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Research Article
(Araştırma Makalesi)

Devrim OSKAY[1*] 0000-0002-3410-2780
Gökhan BAYRAK[2] 0000-0001-6553-7085

[1] Tekirdağ Namık Üniversitesi, Ziraat Fakültesi, Tarımsal Biyoteknoloji Bölümü, Tekirdağ, Türkiye
[2]Tekirdağ Namık Kemal Üniversitesi, Fen Bilimleri Enstitüsü, Tarımsal Biyoteknoloji Anabilim Dalı,Tekirdağ, Türkiye

Corresponding author: doskay@nku.edu.tr

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Anahtar Kelimeler:
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Investigation of Yield and Some Quality Features of Royal Jelly Harvested from Honeybee Colonies Fed with Food Substitutes*

İkame Yemlerle Beslenen Bal Arısı Kolonilerinden Hasat Edilen Arı Sütlerinin Miktar ve Bazı Kalite Özelliklerinin İncelenmesi

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ABSTRACT

Objective: In several nations, royal jelly is used in apitherapy, healthful foods, and cosmetics. The production and quality of royal jelly collected from honeybee colonies are being worked on by researchers and beekeepers. This study investigated the effect of honey and pollen substitute feeding on the production efficiency of royal jelly. In addition, analyses were conducted to determine the differences between fed and unfed groups in terms of 10-hydroxy-2-deconoic acid (10-HDA), protein and pH characteristics, honey, and pollen substitute.

Material and Methods: In this research, the influence of honey and pollen substitute feeding on royal jelly production was examined using the ANOVA test for multiple comparisons by forming 3 different groups and conducting 2 replications. Also, features of the 10- hydroxy-2-deconoic acid (10-HDA), protein and pH of the royal jelly was analyzed for understanding differences among the non-feeding pollen and honey substitute feeding groups.

Results: The average RJ quantity per queen cup for non-feeding colonies (Group A) was 420 mg, while the content of 10-HDA, protein and pH were 2.40%, 14.06% and 4.20%, respectively. For the colonies fed with sugar syrup (Group B), these values were 470 mg, 2.51%, 12.88%, 4.25 and for the colonies fed with syrup+pollen substitute (Group C) 530 mg, 4.05%, 13.13% and 4.18. The statistical test and contents analysis highlighted the impact of substitute feeding on average quantity amounts and 10-HDA. According to the results of the research, average RJ amounts in queen cell cup was significantly different in three honey bee colonies feeding groups ($p<0.05$). Colonies fed with sugar syrup +pollen substitute (Group C) colonies were filled the queen cell cups more amount of RJ than non-feeding (Group A) and fed with sugar syrup (Group B) colonies were filled RJ in the queen cell cups with significantly different. In the study, the colony fed with the sugar syrup +pollen substitute showed almost double the amount of 10-HDA value in RJ than non-feeding and fed with sugar syrup colonies. Protein and pH values shows no differences among the groups.

Conclusion: Even at times when nectar and pollen were available in nature, it was observed in this research that providing pollen substitution feed to honey bee colonies supported the output and quality of royal jelly.

ÖZ

Amaç: Birçok ülkede arı sütü apiterapide, sağlıklı gıdalarda ve kozmetikte kullanılmaktadır. Bal arısı kolonilerinden toplanan arı sütünün üretimi ve kalitesi, araştırmacılar ve arıcılar tarafından araştırılmaktadır. Bu çalışmada, bal ve polen ikamesi beslemesinin, arı sütü üretim verimi üzerindeki etkisi araştırılmıştır. Ayrıca, arı sütü 10- hidroksi-2-dekonoik asit (10-HDA), protein ve pH'ın özellikleri, polen, bal ikame ile beslenen ve beslenmeyen gruplar arasındaki farklılıkları anlamak için analizler yapılmıştır.

Materyal ve Metot: Bu çalışmada, bal ve polen ikame beslemesinin arı sütü üretim miktarı üzerindeki etkisi 3 ayrı grup oluşturulup, 2 tekerrür yapılarak ANOVA çoklu karşılaştırma istatistiksel testi kullanılarak araştırılmıştır. Ayrıca, bal ve polen ikamesiyle beslenen ve beslenmeyen gruplar arasındaki farkları anlamak için arı sütünde 10-hidroksi-2-dekonoik asit (10-HDA), protein ve pH'ının özellikleri analiz edilmiştir.

