

## Overwintering Performance and Spring Development of Honeybee Colonies Fed with Spirulina (*Arthrospira platensis*)

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**Abstract:** With this study, spirulina, which has become quite popular in recent years and has also yielded successful results in cage trials in beekeeping, was used as an alternative protein source instead of pollen in autumn feeding, and the wintering performances of the colonies were determined. For this purpose, Anatolian Bee Muğla Ecotype sister queen bee colonies were created. Each group consisted of 4 colonies and the colonies were fed with bee cakes prepared with rockrose pollen (LP) and different doses of spirulina (S10%, S20% and S30%) once a week during October and November. After wintering, the adult bee population (ABP) in the colonies was determined as  $4.6 \pm 0.47$ ;  $4.7 \pm 0.43$  and  $3.8 \pm 0.31$  and  $3.7 \pm 0.43$  frames in S10%, S20%, S30% and LP groups, respectively. In spring, the highest ABP and brood area (BA) were obtained in S20% ( $9.0 \pm 0.41$  frames and  $6162 \pm 492 \text{ cm}^2$ ) and the lowest ABP and BA were obtained in LP group ( $6.5 \pm 0.65$  frames and  $4722 \pm 289 \text{ cm}^2$ ) and the differences between the groups were found to be statistically insignificant. Considering the high protein content of spirulina and the fact that honey bee colonies enjoy consuming spirulina cake (except S%30), it can be recommended to add spirulina to bee diets during periods when pollen is insufficient. Especially in the future, it will be important to obtain detailed information about the positive and negative effects of spirulina with this and similar studies to be conducted more frequently.

**Keywords:** Rockrose pollen, bee cake, autumn, brood area, adult bee population

**Spirulina (*Arthrospira platensis*) ile Beslenen Bal Arısı Kolonilerinin Kışlama Performansı ve İlkbahar Gelişimleri**

**Öz:** Bu çalışma ile son yıllarda oldukça popüler olan ve arıcılıkta da kafes denemelerinde başarılı sonuçlar veren spirulina, sonbahar beslemesinde polen yerine alternatif protein kaynağı olarak kullanılmış ve kolonilerin kışlama performansları saptanmıştır. Bu amaç için Anadolu Arısı Muğla Ekokipi kız kardeş ana arı kolonileri oluşturulmuştur. Her grupta 4'er koloni olacak biçimde, koloniler lazen poleni (LP) ve farklı dozlarında spirulina (S%10; S%20 ve S%30) ile hazırlanan arıkekleri ile haftada 1 kez Ekim-Kasım ayı boyunca beslenmiştir. Kışlama sonrası kolonilerde ergin arı populasyonu (EAP) S%10, S%20, S%30 ve LP grubunda sırasıyla;  $4.6 \pm 0.47$ ;  $4.7 \pm 0.43$  ve  $3.8 \pm 0.31$  ve  $3.7 \pm 0.43$  adet olarak belirlenmiştir. İlkbaharda ise, en yüksek EAP ve yavruları (YA) S%20'de ( $9.0 \pm 0.41$  adet ve  $6162 \pm 492 \text{ cm}^2$ ) en düşük EAP ve YA ise; LP ( $6.5 \pm 0.65$  adet ve  $4722 \pm 289 \text{ cm}^2$ ) grubunda elde edilmiş, gruplar arasındaki farklar istatistiksel açıdan öneksiz bulunmuştur. Spirulinanın içeriğinde yüksek protein ve bal arısı kolonisinin spirulinalı keki (S%30 hariç) severek tüketmesi göz önünde bulundurulduğunda, polenin yetersiz olduğu dönemlerde spirulinanın arı diyetlerine eklenmesi önerilebilir. Özellikle gelecekte daha çok sayıda yapılacak bu ve benzer çalışmalar ile spirulinanın olumlu ve olumsuz etkileri hakkında detaylı bilgi edinmek arı beslenmesi açısından önemli olacaktır.

