How to cite: Oskay, D., Oğuz, A., The Effect of Substitute Feeding On Male Larvae Production Performance in Honeybee Colonies, J. Anim. Prod., 2022, 63 (2): 84-89, https://doi.org/10.29185/hayuretim.1184151

Research Article
(Araştırma Makalesi)

J. Anim. Prod., 2022, 63 (2): 84-89
https://doi.org/10.29185/hayuretim.1184151

The Effect of Substitute Feeding On Drone Larvae Production Performance in Honey Bee Colonies *

Bal Arısı Kolonilerinde Beslemenin Erkek Arı Larvası Üretim Performansı Üzerine Etkisi

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Alınış (Received): 04.10.2022 Kabul (Accepted): 16.11.2022

* Bu makale ikinci yazarın yüksek lisans tezinden elde edilmiştir

Keywords:
Apilarnil, honey bee, drone larvae, substitute feed, nutrition

ABSTRACT

Objective: In recent years, there has been an increase in interest in functional honey bee products. Due of their high nutritional content, drone larvae (Apilarnil) is capable of a wide range of biological activities. In this study, we compared the individual and total weights of drone larvae obtained from colonies fed with different substitute foods.

Material and Methods: 12 out of 100 colonies comparable in queen age, brood status and colony size were used as material. The colonies were divided into three groups: control, sugar syrup and pollen substitute diet. Each group underwent three replications.

Results: There was no statistically significant difference in the weight averages of individual drone larvae taken from colonies (p>0.05). The difference in the average weight of total drone larvae collected between the groups was statistically significant (p<0.05). The average weight of total drone larvae produced by the colonies was 23.80 g in the control group, 34.70 g in the honey substitute group, and 44.42 g in the pollen substitute group.

Conclusion: In this research, the high average weight of total drone larvae produced in colonies that fed with pollen substitute feed showed that the use of appropriate substitute feeds in colony feeding management in beekeeping practices promotes drone brood production.

Anahtar Kelimeler:
Apilarnil, bal arısı, erkek arı larvası, ikame yem, besleme

ÖZ

Amaç: Son yıllarda fonksiyonel arı ürünlerine olan ilgi artmıştır. Yüksek besin içeriği sayesinde erkek arı larvası (Apilarnil) çok çeşitli biyolojik aktivitelere sahiptir. Bu çalışmada, farklı ikame yemler ile beslenen kolonilerden elde edilen erkek arı larvalarının bireysel ve toplam ağırlık miktarları karşılaştırılmıştır.

Materyal ve Metot: Ana arı yaşı, kuluçka durumu ve koloni boyutu bakımından karşılaştırılabilir 100 koloniden 12'si materyal olarak kullanılmıştır. Tesadüfi olarak 3 gruba ayrılan koloniler, kontrol grubu, şeker şurubu ile beslenen grup ve polen ikame yemi ile beslenen grup olarak ayrılmıştır. Her grupta 3 tekerrür gerçekleştirilmiştir.

Bulgular: Gruplara göre kolonilerden elde edilen bireysel erkek arı larvaları ağırlık ortalamaları arasında istatistiki olarak fark bulunamamıştır (p>0.05). Elde edilen toplam erkek arı larvası ağırlık ortalamalarında gruplar arası fark istatistiki olarak önemli çıkmıştır (p<0.05). Kolonilerin ürettiği toplam erkek arı larvalarının ortalama ağırlığı kontrol grubunda 23.80 gr, bal ikame grubunda 34.70 gr ve polen ikame grubunda 44.42 gr olarak belirlenmiştir.

Sonuç: Bu araştırmada; polen ikame yemi ile beslenen kolonilerde üretilen toplam erkek arı larvalarının ortalama ağırlığının yüksek olması, arıcılık uygulamalarında koloni besleme yönetiminde uygun ikame yemlerin kullanımının erkek arı kuluçka üretimini teşvik ettiğini göstermiştir.

