler [19] was lower than the average emergence weight in our study (informed as 187.6 mg), it was similar to the average emergence weight reported by Dhaliwal et al.[20] (informed as 201.88 mg). The weight of queen bees might be affected by supplemental feeding of starter hives, bee density in starter hives, genetic factors and season. It can be suggested that the transfer of the egg, which is seen to be effective in the emergence weight of queen, should considered for queen breeding. It can be said that it would be beneficial to apply feeding along with egg transfer, a very important queen quality criterion that positively affects the live weight.

In this study, average spermatheca diameters of 1.10±0.019 mm and 0.98±0.025 mm from one-day-old larvae were obtained from the groups with and without supplemental feeding, respectively. The average spermatheca diameter obtained in a study by Dodoloğlu et al.[32] was found to be similar to that of the non-fed group but lower than the fed group (informed as 0.98±0.01 mm). However, average spermatheca diameters from one-day-old larvae was lower than findings of Akyol et al.[4] for heavy grups (informed as 1.258±0.2 mm). The spermatheca diameter obtained from egg transfer higher than Şahinler [19] findings which indicate that the average spermatheca diameter obtained from egg transfer 1.132±0.040 mm. The average spermatheca diameter of 1.23±0.021 mm the queen bees obtained from egg transfer was found compatible with the spermatheca diameter reported by Akyol et al.[4] for heavy grup (informed as 1.258±0.2 mm). The spermatheca diameters queen bees might be affected by genetic factors and season. In terms of influencing the spermatheca diameter of queens raised with supplemental feeding, our findings were similar to the results of Njeru et al.[8] and Mahbobi et al.[15]. In this study, it was observed that egg transfer promoted a more effective diameter of spermathecae in the queen than other transfers. These results highlight the transfer of eggs as grafting material.

The average number of spermatozoa in the spermathecae 4.44±0.429 million/queen obtained from one-day-old larvae was found to be lower than the finding of Güler and Alpay [33] (informed as 5.08±0.18). However, our findings were consistent with the reports of Koç and Karacaoğlu [6] for Caucasian bees (informed as 4.24±0.599). The average number of spermatozoa in the spermathecae of the queens obtained from the two-day-old egg transfer was similar to the values reported by Akyol et al.[4] for medium grup and Kahya et al.[34] (informed as 4.75±0.2 and 4.87±78). It is estimated that the number of different spermatozoa reported by the researchers is due to the season and the number of adult drones. In our study, it was seen that more spermatozoa were obtained from the fed groups than the other groups. This result is consistent with the results reported by Njeru et al.[8] and Mahbobi et al.[15].

It has been shown that supplemental feeding increases the acceptance rate in all groups; therefore, supplemental feeding to queen rearing colonies is important in increasing the success rate. Although the rate of acceptance of egg transfer is low, it is thought to be an important application in terms of increasing the emerge weight of queen bees. In commercial queen rearing, we recommend that bee-keepers rearing queen bees by supplemental feeding from one day old larvae. As a result, the feeding and transfer age of larvae were found to impact the live weight of queens, the diameter of spermathecae, and the number of spermatozoa in the spermatheca. This finding is supported by studies conducted by many researchers. It can be said that it is possible to produce better quality queens by means of supplemental feeding of starter colonies and use of eggs as transfer material.

REFERENCES

1. Lee KV, Goblirsch M, McDermott E, Tarpy DR, Spivak M: Is the brood pattern within a honey bee colony a reliable indicator of queen quality? *Insects*, 10 (1):12, 2019, DOI: 10.3390/insects10010012

2. Wu X, Zhou L, Zou C, Zeng Z: Effects of queen cell size and caging days of mother queen on rearing young honey bee queens *Apis mellifera* L. *J Apic Res*, 62 (2): 215-222, 2018. DOI: 10.2478/JAS-2018-0025

3. Walsh E, Rangel J: Local honey bee queen production and quality. *Bee World*, 93 (2): 30-32, 2016. DOI: 10.1080/0005772X.2016.1211501