**Anahtar kelimeler:** Lazen poleni, arı keki, sonbahar, yavruları, ergin arı populasyonu

### INTRODUCTION

Honeybees (*Apis mellifera* L.) constitute a vital aspect of human food security in the modern world with their contribution to pollination, while they play an important economic role with their valuable products such as honey, pollen, royal jelly, bee bread, propolis, and bee venom (Khalifa et al., 2021). Managed bee colonies contribute to 35% of global food production, while their services and products are a multi-billion dollar global industry, providing economic benefits to the agricultural, food, and medical sectors (Klein et al., 2006; Van der Sluijs and Vaage, 2016). However, in recent years, honeybees have been facing high colony mortality rates, habitat loss, and climate change that threaten their populations and the services they provide to humans (Potts et al., 2010). Climate change, habitat loss, and monoculture farming, etc. also directly negatively affect the

nectar and pollen resources that bees benefit from. Many reasons, such as climate change, habitat loss, pesticides and herbicides, and monoculture farming, limit access to sustainable feed sources for bee colonies throughout the season (Prevey, 2020). This situation forces beekeepers to intervene in their colonies. The best protein sources for bee colonies are pollen and bee bread, but the sale of these products provides additional income to the beekeeper (Ellis and Hayes, 2009; Vasquez and Olofsson, 2009). In addition, these products are difficult to store because they tend to lose their nutritional value over time, and problems can

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occur in bee colonies in the spring, when there is a high demand for protein. Therefore, different recipes for alternative food for bees have been developed according to the composition, acceptability, flavor content, and digestibility of nutrients of honey and pollen. As alternative protein sources for bees, soybean meal, pea paste, rice bran, brewer's yeast, guar flour, skimmed milk powder, egg yolk powder, casein and fish meal have generally been used (Dastouri et al., 2007; Irandoust and Ebadi, 2013; El-Wahab et al., 2016; Kumari and Kumar, 2020; Paray et al., 2021). Artificial diets and feed supplements derived from various plant and animal products show mixed results in their capacity to support colony growth and productivity (Noordyke and Ellis, 2021; Paray et al., 2021; Tsuruda et al., 2021). Current research focusing on artificial diets suggests that there are opportunities to improve bee feed to meet the increasing demands of beekeepers who are increasingly dependent on supplementary feeding (Ricigliano et al., 2022). In recent years, algae have come to the fore as an alternative protein source. Single-celled microalgae or simple multicellular organisms, mostly photosynthetic, have recently attracted attention as a feed source for terrestrial farm animals, including aquaculture and honeybees (Jehlik et al., 2019; Ricigliano and Simone-Finstrom, 2020). Algal biomass has been shown to be promising as an alternative feed source and health-regulating natural product source for honeybees (Ricigliano and Simone-Finstrom 2020), and due to their nutritional content, sustainability, and suitability for some specific manipulations, algae can support bee populations against current stress factors and can be transformed into new nutritional supplements (Stengel et al., 2011; Vigani et al., 2015; Ricigliano and Simone-Finstrom, 2020). While the nutritional activity of microalgae (*Arthrospira platensis*) is well documented in other animals, their effectiveness as a food source for honey bees is largely unknown. Blue-green microalgae *Arthrospira platensis* is cultivated on an industrial scale as a dietary supplement for humans and livestock (Soni et al., 2017). This microalgae, commonly called spirulina, is considered a complete food source and contains essential amino acids, functional lipids, complex carbohydrates, vitamins, and minerals (Ciferri, 1983). In modern beekeeping, beekeepers intervene in colony management in the form of disease control and supplementary feeding at certain periods to overcome factors such as changing weather conditions, lack and diversity of food sources, parasites, pathogens and pesticides (Potts et al., 2010; Vaudo et al., 2015; Dolezal and Toth, 2018). In technical beekeeping, whether

supplementary feeding is necessary is generally determined by whether sufficient nectar and pollen are received, the colony's brood rearing activity, and whether there are sufficient honey and pollen stocks in the colony. In addition, colonies are usually fed with sugar syrup in the spring months to encourage the queen to lay eggs and field bees to collect more pollen so that they can enter the main nectar flow of the region with a strong population.