INTRODUCTION

Honeybees are negatively affected by climate change, diseases and pests, agrochemicals, and habitat degradation (Brown et al., 2016). When honeybee colonies are unable to obtain sufficient nectar and pollen in nature, they should be supplemented until the honey flow time (Tunç et al., 2020). Honey bees have difficulties finding nectar and pollen that are not suitably chemically contaminated (Hladun et al., 2012; Hladun et al., 2015). Pollen with a high nutritional content and no chemical residues is crucial for the survival and growth of honey bee colonies (Pernal and Currie, 2000). Under these circumstances, beekeepers feed their colonies with substitute pollen and honey (Oskay, 2021). According to Herbert (1992), Goodwin et al. (1994), Koç and Karacaoğlu (2004), additional diets are required to boost honey bee colony population, brood, queen, drone breeding, effective wintering, and honey production.

Honey bees generate nutrient-dense, physiologically active, and biochemically diverse natural products, such as flavonoids and phenolic compounds, with high nutritional value (Suleiman et al., 2021). Since ancient times, these items have been widely used as food, cosmetics, and for the prevention or cure of illnesses (Martinello and Mutinelli, 2021). Honey, pollen, bee bread (perga), propolis, royal jelly, drone larvae (apilarnil), and honey bee products such as bee venom, antioxidant, antibacterial, anti-inflammatory, anticancer, and antiviral agents, among others. Numerous biological activities have been attributed to this substance (Bartkiene et al., 2020; Nainu et al., 2021).

Apilarnil is a natural bee product with a homogenous, yellowish-gray hue and a bitter flavor, produced by collecting drone larvae aged 3 to 7 days (Silici, 2019). 25–35% dry matter, 9–12% protein, 6–10% carbs, 5–8% lipids, vitamins (A, B1, B6, and choline), and minerals make up the nutrient composition of Apilarnil (Ca, P, Na, Zn, Mn, Cu, Fe, and K) (Hashem et al., 2021). It is also high in sex hormones, including testosterone, prolactin, progesterone, and estradiol (Erdem and Özkok, 2018). Apilarnil has been shown to boost spermatogenesis, sexual performance, and testosterone production (Altan et al., 2013). Numerous studies have demonstrated the antioxidant and antimicrobial activities of apilarnil (Hroshovyi et al., 2021), renoprotective (İnandiklioğlu et al., 2021), protective against oxidative stress and DNA damage (Doğanyiğit et al., 2020), preventive of testicular damage (Doğanyiğit et al., 2019), androgenic (Yücel et al. 2011) and neuroprotective effects (Hamamci et al., 2020). If post-harvest cold chain storage is taken into consideration, apilarnil may be eaten fresh. Alternatively, it can be used in long-term storage using procedures such grinding, homogenization, filtering, and lyophilization (Topal et al., 2018).

Apilarnil stands apart from other bee products due to its androgenic characteristics. When colonies raise drones in the spring, beekeepers kill drones after they have entered the pupal stage, both in the fight against varroa and in order to reduce honey consumption, because apilarnil's importance has not been fully understood by consumers and cannot take its place in the market for bee products at the desired level. The demand on beekeepers will increase as awareness of the use of apilarnil in apitherapy procedures and wholesome human diet spreads. Beekeepers will be able to increase their income from their enterprises by producing more apilarnil from honey bee colonies. There is an export potential in this area as shown by the fact that apilarnil is consumed in other countries and has a market (Isidorov et al., 2016).

In this study, drone larvae produced by honey bee colonies fed substitute foods of honey and pollen were collected and their individual and average weights were compared. Furthermore, the effect of feeding on the production performance of drone larvae (apilarnil) was investigated.

MATERIAL and METHOD

In the month of May, research was conducted on 12 colonies with one-year-old queen bees and each have eight bee frames from an apiary in the Aydos woodland in the Maltepe-Kayışdağı district of Istanbul province. The synchronization of colonies made with four frames of brood. In addition, colonies contain queens of the same age and equal brood areas. Colonies were divided into three groups one week prior to the study as detailed below.

Control: Honey bee colonies in this group were permitted to obtain pollen and nectar from the environment. This group was not fed supplementary with any other nutrition.

