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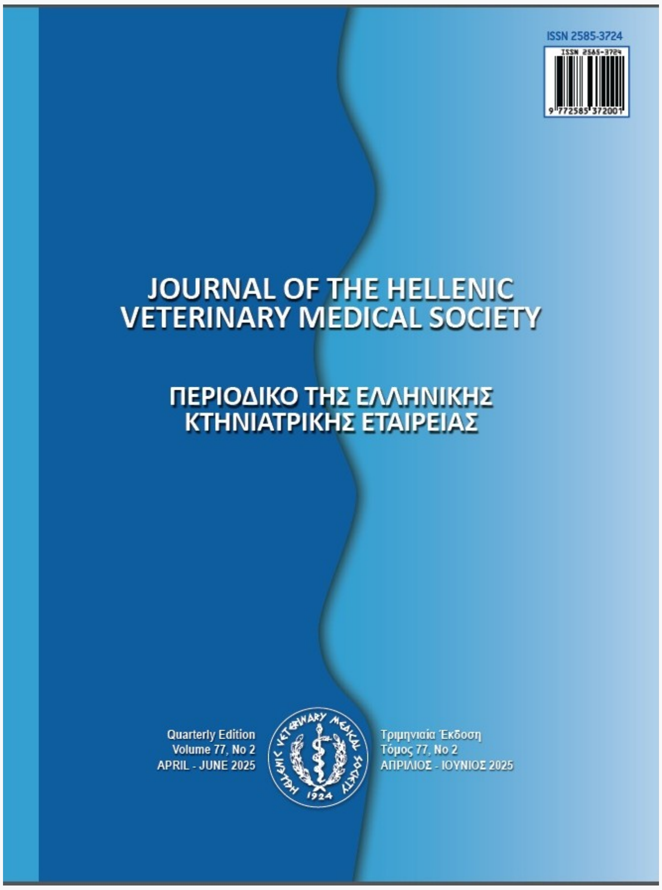

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Comparison of colony initiation success of queen bees (*Bombus terrestris* L.) fed diets with different pollen sources

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ABSTRACT: This study examined the effect of feeding diets with different pollen sources to queen bees (*Bombus terrestris* L) on the initiation of the colony. 120 fertile queen bees that healthily emerged from diapause were randomly distributed to six diet groups of different pollen sources: i) Poppy pollen (PP), ii) Chestnut pollen (CP), iii) Labdanum pollen (LP), iv) Poppy-Chestnut pollen (PPCP), v) Poppy-Labdanum pollen (PPLP) and vi) Chestnut-Labdanum pollen (CPLP). In addition, groups were fed sugar syrup (50 brix) *ad libitum*. During the rearing of queens, the room temperature and relative humidity were 27-28 °C and 50%, respectively. It was found that pollen consumption differed among the groups ($P<0.01$); highest and lowest in the PPCP and CP groups, respectively. The worker bee emergence time was earliest and latest in the LP and PP groups, respectively ($P<0.05$). Furthermore, the number of worker bees in the first brood varied among the groups ($P<0.01$), higher in the PPCP compared with the rest of the groups. On the other hand, the weight of queen and worker bees, first egg-laying time, and survival rate did not differ among the groups ($P>0.05$). In conclusion, this study suggested that supplementing bee diets with multi-floral poppy-chestnut pollen could enhance colony initiation of queen bumblebees through increasing pollen consumption and the number of worker bees in the first brood.

Keyword: Pollen; Colony initiation; *Bombus terrestris* L; Queen bees.

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INTRODUCTION

It is well-known that insects forage on plants to meet their pollen and nectar needs and ensure pollination in the meantime (Belsky et al., 2020; Korkmaz, 2016). Bumblebees constitute the most important pollinator insect species after honeybees (Aktürk et al., 2022; Velthuis and van Doorn, 2006). Among the approximately 250 species of bumblebees identified, *Bombus terrestris* is the most commercially raised species because it is easier to keep and there is a high potential of crowding its colonies in apiaries (Velthuis and van Doorn, 2006). The commercial rearing of *Bombus terrestris* bees, whose importance in pollination has been known since the early 20th century, has been increasing over the last 35 years (Aktürk, 2022; Gürel et al., 2011).

Similar to other honeybee species, bumblebees need different food sources, acquiring them from the pollen and nectar they collect from flowers (Piko et al., 2021; Gösterit and Çiçek, 2017). While nectar sources meet their carbohydrate needs, they obtain most of the other nutrients from pollen (Ruedenauer et al., 2015). In commercial rearing, they are fed with sugar syrup and fresh pollen obtained from honeybee colonies with pollen traps (Baloğlu and Gürel, 2015; Riberio et al., 1996). Studies indicate that bumblebees are more selective than honeybees in nature when choosing pollen, which is the only protein source (Ghosh and Jung, 2020; Brodschneider and Crailsheim, 2010), and they focus on the quality rather than the quantity of pollen (Baloğlu and Gürel, 2015; Leonhardt and Bluthgen, 2012). Usually, bumblebees prefer pollen with higher quality in terms of protein content, essential amino acid, sterol values, and protein: lipid ratio (Vaudo et al., 2015). Also, bumblebees mix the pollen they obtain from different plant species to regulate their food intake, which is important for the improvement of nutrition and life activities (Piko et al., 2021; Bortolotti et al., 2020; Roger et al., 2017; Ruedenauer et al., 2015; Vaudo et al., 2015).