Similar feeding in the fall is also done to encourage the queen bee to lay eggs and the field bees to collect pollen in order to ensure that the colonies spend the winter with young worker bees (Haydak and Dietz, 1965). However, in recent years, especially in the Aegean Region, the drought in the fall due to climate change has caused a shortage of pollen in the colonies. Carbohydrate feeding during this period is not sufficient. Therefore, it is important to research new substitute protein sources that can replace pollen. In this study, the effect of adding spirulina, which has become quite popular in recent years and has also yielded successful results in cage trials in beekeeping, to the diets in the fall feeding on the wintering performance and spring development of the colonies was investigated.

## MATERIAL AND METHODS

The material of the study consisted of Anatolian bee Muğla Ecotype colonies. Sister queen bees were raised from a breeding colony. The queen bees that were left to mate naturally were given to the newly established colonies after they started laying eggs. The newly established colonies were maintained and fed throughout the summer. The colonies were fed with sugar syrup (1:1.5) once a week in September-October, and chemical control was applied against varroa. The colonies were equalized and random groups were formed. The number of bee frames, brood areas and hive weights were determined in the colonies on October 24, 2023, with 4 colonies in each group, and the feeding program was started the next day (October 25, 2023). In this study, rockrose pollen was used as a control group to determine the potential of spirulina as an alternative feed to pollen, especially in autumn feeding. Monofloral pollen was preferred as the control group since it has a standard content. Rockrose pollen is collected intensively by colonies in certain parts of the region, especially in May and June. In a study conducted in the region, it was reported that rockrose pollen can be evaluated as a monofloral pollen (Yağcıoğlu and Uçak Koç, 2024). The control group dried rockrose pollen has approximately 20% protein content (LP) and the Spirulina group was prepared in approximately three different ratios as 30% spirulina added bee cake (S30), 20% spirulina added bee cake (S20), 10% spirulina added bee cake (S10). For colonies fed with pollen;

for 100 g pollen cake, 20 g pollen, approximately 80 g powdered sugar (by grinding crystal sugar), approximately 10-15 g of strained honey and approximately 40-50 mL of drinking water were added to get the consistency of the bee cake and the consistency was obtained. To obtain spirulina cake, 10 g for 10%, 20 g for 20% and 30 g for 30% cake were added to 100 g of powdered spirulina (commercial name: Naturiga organic spirulina powder 66% protein) using the same method and completed to 100 g of powdered sugar. Feeding was started once a week for 5 weeks (October 25-November 22, 2023) with 150 g, but since it was seen that they did not consume, 100 g of bee cake was added to the frames (Figure 1) and drinking water was added to the feeders in the second feeding. No maintenance feeding was done in the colonies during December and until the second week of January, and the colonies were wintered. However, since December and January of 2023 were mild compared to other years, the colonies were weighed in the third week of

January for their wintering exit performance and the number of frames with bees was determined and the colonies were fed in the same way for 5 weeks. In addition, 100 cc sugar syrup (1:1.5) was given to the colonies instead of water along with the cake. At the end of the feeding, the number of frames with bees and brood areas were determined in the colonies (in March). The number of frames with bees was counted as 1 frame completely covered with bees and the brood area was calculated with the help of a ruler using the Puchta method for short and long side measurements. The colonies were housed in hives with standard pollen traps and the hive covers were removed when the hive was weighed.

#### STATISTICAL ANALYSIS

Minitab package program was used for variance analysis and multiple comparison test to determine the effect of bee feed (LP, S10, S20 and S30) on wintering performance and spring development.



Figure 1. Image of spirulina cake in one of the experimental colonies.

#### RESULTS

The pre-feeding (24.10.23), post-feeding (17.01.24) and spring development (01.03.24) of the colonies fed with spirulina are presented in Table 1. According to the variance analysis applied to the data the effect of feeding on the groups is insignificant ( $p>0.05$ ). When the experimental colonies started feeding, they had an average of 5 bee frames and an average brood area of 2300-2400  $\text{cm}^2$ . The colonies (hive) weighed an average of 20-23 kg. After the colonies were wintered for approximately 1.5 months, the colony weights at winter exit were determined as  $20.8\pm1.94$  kg,  $19.7\pm0.53$  kg,  $18.6\pm0.62$  kg and  $20.0\pm1.28$  kg for the S10, S20, S30 and P groups, respectively. In colonies fed after winter, the highest number of frames with bees was obtained in the S20 group ( $9.0\pm0.41$  frames), while the

lowest was obtained in the LP ( $6.5\pm0.65$ ) group. Again, when the groups were evaluated in terms of brood area, the highest brood area was obtained in the S20 group ( $6162\pm492$   $\text{cm}^2$ ), while the lowest brood area was obtained in the LP group ( $4722\pm289$   $\text{cm}^2$ ). However, the differences between the groups were statistically insignificant.