Preparation of sugar syrup (honey substitute feed): Beet plant water and granulated sugar were combined in a ratio of 1:1 to produce sugar syrup. During the preparation of the syrup, the water was first heated (110 °C) and cooled (50°C), then sugar was added gently ad dissolved uniformly in the water (Frizzera et al., 2020) Using bag-shaped feeders, sugar syrup was distributed to the beehives.

Preparation of pollen substitute food: Using inactive baker's yeast with 40% protein content, powdered sugar, and floral honey, 10% protein pollen substitute diet was created (Oskay, 2021). After thoroughly combining the powdered sugar and inactive baker's yeast, honey was added and kneaded by hand until dough formed. The five hundred grams of replacement feed was put on the frames after being packed in plastic bags, taking into mind that it would not spill over the bees. Throughout the experiment, colonies were fed weekly.

Preparation of honeycombs for apilarnil production

In the lowest portions of the half-height (485x110mm) frame-type plastic mangers in the apiary's existing beehives, the honeycombs holding the drone cells that the bees weave naturally were removed and used. Honeycombs containing drone cells used in the research were maintained at -18 degrees Celsius in honeycomb storage containers to prevent infestation by moths.

Apilarnil production

The colonies in the control group were not fed, so they were free to obtain pollen and nectar from nature. Honey and protein replacement diets were simultaneously administered to the other groups. During daily controls, colonies that had consumed all their food were fed again with a substitute. The colonies used in the study were provided with feeders containing drone honeycomb cells. The colonies were inspected daily, and the egg production of the queen was monitored. Colonies with queens that deposit eggs were documented. The practice of replacement feeding proceeded throughout the study period of 1-30 May.

Apilarnil harvest and storage

After four days, drone broods that reached the larval stage were collected from the colonies. The harvest dates were scheduled and executed on the seventh day after the queen bee laid her egg on the drone combs. At the same time as the combs were taken from the hives, the harvesting of drone larvae began. During the collection of larvae from drone cells, forceps were used. On precision scale (KERN, ABJ 220-4NM), larvae were weighed, and the larval weights and overall apilarnil weights were recorded (g). Colonies of larvae were placed in glass jars and kept at -18°C to avoid degradation.

Statistical analysis

Individual larval weights were determined by randomly choosing 9 (n=9) larvae from each colony and weighing them. The total weight of the larvae is determined by simultaneously collecting all larvae from each group. Each experiment was conducted three times. Using IBM-SPSS 15 (1999) Statistics and the ANOVA-Tukey multiple comparison test, the difference between the groups was established. Experiment outcomes were deemed significant if the p-value was less than 0.05.

RESULTS and DISCUSSION

Individual weights of drone larvae

Table 1 and Figure 1 show the average individual drone larval weights produced by groups of honeybee colonies fed with various substitute foods. In all three replications, the difference between the groups did not found significant, statistically ($F=1.217$; $p>0.05$-$F=1.095$; $p>0.05$-$F=2.415$; $p>0.05$).

Table 1. Comparison of the average weight of individual drone larvae reared by honey bee colonies fed with different substitute foods.

Tablo 1. *Farklı ikame yemlerle beslenen bal arısı kolonilerinden üretilen bireysel erkek arı larva ağırlık ortalamaları ve standart hataları karşılaştırılması*

Groups	n	Mean (g)	Standard Error	Minimum	Maximum
Control	27	0.1637	0.0065	0.13	0.19
Sugar Syrup	27	0.1567	0.00681	0.126	0.186
Pollen Substitute Food	27	0.1729	0.01246	0.12	0.233
General Mean	27	0.1644	0.00664	0.125	0.203

According to Table 1, the average weight of each individual drone larvae in the control group was 0.1637 g, 0.1567 g in the group provided honey substitute feed, and 0.1729 g in the group supplied pollen substitute feed. Although the average weight of individual larvae obtained from colonies given pollen substitute feed was greater than those obtained from colonies provided honey substitute feed and the control group, the difference was not statistically significant (p>0.05).