4. Akyol E, Yeninar H, Kaftanoğlu O: Live weight of queen honey bees *(Apis mellifera* L.*)* predicts reproductive characteristics. *J Kans Entomol Soc*, 81 (2): 92-100, 2008. DOI: 10.2317/JKES-705.13.1

5. Hatjina F, Bienkowska M, Charistos L, Chlebo R, Costa C, Drazic´ MM, Filipi J, Gregorc A, Ivanova EN, Kezıc N, Kopernicky J, Kryger P, Lodesani M, Lokar V, Mladenovıc M, Panasiuk B, Petrov PP, Rasic S, Skerl MIS, Vejsnæs F, Wilde J: A review of methods used in some European countries for assessing the quality of honey bee queens through their physical characters and the performance of their colonies. *J Apicult Res*, 53 (3): 337–363, 2014. DOI: 10.3896/IBRA.1.53.3.02

6. Uçak Koç A, Karacaoğlu M: Effects of queen rearing period on reproductive features of Italian *(Apis mellifera ligustica)*, Caucasian *(Apis mellifera caucasica)*, and Aegean ecotype of Anatolian honey bee *(Apis*

mellifera anatoliaca) queens. *Turk J Vet Anim Sci*, 35 (4): 271-276, 2011. DOI: 10.3906/vet-1007-375

7. De Souza DA, Huang MH, Tarpy DR: Experimental improvement of honey bee *(Apis mellifera)* queen quality through nutritional and hormonal supplementation. *Apidologie*, 50 (1): 14-27, 2019. DOI: 10.1007/s13592-018-0614-y

8. Njeru LK, Raina SK, Kutima HL, Salifu, D, Cham DT, Kimani JNAA, Muli EM: Effect of larval age and supplemental feeding on morphometrics and oviposition in honey bee *Apis mellifera scutellata* queens. *J Apicult Res*, 56 (3): 183-189, 2017. DOI: 10.1080/00218839.2017.1307714

9. Emsen B, Dodologlu A, Gene F: Effect of larvae age and grafting method on the larvae accepted rate and height of sealed queen cell *(Apis mellifera* L.). *J Appl Anim Res*, 24 (2): 201-206, 2003. DOI: 10.1080/09712119.2003.9706457

10. Tarpy DR, Simone-Finstrom M, Linksvayer TA: Honey bee colonies regulate queen reproductive traits by controlling which queens survive to adulthood. *Insect Soc*, 63 (1): 169-174, 2016. DOI: 10.1007/s00040-015-0452-0

11. Okuyan S, Akyol E: The effects of age and number of grafted larvae on some physical characteristics of queen bees and acceptance rate of queen bee cell. *Turk J Agric Food Sci Technol*, 6 (11): 1556-1561, 2018.

12. Avni D, Dag A, Shafir S: The effect of surface area of pollen patties fed to honey bee *(Apis mellifera)* colonies on their consumption, brood production and honey yields. *J Apicult Res*, 48 (1): 23-28, 2009. DOI: 10.3896/IBRA.1.48.1.06

13. Estegamat O, Gholami M: Acceptance of queen transplantation in honey bee colonies influenced with artificial feeding (syrup, nectar or pollen). *Acad J Entomol*, 3 (2): 52-54, 2010.

14. Gençer HV, Shah SQ, Firatli Ç: Effects of supplemental feeding of queen rearing colonies and larval age on the acceptance of grafted larvae and queen traits. *Pak J Biol Sci*, 3 (8): 1319-1322, 2000.

15. Mahbobi A, Farshineh-Adl M, Woyke J, Abbasi S: Effects of the age of grafted larvae and the effects of supplemental feeding on some morphological characteristics of Iranian queen honey bees *(Apis mellifera meda* Skorikov, 1929*)*. *J Apicult Res*, 56 (1): 93-98, 2012. DOI: 10.2478/v10289-012-0010-1

16. Doolittle GM: Scientific queen-rearing. Thomas G. Newman & Son, Chicago, Illinois, 1899.

17. Şahinler N, Gül A, Şahin A: Vitamin E supplement in honey bee colonies to increase cell acceptance rate and royal jelly production. *J Apicult Res*, 44 (2): 58-60, 2005. DOI: 10.1080/00218839.2005.11101149

18. Goodman R: Raising Queen Honey Bees. Agriculture notes, ID: AG1194. State of Victoria, Department of Primary Industries, 2008.