Bulgular: Beslenmeyen koloniler (A Grubu) için ana arı yüksüğü başına ortalama arı sütü verimi, 420 mg iken 10-HDA, protein ve pH içeriği sırasıyla %2,40 %14,06 ve %4,20 olarak bulunmuştur. Şeker şurubu ile beslenen kovanlar için (Grup B) bu değerler 470 mg; %2,51, %12,88, %4,25 ve şeker şurubu + polen ikamesi (Grup C) ile beslenen kovanlar için 530 mg; %4,05, %13,13 ve %4,18 olarak bulunmuştur. İstatistik analizler sonucunda, ikame beslemenin ortalama verim miktarları ve 10-HDA üzerinde etkisi önemli bulunmuştur. Araştırma sonuçlarına göre, ana arı yüksüklerindeki ortalama arı sütü miktarları üç bal arısı kovanı besleme grubunda anlamlı olarak farklı bulunmuştur ($p <0.05$). Şeker şurubu + polen ikamesi (Grup C) ile beslenen kovanlar, ana arı yüksüklerine beslenmeyen (A Grubu) ve şeker şurubu (Grup B) ile beslenen kovanlara kıyasla daha fazla miktarda arı sütü doldurulmuş ve aralarında önemli ölçüde fark bulunmuştur. Çalışmada, şeker şurubu + polen ikamesi ile beslenen kovanlardan elde edilen arı sütlerindeki 10-HDA içeriği şeker şurubu ile beslenen ve beslenmeyen kovanlara göre neredeyse iki kat daha fazla bulunmuştur. Protein ve pH değerlerinde gruplar arasında önemli fark bulunmamıştır.

Sonuç: Yapılan bu çalışmada, doğada nektar ve polenin bulunduğu dönemlerde dahi arı sütü üretimi yapılan bal arısı kolonilerinin polen ikame yemiyle beslemenin arı sütü verimi ve kalitesini desteklediği görülmüştür.

INTRODUCTION

Honey bees are very important to our civilization because of the role they play in the pollination of plants in both agriculture and the natural environment. Honey, propolis, pollen, and beeswax are natural honey bee products that have been extensively utilized in traditional medicine, food, and cosmetics since antiquity (Viuda-Martos et al., 2008). Royal Jelly (RJ) is one of the most popular bee products. It is a yellowish-white, homogenous, acidic material released by the mandibular and hypopharyngeal glands of immature worker bees (Fontana et al., 2004). It is the primary source of nutrition for the queen bee (Fujiwara et al., 1990). Reportedly, it contributes to the special traits of the queen bee, such as her lifespan, high fecundity, learning and memory abilities (Khan et al., 2021). Royal jelly contains 60–70% water, 12–15% protein, 10–16% carbohydrate 3–6% fats, minerals, and vitamins, free amino acids, and volatile substances (Bogdanov, 2011; Collazo. et al., 2021; Guo et al., 2021). Proteins (MRJPs) and trans-10-hydroxy-2-decenoic acid (10-HDAs) are the main active compounds known to be present in royal jelly (Ali and Kunugi, 2020a). There are many *in vitro* and *in vivo* studies on the biological activities and bioactive components of royal jelly (Pavel et al., 2011; Khazaei et al., 2018; Strant et al., 2019; Shakib Khoob et al., 2022). In these studies, royal jelly has been shown to have antioxidant (Ghanbari et al., 2016), antimicrobial (Park et al., 2019), antibacterial (Fratini et al., 2016), anti-inflammatory (Chen et al., 2016), anticancer (Miyata and Sakai, 2018), antitumor (Albalawi et al., 2022), anti-aging (Ali and Kunugi, 2020b) effects.

Due to its extensive biological qualities, royal jelly has a greater commercial value than other bee products. Therefore, royal jelly has become a significant source of revenue for beekeepers worldwide. Although there are no official market statistics for royal jelly, it is known that China accounts for more than 90 % percent of worldwide royal jelly production (Ahmad et al., 2020). According to reports, several nations export royal jelly, in particular (Cao et al., 2016).

Climate, botanical source, bee species, and artificial feeding of the honeybee all affects royal jelly composition (Virgiliou et al., 2020), and there are currently no national quality requirements for royal jelly in many countries (Arfa et al., 2021). Current requirements for royal jelly quality include moisture, total proteins, sugars (fructose, glucose, and sucrose), and 10-hydroxy-2-decenoic acid (10-HDA) (Kanelis et al., 2015). The purpose of this research was to examine the quantity and quality characteristics of royal jelly harvested from bee colonies given honey and pollen replacement feeds to those not fed.