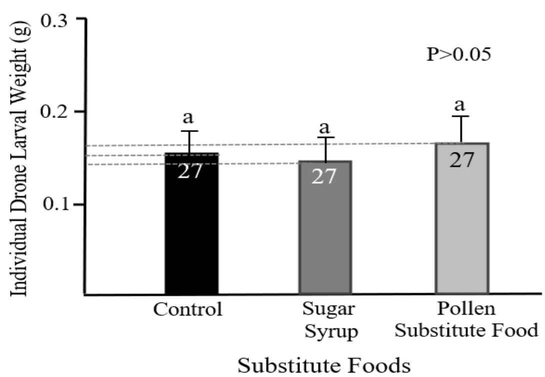


Figure 1. Weight of individual drone larvae produced by honey bee colonies given different substitute foods.

Şekil 1. *Farklı ikame yemlerle beslenen bal arısı kolonilerinden üretilen bireysel erkek arı larva ağırlık ortalamaları*

Total drone larval weights

Table 2 and Figure 2 show the average total weights of drone larvae acquired by groups of honey bee colonies fed with various substitute foods. In all three replications, the difference between the groups was statistically significant (F=5.209; p< 0.05-F=5.560; p<0.05-F=6.788; p<0.05).

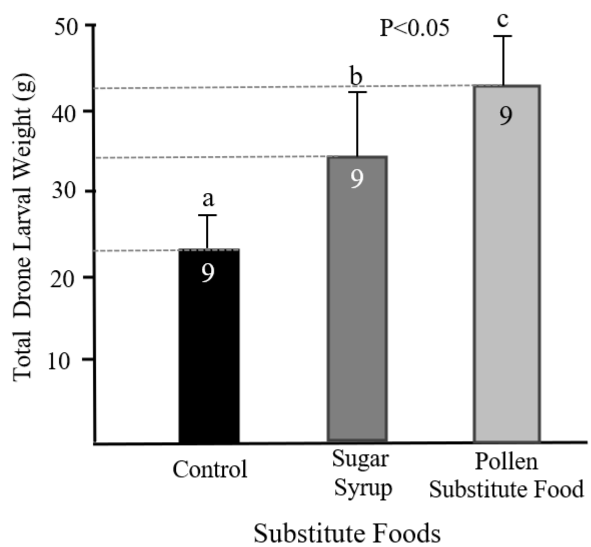
Table 2. Total weights and standard deviations of drone larvae harvested from honeybee colonies fed with different substitute feeds.

Tablo 2. *Farklı ikame yemlerle beslenen bal arısı kolonilerinden hasat edilen toplam erkek arı larva ağırlıkları ve standart hataları*

Groups	n	Mean (g)	Standard Error	Minimum	Maximum
Control	9	23.80	1.14	21.83	25.66
Sugar Syrup	9	34.70	7.37	22	47.42
Pollen Substitute Food	9	44.42	1.08	42.66	46.60
General Mean	9	34.30	3.19	28.83	39.89

The average weight of total drone larvae produced by the colonies was 23.80 g in the control group, 34.70 g in the honey substitute group, and 44.42 g in the pollen substitute group. It was determined that the difference between the groups was statistically significant (p<0.05). The average weight of drone larvae from colonies given pollen substitute was significantly greater than that of the control group (p<0.05). Although the overall larval weights acquired from the group given honey substitute foods were greater than those obtained from the control group, the difference was not statistically significant (p>0.05). The average weight of total larvae produced by colonies fed pollen substitute was statistically significantly higher (p<0.05). Individual larval weights did not significantly vary across colonies of honey bees given alternative diets, as established by the investigation. However, the total number of drones acquired from colonies given pollen feed was significantly greater than those obtained from colonies provided honey replacer feed and the control group. This situation; shows that pollen substitute feed intake is effective on the brood amount of drone larvae in the colony.

This is the first research to investigate the effects of pollen substitution and sugar syrup feeding on colony apilarnil production. Numerous studies have shown that the use of pollen substitutes in honey bee nutrition increases brood development performance (Saffari et al., 2004; Avni et al., 2009; Brodschneider and Crailsheim, 2010; Kumar et al., 2013; Pande and Karnatak, 2014).