19. Şahinler N: Yumurta ve larva transferi ile yetiştirilen ana arıların performanslarının belirlenmesi. *Yüksek Lisans Tezi*¸ Çukurova Üniv. Fen Bil. Enst., 1992.

20. Dhaliwal NK, Singh J, Chhuneja PK: Weight of *Apis mellifera* queen bees reared from different development stages of brood. *J Exp Zool*, 19 (1): 397-399, 2016.

21. Woyke J: Correlations between the ages at which honeybee brood was grafted, characteristics on the resultant queens and result of insemination. *J Apicult Res*, 10, 45-55, 1971. DOI: 10.1080/00218839.1971.11099669

22. Cengiz M, Emsen B, Dodoloğlu A: Some characteristics of queenbees *(Apis mellifera* L.) rearing in queen right and queenless colonies. *J Anim Vet Adv*, 8 (6): 1083-1085, 2009.

23. Güler A, Korkmaz A, Kaftanoğlu O: Reproductive characteristics of Turkish honeybee *(Apis mellifera* L.) genotypes. *J Anim Prod*, 39-40, 113-119, 1999.

24. Arslan S, Güler A, Arslan, HS: Quality criteria and standards compliance with grown queen bee at Mediterranean region in Turkey. *International Conference on Engineering and Natural Science.* 211-220, 21-23 January Thailand, 2015.

25. Arslan S, Arslan, HS, Cengiz MM, Karakuş B: Determination of the standards compliance of the quality criteria of the queen bees grown in the early period of the Mediterranean Region. *6th International Muğla Beekeeping and Pine Honey Congress.* 42-48, 15-19 October Muğla, 2018.

26. Woyke J: Effect of the access of worker honey bees to the queen on the results of instrumental insemination. *J Apicult Res*, 18 (2): 136-143, 1979. DOI: 10.1080/00218839.1979.11099957

27. Koç AU, Karacaoğlu M: Anadolu arısı Ege ekotipi *(Apis mellifera Anatolica)* ana arılarında üreme özellikleri. *ADÜ Ziraat Derg*, 2 (1): 73-77, 2005.

28. Ergün G, Aktaş S: ANOVA modellerinde kareler toplamı yöntemlerinin karşılaştırılması. *Kafkas Univ Vet Fak Derg*, 15 (3): 481-484, 2009. DOI: 10.9775/kvfd.2009.213

29. Sagili RR, Metz BN, Lucas HM, Chakrabarti P, Breece CR: Honey bees consider larval nutritional status rather than genetic relatedness when selecting larvae for emergency queen rearing. *Sci Rep*, 8:7679, 2018. DOI: 10.1038/s41598-018-25976-7

30. **Gene F, Emsen B, Dodologlu A:** Effects of rearing period and grafting method on the queen bee rearing. *J Appl Anim Res*, 27 (1): 45-48, 2005. DOI: 10.1080/09712119.2005.9706535

31. Vaziritabar S, Esmaeilzade SM: Preliminary attempts to rear larvae of the Iranian honeybee *(Apis mellifera meda)* colony and effect of different factors on graft acceptance in honeybee colonies in Karaj apiary. *J Entomol Zool Stud*, 6 (3): 683-692, 2018.

32. Dodologlu A, Emsen B, Gene F: Comparison of some characteristics of queen honey bees *(Apis mellifera* L.) reared by using Doolittle method and natural queen cells. *J Appl Anim Res*, 26 (2): 113-115, 2004. DOI: 10.1080/09712119.2004.9706518

33. Güler A, Alpay H: Reproductive characteristics of some honeybee *(Apis mellifera* L.) genotypes. *J Anim Vet Adv*, 4 (10): 864-870, 2005.

34. Kahya Y, Gençer HV, Woyke J: Weight at emergence of honey bee *(Apis mellifera caucasica)* queens and its effect on live weights at the pre and post mating periods. *J Apicult Res*, 47 (2): 118-125, 2008. DOI: 10.1080/00218839.2008.11101437