#### DISCUSSION

In this study, before the bee colonies started to be fed, they were formed from colonies with an average of 5 bee frames, approximately 2-3 frames with brood, and as equal as possible in terms of honey and pollen stocks. The colonies that were fed for 5 weeks in the autumn under the conditions of the Aegean Region were wintered and after wintering, S10 and S20 completed the winter (half of

December and January) with approximately 1 frame more bees than S30 and LP in terms of bee frame numbers.

Table 1. Parameters of feeding groups in the autumn period

Groups	NFBBF*	NFBAW	NFBS	PFBA(cm <sup>2</sup> )	SBA (cm <sup>2</sup> )	PFHW (kg)	PWHW (kg)
	(24.10.23)	(17.01.24)	(01.03.24)	(24.10.23)	(01.03.24)	(24.10.23)	(17.01.24)
S10	5,1±0,83	4,6±0,47	8,8±1,65	2453±107	5496±773	23,9±2,37	20,8±1,94
S20	5,2±0,60	4,7±0,43	9,0±0,41	2381±152	6162±492	21,7±0,64	19,7±0,53
S30	5,0±0,35	3,8±0,31	7,3±0,75	2254±195	5436±813	20,6±0,65	18,6±0,62
LP	5,0±0,70	3,7±0,43	6,5±0,65	2342±152	4722±289	22,6±1,57	20,0±1,28

\* Number of Frames with Bees Before Feeding (NFBBF); Number of Frames with Bees After Wintering (NFBAW); Number of Frames with Bees in Spring (NFBS); Pre-Feeding Brood Area (PFBA); Spring Brood Area (SBA); Pre-Feeding Hive Weights (PFHW); Post-Wintering Hive Weights (PWHW)

In addition, at the end of the 5-week feeding in early spring, S10 and S20 had more adult bees than S30 and LP. In addition, in this study, bee cakes containing approximately 10% and 20% spirulina were consumed by the bee colonies quickly and with pleasure, although not as much as bee cake with pollen, but the consumption rate decreased as the ratio increased (30%). In our study, rockrose pollen (*Cistus creticus*) was preferred in bee feeding due to its standard content and being more economical than spring pollen with rich content. In nutrition, the amino acid profile of the protein is more important than the total protein content. Since honey bees cannot synthesize arginine, histidine, lysine, tryptophan, phenylalanine, methionine, threonine, leucine, isoleucine and valine, the presence of these amino acids in the diet is critical (De Groot, 1953). Amino acids are obtained from pollen and are supplemented with pollen substitute diets. It has been reported that the essential amino acids detected in Spirulina exceed those of other tested diets, except for histidine and lysine, which are two times higher in pollen (Ricigliano and Simone-Fistrom, 2020). In a study (Sarioğlu-Bozkurt et al., 2022), the amino acid content of rockrose pollen compared to mixed pollen was found to be low in glycine, leucine, isoleucine, threonine, serine, aspartic acid, glutamic acid, phenylalanine, lysine, histidine and tyrosine, and high only in proline and methionine. In our study, it is highly probable that the amino acid content in rockrose pollen affected the offspring production in the LP group. However, this can be demonstrated by comparing mixed fresh pollen collected in the spring and stored in a deep freezer with spirulina and using it in autumn feeding. In recent years, as a result of climatic changes, agricultural pollutants and the damage occurring in nature, bee colonies have been experiencing pollen shortages, especially in the Aegean Region due to drought in autumn. Years ago, studies were conducted on

substitute feeds that could replace pollen in bee colonies, and since bees do not consume these feeds very fondly, studies in this direction have been abandoned from time to time. However, in recent years, studies on pollen substitute feeds have been refocused as an alternative to pollen shortages, and the number and variety of commercially available bee feeds have also increased.