Graphic 2. Total weights of drone larvae harvested from honeybee colonies fed with different substitute foods

Şekil 2. *Farklı ikame yemlerle beslenen bal arısı kolonilerinden hasat edilen toplam erkek arı larva ağırlıkları*

Avni et al. (2009), investigated the effects of pollen patties on consumption, brood production, and honey yield. Comparing three pollen-patties sizes of equal weight, the research showed that consumption rose as surface area increased. However, brood production tended to increase with pollen patty size, and colonies fed patties with the largest surface area produced significantly more brood than those fed a control patty containing only carbohydrates. The difference in honey production between the groups was not statistically significant. It has been reported that honey bee larvae, in especially, need more protein, and that protein deficiency during brood production has a significant negative impact on the larvae (Brodschneider and Crailsheim, 2010). Kumar et al. (2013), used defatted soy flour, roasted grams, brewer's yeast, soy protein hydrolysate, spirulina, skimmed milk powder, and natural pollen to formulate a protein-rich pollen substitute. The defatted soy four, brewer's yeast, and soy protein hydrolysate powder composition was the most successful diet, with a closed hatchery area of 2155.3 cm^2, a total of 5.8 bee-covered frames, and a population of 11509 bees. In another study, honeybee colonies were fed four different pollen substitutes and their effects on honeybees colonies were compared to a control (no food). After feeding in all dietary combinations, including ger Chickpea, ger Greengram, and ger Horse gram, a significant increase in brood area, honey store, pollen store, and foraging activity was observed (Pande and Karnatak, 2014). In different study, hatching areas were estimated as 1357.0 cm^2, 1567.3 cm^2, 1251.8 cm^2, and 1456.3 cm^2, respectively, while studying the effect of four different pollen diets on brood production and colony strength in honeybees (Israr et al., 2022). Noordyke et al. (2022) noted that beekeepers in tropical locations may benefit from feeding stressed honeybee colonies pollen substitutes during the winter to reduce total colony biomass loss. In a research containing eight types of feeds: commercial diets, a beekeeper-formulated diet, and sugar negative control, honeybee colonies were analyzed for population size, mean bee weight, nutritional gene expression, gut microbiota abundance, and pathogen levels. According to the findings of the research, two pollen-containing diets (commercial and beekeeper developed) produced the largest colonies and the heaviest bees per colony (Ricigliano et al., 2022).

Care for drone larvae requires 2.78 times longer than care for worker larvae (Calderone and Kuenen, 2003). It has been found that 325-487.5 mg of pollen are required to produce apilarnil (Hrassnigg and Crailsheim, 2005). This is more than three times the amount of pollen required to raise worker bees. The equivalents in the colony are honey and pollen. The fact that pollen and honey replacement feeding increases the overall number of apilarnil without altering the weight of individual larvae demonstrates the significance of supplementing and substituting the colony's nutrition. Maintaining a systematic approach in pollen substitute research will improve the feeding management of honeybee colonies and economically benefit beekeepers, despite the importance of feeding honeybee colonies with pollen during critical periods in order to minimize the problem of poor quality and insufficient nutrition (Topal et al., 2019).

CONCLUSION

The optimal honeybee diet affects the amount of brood produced, the longevity and health of adult bees in a colony, as well as the quantity and quality of products produced.

In this study, feeding with pollen substitute and sugar syrup did not affect the weight of individual drone larvae, but it increased the weight of total drone larvae produced by the colonies. According to the results, beekeepers should prefer beekeeping in regions with rich pollen and nectar sources in order to increase the drone larvae production performance of honey bee colonies. In recent years, factors such as climate change, habitat loss, pesticides, environmental pollution, diseases and pests have negative impact on colony performance and caused colony losses. Furthermore, it becomes more difficult for honeybees to access sufficient and clean food sources. These findings indicate that beekeepers may utilize substitute food to meet their bees' nectar and pollen sources in the spring and autumn. In the future, further research will be needed on the development of substitute honey bee foods.