MATERIAL and METHOD

The research was conducted by Içtaş apiary in the Biga region of Çanakkale province. Among the 200 beehives in the company's beekeeping operation, thirteen honey bee colonies with one-year-old sister queens with a strong population, a double super, and similar brood frame numbers were chosen. Six of these colonies were designated as starters, six as finisher, and one as a larval provider.

We used the procedure suggested by Doolittle, who suggested moving the first instar larvae from their original cell in the combs to grafting cells for the artificial cups of queens, to produce royal jelly (Büchler et al 2013).

As a reference for the experiment, dark-colored plastic queen bee production queen cups were utilized as a replacement for beeswax queen cups in order to properly estimate the quantity of royal jelly per queen cup.

Queen cups were accurately weighed on the scales, and numbers were assigned according on the groups that would be created and put in the frames. For each group, 45 queen cells were obtained. Statistics were used to determine the difference among the groups based on the weights of the royal jelly obtained from 10 randomly chosen queen cells from each group. The research was constructed with two replications for each of the three feeding groups.

Selection of larval provider colony and production of larvae

The whole transfer was performed out from a larval provider colony used for reproduction to guarantee that the grafted larvae had a similar genetic structure. The age of the grafted larvae influences both the output of royal jelly production and the acceptance rate of the grafted larvae. In order to collect 0-24 hour larvae for the graft, the queen bee was caged in combs with acceptable egg-laying regions four days prior to inoculation. The grafting of larvae began with the development of larvae in the brood after the detection of the first eggs. (Chen et al. 2002).

Preparation of cell starter colonies

It was chosen among colonies with queen bees of the same age, double supers, brood areas, strong bee populations, and equivalent strength. Two days prior to larval grafting, the queen of the starter colonies was removed. To increase the number of bees per frame, both brood and adult bee-covered combs were placed in the brood nest.

In order for the honey bees to adapt to the plastic queen cups, the frames on which the plastic queen bee

cups were placed were soaked in sugar syrup at a ratio of 1:1 and kept in the starter colonies one day before to the larvae grafting. One week prior to the initiation of the grafting procedure, the starter colonies in each group were fed.

Feeding groups:

Group A: (the control group): unfed colonies

Group B Consists of colonies fed with sugar syrup

Group C: Colonies fed with sugar syrup and pollen substitute diet

Sugar syrup was made by combining equal volumes of crystallized beet sugar and water.

Pollen substitute food preparation: Powdered sugar made from beet sugar, inactive baker's yeast, honey, vegetable oil, and pollen was utilized to create a 10% protein pollen alternative. Pollen substitute + sugar syrup group (500ml 1:1 sugar syrup 2 times per week + 500gr pollen substitute bee feed), sugar syrup group (500ml 1:1 sugar syrup 2 times per week), and control group (no feeding) (Oskay, 2021; Rangel et al. 2013; Genç and Dodoloğlu, 2002).

Preparation of finishing colonies

Queen bee cells that had been stored in the starter colonies for 24 hours were transferred to the finishing colonies and kept there until harvest day (48 hours). Colonies consisting of six double-super beehives with close populations of adult bees, honey, pollen, brood frames and same age queen bees were chosen among 200 colonies. Using a queen bee excluder, the queens of the colonies intended for use as a finisher were restricted to the brood nest. Thus, the queen was prevented from entering the area containing the queen bee cells (Doğaroğlu, 2009). A week ago, feeding of finisher colonies began. Prior to transferring frames containing queen cells from the starting colony to the finishing colony, the number of queen cells were balanced.

Grafting of larvae

In order to transfer the larvae, the queen of the breeding hive was imprisoned in the hive with two frames four days before to the larvae transfer operation and forced to queen bee lay eggs. On the day of transfer, the appropriate-age larvae-containing combs were transferred to a room with a temperature of 35 °C and relative humidity of 60%. Before putting the larvae in the queen cups, a drop of 1:1 diluted royal jelly was placed on the bottom of the queen cups, and the larvae were transferred using a Chinese spoon onto this royal jelly (Moritz 1984).