Some studies have reported that commercial feeds significantly enriched in protein increased protein titers in bees by 2.65 times, and pollen by 1.76 (De Jong et al., 2009). Şahinler and Kaya (2001) reported that feeding honey bee colonies with cake (4 parts soy flour, 1 part milk powder, 1 part sugar syrup, 1 part honey) and syrup increased the colony brood area by 1.15 times compared to the cake-fed group, 1.85 times compared to the syrup-fed group, and 2.18 times compared to the control group.

Gregory (2006) reported that colonies fed fresh pollen had a longer lifespan compared to commercial feeds and pollen with extended shelf life, and Kösoğlu et al. (2019) obtained higher values in terms of the number of bee frames, brood area and pollen storage area in colonies fed with mixed spring pollen compared to commercial bee cake. Adanacioğlu et al. (2022) stated that feeding with poppy pollen was slightly more advantageous than other feeding groups in their analysis of whether the bee frame values per hive covered the production costs in poppy (*Papaver somniferum* L.) pollen, rockrose (*Cistus creticus* L.) pollen, mixed pollen, bee cake, syrup and control group colonies. In a study by Khan and Ghramh (2022), the effectiveness of different dietary supplements, bee pollen, ajeena (commercially sold pollen substitute) and date paste, alone or mixed with varying percentages of spirulina (*Arthospira platensis*), was compared for honey bees (*Apis mellifera jemenitica*) in cages. The same researchers reported that honey bees consumed bee pollen (11.51 ± 2.22 mg/bee) and

ajeena diet ( $10.68 \pm 1.29$  mg/bee) significantly more than the other diets, while the maximum consumption was preferred to date paste mixed with 2.5% spirulina.

Another study on spirulina compared dry and fresh laboratory-grown spirulina with bee-collected pollen and sucrose syrup as a control and a commercial pollen substitute. The diets were given ad libitum as a paste to newly emerged bees in cages (10–13 cage replicates), and samples were taken from the bees on days 5 and 10 for physiological and molecular measurements. Spirulina diets showed higher values for worker bees' thoracic weight, head protein content, and beneficial gut bacteria despite reduced consumption. Spirulina diets also significantly increased fat body lipid content and central storage lipoprotein vitellogenin mRNA levels, concluding that Spirulina has significant potential as a pollen substitute or prebiotic dietary additive to improve honeybee health (Ricigliano and Simone-Finstrom, 2020). In a study by Guldas et al. (2022), they reported that the total phenolic content of green-colored algae honey obtained from honeybee colonies fed with spirulina increased by 28.53% compared to the control group, and among the phenolic compounds detected (15 phenolics), acacetin (48.55%) and pinocembrin (47.13%) were higher than the control group, and they stated that it could be enriched up to 20% by feeding honeybees with 10% Spirulina.

## CONCLUSION

The search for alternative protein sources in the diet of bees is a current issue because the deficiency of some amino acids in bee nutrition can weaken the bee colony. In this study, unlike cage studies, bee colonies were fed with spirulina bee cakes. Although the colonies consumed the spirulina cake as quickly and fondly as the pollen cake, it had positive effects on the development of the colonies' young and adult bees. It can be used as a pollen replacement feed by adding 10–15% to the bee cake in the autumn feeding when there is a pollen shortage. In addition, it is necessary to compare the cakes prepared with mixed fresh pollen collected in the spring season with spirulina and to perform their economic analyses.

Honey bees have developed many strategies to cope with parasites and pathogens, but if they are under nutritional stress, they face a great challenge. Therefore, future research should consider the interaction of possible nutritional effects with other factors, such as the effect of nutrition on the susceptibility or tolerance of honey bees to parasites, pathogens and pesticides, the energetic stress of bees caused by parasites and the role of nutrition in strengthening the honey bee immune system, how honey bees are impaired in their ability to defend against pathogens when they are undernourished and how this ability can be improved by adequate nutrition. Future

research on this topic will provide more detailed information.

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