REFERENCES

Altan Ö, Yücel B, Açikgöz Z, Şeremet Ç, Kösoğlu M, Turgan, N, Özgönül, A M. 2013. Apilarnil reduces fear and advances sexual development in male broilers but has no effect on growth. British poultry science, 54(3): 355-361.

Avni D, Dag A, Shafir S. 2009. The effect of surface area of pollen patties fed to honey bee (*Apis mellifera*) colonies on their consumption, brood production and honey yields. Journal of apicultural research 48(1):23-28.

Bartkiene E, Lele V, Sakiene V, Zavistanaviciute P, Zokaityte E, Dauksiene A, ve Ruzauskas M. 2020. Variations of the antimicrobial, antioxidant, sensory attributes and biogenic amines content in Lithuania-derived bee products. LWT 118:108793.

Brodschneider R, Crailsheim K. 2010. Nutrition and health in honey bees. *Apidologie*, *41*(3):278-294.

Brown M J, Dicks L V, Paxton R J, Baldock K C, Barron A B, Chauzat M P, Li J. 2016. A horizon scan of future threats and opportunities for pollinators and pollination. Peer J 4: e2249.

Calderone N W, Kuenen L P S. 2003. Differential tending of worker and drone larvae of the honey bee, *Apis mellifera*, during the 60 hours prior to cell capping. Apidologie, *34*(6): 543-552.

Doğanyiğit Z, Okan A, Kaymak E, Pandır D, Silici S. 2020. Investigation of protective effects of apilarnil against lipopolysaccharide induced liver injury in rats via TLR 4/HMGB-1/NF-κB pathway. Biomedicine & Pharmacotherapy 125:109967.

Doğanyīğīt Z, Sīlīcī S, Kaymak E, Okan A, Akin A T, Pandir D. 2019. Apīlarnīlīn'īn erkek sıçanlarda līpopolīsakkarīte (lps) bağlı testīs toksīsītesīne karşı koruyucu rolünün belīrlenmesī. Bozok Tıp Dergisi, 9(2):146-154.

Erdem B, Özkök A. 2018. Can food supplement produced from apilarnil be an alternative to testosterone replacement therapy? Hacettepe Journal of Biology and Chemistry 45(4): 635-638.

Frizzera D, Del Fabbro S, Ortis G, Zanni V, Bortolomeazzi R, Nazzi F, Annoscia D. 2020. Possible side effects of sugar supplementary nutrition on honey bee health.Apidologie,*51*(4), 594-608.

Goodwin, R.M., Ten Houten, A. ve Perry, J.H. 1994. Effect of feeding pollen substitutes to honey bee colonies used for kiwifruit pollination and honey production. *New Zealand J. Crop. Hort* 22: 459-462.

Hamamci M, Doganyigit Z, Silici S, Okan A, Kaymak E, Yilmaz S, Tokpinar A, İnan L.E. 2020. Apilarnil: a novel neuroprotective candidate. Acta Neurologica Taiwanica 29, (2): 33-45.

Hashem N M, Hassanein E M, Simal-Gandara J. 2021. Improving reproductive performance and health of mammals using honeybee products. Antioxidants 10(3):336.

Herbert E.W. 1992. Honey bee nutrition. In Graham J. M. (ed.), The hive and the honey bee. Dadant and Sons Hamilton IL. pp. 197-233.

Hladun K R, Smith B H, Mustard J A, Morton R R, Trumble J T 2012. Selenium toxicity to honey bee (*Apis mellifera* L.) pollinators: effects on behaviors and survival. PloS one, 7(4): e34137.

Hladun K R, Parker D R, Trumble J T 2015. Cadmium, copper, and lead accumulation and bioconcentration in the vegetative and reproductive organs of Raphanus sativus: implications for plant performance and pollination. Journal of Chemical Ecology 41(4): 386-395.

Hrassnigg N, Crailsheim K, 2005. Differences in drone and worker physiology in honey bees (*Apis mellifera*). Apidologie 36(2): 255-277.

Hroshovyi T, Dobrynchuk M, Pavliuk B, Chubka M, 2021. Pharmaceutical Sciences. Biological Sciences 2(63): 36.