Four days prior to the larvae grafting procedure, the queen of the larval provider colony and two honeycombs were kept at the center of the brood nest and the queen was forced to lay eggs. On the day of transfer, combs containing larvae of the proper age were transported to a room with a temperature of 35 degrees Celsius and a relative humidity of 60 percent. Before inserting the larvae into the queen cups, the bottom of the queen bee cups are coated with royal jelly diluted 1:1. Moritz (1984) used a Chinese spoon to attach a one-day-old larvae to a drop of diluted royal jelly (Moritz 1984).

Royal jelly harvesting, weighing, and storage

After 72 hours, inoculated queen cells were extracted from the hives and larvae-containing royal jelly samples were weighed using precision scales. The larvae were then removed with forceps and royal jelly measurements were taken. Samples of royal jelly that were collected up to the day of analysis were kept for six months in dark jars, away from light and oxygen, at -18 degrees Celsius in a deep freezer (Yaochun 1993; Kösoğlu and Doğaroğlu 2012).

pH, Protein, and 10-HDA Analysis of Royal Jelly

Purchasing services from the accredited labs of the Scientific and Technical Research Council of Turkey (TUBITAK) Marmara Research Center, pH, protein, and 10-HDA analyses were performed on the royal jelly samples generated for the study (MAM). For pH analysis, AOAC 960.52, 10-HDA, and D.05.G432.-HPLC UV methods were used.

Statistical analysis

Using the ANOVA-Tukey multiple comparison test in the JMP 13 (SAS) statistical software, the mean royal jelly per queen cell was compared across groups.

RESULTS and DISCUSSION

Royal Jelly Weight Ratios

Figure 1 indicates the average royal jelly weights recovered from the queen cells of honeybee colonies fed with different substitute foods. There was a statistically significant difference between the groups ($F= 10.82$; $p < 0.05$- $F= 5.24$; $p < 0.05$).

The average weight of royal jelly produced by queen cells was 440 mg in the control group (A), 470 mg in the sugar syrup group (B), and 545 mg in the sugar syrup with pollen substitute group (C).

Despite the fact that the average weight of royal jelly produced by colonies fed sugar syrup was greater than that of the control group, the difference was not statistically significant ($p> 0.05$). The average weight of royal jelly produced by colonies fed with sugar syrup + pollen substitute was greater than the average weight of royal jelly produced by colonies given sugar syrup alone ($p < 0.05$).

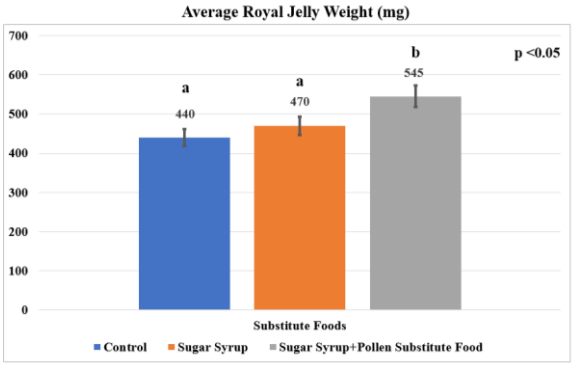

Figure 1. The average quantity of royal jelly produced in queen cells by honey bee colonies fed with different substitute foods.

Şekil 1. *Farklı ikame yemlerle beslenen bal arısı kolonileri tarafından ana arı gözlerinde üretilen ortalama arı sütü miktarı (mg)*

pH analysis of royal jelly

Figure 2 shows the pH analysis findings of royal jelly collected from the queen cells of honey bee colonies fed with different substitute foods. Accordingly, the pH of the royal jelly produced in the queen cells was 4.20 for the control group (A), 4.25 for the sugar syrup group (B), and 4.18 for the sugar syrup + pollen-substitute group (C).

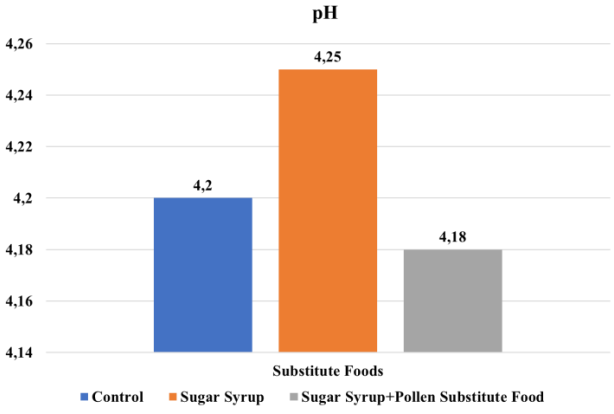

Figure 2. The pH measurements of royal jelly harvested from colonies fed with different substitute foods.