Inandiklioglu N, Doganyigit Z, Okan A, Kayma E, Silici S. 2021. Nephroprotective effect of apilarnil in lipopolysaccharide-induced sepsis through TLR4/NF-κB signaling pathway. Life Sciences, 284:119875.

Isidorov VA, Bakier S, Stocki M, 2016. GC-MS investigation of the chemical composition of honey bee drone and queen larvae homogenate. Journal of Apicultural Science 60(1): 111-120.

Israr M, Malik S, Ali J, Khan M N, Khan K, Tariq K, Alam S 2022. Effect of artificial pollen substitute diets on diets consumption, brood area, and colonies strength of Apis mellifera L. colonies during dearth period. Journal of Applied Entomologist, 2(2), 09-11.

Koç A U, Karacaoğlu M. 2004. Effects of rearing season on the quality of queen honeybees (*Apis mellifera L.*) raised under the conditions of Aegean Region. Mellifera 4(7):34-37.

Kumar R, Mishra R C, Agrawal O P. 2013. Effect of feeding artificial diets to honey bees during dearth period under Panchkula (Haryana) conditions. Journal of Entomological Research, 37(1):41-46.

Martinello M, Mutinelli F 2021. Antioxidant activity in bee products: A review. *Antioxidants*, 10(1), 71.

Nainu F, Masyita A, Bahar M, Raihan M, Prova S R, Mitra S, Simal-Gandara J. 2021. Pharmaceutical prospects of bee products: special focus on anticancer, antibacterial, antiviral, and antiparasitic properties. Antibiotics 10(7):822.

Noordyke E R, van Santen E, Ellis J D. 2022. Evaluating the strength of western honey bee (Apis mellifera L.) colonies fed pollen substitutes over winter. Journal of Applied Entomology,*146*(3), 291-300.

Oskay, O. 2021. Effects of diet composition on consumption, live body weight and life span of worker honey bees. Applied Ecology and Environmental Research 19(6):4421-4430.

Pande R, Karnatak A K. 2014. Germinated pulses as a pollen substitute for dearth period management of honey bee colonies. Current Biotica 8(2):142-150.

Pernal S F, Currie R W. 2000. Pollen quality of fresh and 1-year-old single pollen diets for worker honey bees (*Apis mellifera* L.). Apidologie 31: 387-409.

Ricigliano V A, Williams S T, Oliver R. 2022. Effects of different artificial diets on commercial honey bee colony performance, health biomarkers, and gut microbiota. BMC veterinary research, *18*(1), 1-14.

Saffari A M, Kevan P G, Atkinson J L. 2004. A promising pollen substitute for honey bees. American Bee Journal, 144(3):230-231.

Silici S. 2019. Bal arısı ürünleri ve apiterapi. Türk Tarım-Gıda Bilim ve Teknoloji dergisi 7(9):1249-1262.

SPSS 1999. SPSS for windows release 16.0.2., SPSS Inc., Chicago.

Suleiman J B, Bakar A B A, Mohamed M. 2021. Review on bee products as potential protective and therapeutic agents in male reproductive impairment. Molecules, 26(11):3421.

Topal E, Strant M, Yücel B, Kösoğlu M, Margaoan R, Dayıoğlu M. 2018. Ana ve erkek arı larvalarının biyokimyasal özellikleri ve apiterapötik kullanımı. Hayvansal Üretim *59*(2): 77-82.

Topal E, Yücel B, Tunca R İ, Kösoğlu M. 2019. Bal arılarında beslemenin koloni dinamiği üzerine etkileri. Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi 9(4): 2398-2408.

Tunç M A, Cengiz M M, Yazıcı K, Turan M. 2020. The effects of supplemental feeding with sodium humate on the performance of honey bee colonies (*Apis mellifera* L.). Uludağ Arıcılık Dergisi 20(2), 181-188.

Yücel B, Acikgoz Z, Bayraktar H, Seremet C. (2011). The effects of apilarnil (drone bee larvae) administration on growth performance and secondary sex characteristics of male broilers. Journal of Animal and Veterinary Advances *10* (17): 2263-2266.