Şekil 2. *Farklı ikame yemlerle beslenen kolonilerden hasat edilmiş arı sütünün pH analiz sonuçları*

Protein analysis of royal jelly

Figure 3 shows the protein analysis findings of royal jelly produced from queen cells of honey bee colonies fed with different substitute foods. Accordingly, the protein levels of the royal jelly formed in the queen cells were 14.06 g/100 for the control group (A), 12.88 g/100 for the sugar syrup group (B), and 13.13 g/100 for the sugar syrup + pollen substitute group (C).

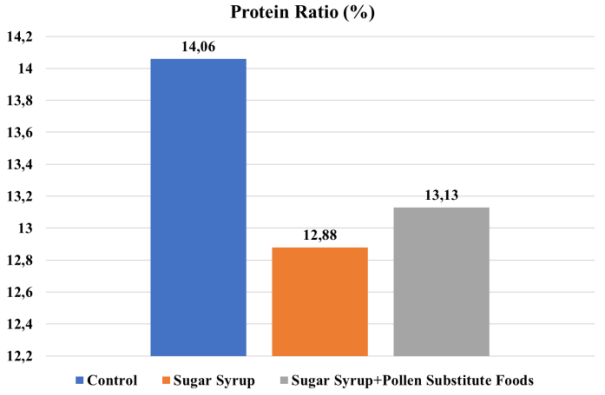

Figure 3. The protein measurements of royal jelly harvested from colonies fed with different substitute foods.

Şekil 3. *Farklı ikame yemlerle beslenen kolonilerden hasat edilmiş arı sütlerinin protein analiz sonuçları.*

10-HDA analysis of royal jelly

The 10-HDA analysis results of the royal jelly obtained from the queen cells of honey bee colonies fed with different substitute foods are shown in Figure 4. Therefore, the 10-HDA value of royal jelly produced in queen cells is 2.40 g/100 for the control group (A), 2.51 g/100 for the group (B) fed with sugar syrup, and 4.05 g/100 for the group (C) fed with sugar syrup + pollen substitute.

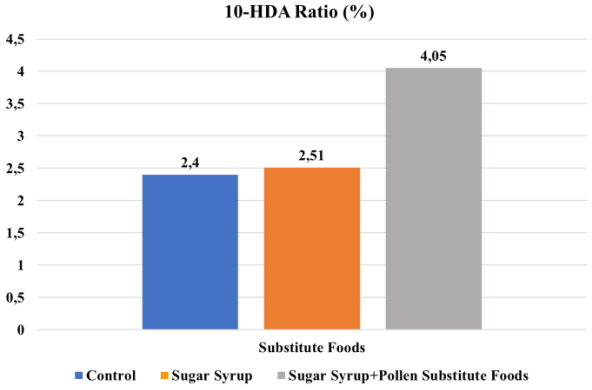

Figure 4: The 10-HDA measurements of royal jelly harvested from colonies fed different substitute foods.

Şekil 4. *Farklı ikame yemlerle beslenen kolonilerden hasat edilmiş arı sütlerinin 10-HDA analiz sonuçları*

The demand for bee products other than honey has surged as a result of significant advancements in apitherapy. Royal jelly's yearly production volume is growing because of the rising worldwide demand for it, which is driven by the fact that it is a high-value bee product and plays a significant part in human nutrition (Cao et al., 2016). Therefore, one of the primary goals

of royal jelly production operations is to increase the quality and quantity per colony via the creation of colony management systems.

Numerous studies have shown the beneficial effects of honey and pollen substitute foods on the biology, brood growth, honey storage efficiency, comb construction, longevity, body weight, queen cell acceptance rate and queen cell weight of honey bee colonies (Herbert et al., 1977; Doull 1980; Winston et al., 1983; Dastouri and Maheri-Sis, 2007; Almaida-Dias et al., 2018; Adgaba et al., 2020; Oskay 2021; Khan and Ghramh 2022).

In this research, the effect of feeding honey bee colonies managed for royal jelly production with honey and pollen substitute on royal jelly production and a number of its quality characteristics was explored. According to the data collected, more royal jelly was made by honey bee colonies that were fed pollen substitute and sugar syrup than by honey bee colonies that were fed only sugar syrup and never fed with pollen substitute or sugar syrup. The amount of 10-HDA in royal jelly samples made by beehives that were fed with pollen substitute and sugar syrup was about double the standard amount of 10-HDA in royal jelly.

The study has shown that providing high protein pollen substitutes to honeybee colonies that produce royal jelly increases the quantity and quality of the royal jelly. According to research by Pattamayutanon et al. (2018), the protein content of royal jelly produced in Thailand by honey bee colonies fed pollen from three different plants (tea, coffee, and bitter bush) was similar, but the 10-HDA content varied. The 10-HDA levels in royal jelly samples from bee colonies fed on bitter bush and coffee pollen were greater compared to those in royal jelly samples from bee colonies fed with tea pollen. In support of our research, Pattamayutanon et al. (2018) indicated that 10-HDA concentration but not protein ratios are influenced by royal jelly derived from honey bee colonies fed with pollen of different species of plants.

Balkanska (2018) also investigated the content of 10-HDA in royal jelly produced in Bulgaria by honey bees fed a substitute food. In the study, 3 different feeding groups were established. Group 1 colonies were fed just sugar syrup, Group 2 colonies were fed sugar syrup containing 10% baker's yeast, and Group 3 colonies were fed sugar syrup with vitamin AD3E. The mean ratio of 10-HDA (%) in royal jelly from the three groups was 1.89, 2.13, and 1.89, respectively. In the study, the group given baker's yeast produced royal jelly with the highest 10-HDA ratio.

Compared to Balkanska (2018), our pollen substitute food includes around twice as much inactive baker's yeast. In addition, pollen, honey, and vegetable oil were added to the pollen substitute foods utilized in our study. This variant may cause a twofold increase in the proportion of 10-HDA in the royal jelly used in this study.

Mureşan et al. (2016) conducted chemical analyses of carbohydrates, lipids, proteins, (10-HDA), and mineral components in samples of royal jelly obtained from Romanian commercial royal jelly producers. In the research results, fructose ranged from 3.4% to 5.87%, glucose from 4.12% to 7.05 %, sucrose from 0.95 % to 2.56%, lipid content from 1.85% to 6.32 %, protein levels from 13.10% to 17.04 %, and 10-HDA 1%. It was reported that the mineral concentration was between 3188.70 mg/kg to 4023.39 mg/kg, with potassium having the highest concentration, followed by magnesium, sodium, and calcium. Our 10-HDA and protein levels were comparable to those obtained in the research by Mureşan et al. (2016).

Sahinler et al. (2005) studied the effect of feeding honey bee colonies with different substitute foods on royal jelly yield per queen cell, queen cell acceptance rate, and total royal jelly production. In the experiment, soy flour, milk powder, sugar syrup, and vitamin E were used to make substitute foods. It was shown that colonies fed with sugar syrup and vitamin E produced more royal jelly than colonies fed with pollen substitute food. In addition, there was no statistical difference in the average royal jelly production per queen bee cell between the groups fed with sugar syrup and pollen substitute food. There are differences between this study's results and our own study. Şahinler et al. (2005) used soy flour and milk powder for pollen substitute, however in our research inactive baker's yeast may have a significant impact. The significant difference between the average amount of royal jelly produced by the queen cells in the Şahinler et.al. (2005) research and the average amount of royal jelly produced by the queen cells in our research may be due to the fact that fewer queen cells were distributed to the colonies in our study.

Sakla and El-Shafeiy (2022) found that the quality of royal jelly produced from colonies fed with pollen and plant-supported supplementary feed formula is superior to that created from colonies fed with sugar syrup; therefore, the quality of the reared queen bees reared is greater.

CONCLUSION

In order to boost the quantity and quality per hive for the production of royal jelly used in human nutrition and apitherapy, various nations have set their own

standards and conducted scientific research. China is the leader in the manufacturing of royal jelly among these nations. Climate, plant, and honey bee genetic diversity are all quite rich in Turkey. For this reason, the import of royal jelly may be ceased, and exports can be made when the number of firms producing royal jelly in the beekeeping sector increases along with the quantity and quality of royal jelly produced per hive. This research shows that when the honey bee colony producing the royal jelly received the proper amount of honey and pollen substitute food, both the amount and quality of the royal jelly significantly increased. Access to sufficient amounts of healthy, safe food; climatic change; illnesses and pests; etc. Honey and pollen substitute research and application studies will continue to be essential as long as negative aspects continue to occur.

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