The nutritional content and pollen quality vary significantly depending on several factors such as the plant species, pollen storage conditions, and humidity rate (Taha and Al-Kahtani, 2020; Percie du Sert, 2009; Riberio et al., 1996). However, the inability of bumblebees to obtain the pollen they need negatively affects their immune system and consequently, their resistance to various diseases, parasites, and environmental factors decreases (Roger et al., 2017; Ruedenauer et al., 2015; Archer et al., 2014; Brodschneider and Crailsheim, 2010). Although bumblebees do not have problems associated with pollen quantity during foraging activities in nature, feeding on a single type of pollen and nectar results in unhealthy individuals in the colony (Praz et al., 2008; Vaudo et al., 2015).

Similarly, the diversity and quality of pollen used in commercial rearing is a factor that directly affects the colony development of bumblebees (Gösterit and Çiçek, 2017; Genissel, 2002). *Bombus terrestris* colonies vary greatly in characteristics such as the number of worker bees, worker bee body mass, time to start egg-laying, and colony initiation rate. Considering that, these features spread to the future of the colony, the pollen quality or pollen they feed on in the period until the social phase and at the beginning of the social phase (first worker emergence) becomes even more important (Gösterit and Çiçek, 2017).

This study therefore investigated the effect of monofloral and multifloral feeding of three different pollen sources on the success of *Bombus terrestris* L. queen bees in colony initiation.

MATERIALS AND METHODS

Production of colonies

The research was carried out in 2022 at the Bombus Bee Research and Application Laboratory within the Directorate of Apiculture Research Institute of the General Directorate of Agricultural Research and Policies of the Republic of Turkey, Ministry of Agriculture and Forestry. The research material consisted of 120 fertile *Bombus terrestris* L. queen bees that had completed the diapause period under controlled conditions. Queen bees were collected from the Institute's stock of the *Bombus terrestris* L. population, initially weighed with a Precisa XB 220A SCS analytical balance with a sensitivity of 0.0001, and placed in ventilated-numbered breeding boxes of 10 ×10 × 10 cm. Red light was used to control the colonies during the collection of queen bees. Queen bees were raised in a controlled environment of 27-28 °C and 50% relative humidity and fed *ad libitum* with 50% brix syrup prepared with granulated sugar and fresh frozen pollen.

Identification of pollen and determination of some of its properties

In the study, pollen sources involved labdanum (L; *Cistus* spp), poppy (P; *Papaver somniferum*), and chestnut (C; *Castanea sativa*) pollens, which are commercially used to produce monofloral honey.

Pollens were obtained from AkdiPolen®Company and samples were examined under a pollen light microscope. After identification, species were determined using a pollen atlas (Sorkun, 2008). Protein and moisture content (%) were determined according to Sorkun et al. (2010) and shown in Table 1. Pollen was frozen fresh and stored at -18 °C throughout the study.

Experimental treatments

There were six dietary groups, with 20 queen bees in each. The groups included; i) Poppy pollen (PP), ii) Labdanum pollen (LP), iii) Chestnut pollen (CP), iv) Poppy-Labdanum pollen (1/1) (PPLP), v) Poppy-Chestnut pollen (1/1) (PPCP), and vi) Labdanum-Chestnut pollen (1/1) (LPCP).

Data collection

At the end of the diapause period, fertile queen bees were taken from the cold weather environment, weighed, and randomly placed in the starting boxes. Daily checks were made to determine the time when the queen bees started egg-laying (days), the rate of beginning to lay eggs (%), the colony initiation rate (%), worker bee emergence time (days), the number of worker bees (number), worker bee weights (g) and the amount of pollen consumed (g) was determined (Amin et al., 2010). The clarification of the collected data is given below:

Queen bee weight (g): Weight of queen bees before being placed in the starter boxes**.**

Egg-laying rate (%): Rate of queens placed in the starter boxes laying eggs.

First egg laying time (days): Time from placing the queen bees in the starter boxes to laying the first egg cluster.

Table 1. Protein and moisture content of different pollen sources (%)

Group	Protein content (%)	Moisture content (%)
PP	22.2	18.96
CP	20.2	18.86
LP	11.8	23.40
PPCP	21.2	18.91
PPLP	17	21.18
CPLP	16	21.13

Abbreviations: PP; poppy pollen, CP; chestnut pollen, LP; labdanum pollen, PPCP; poppy-chestnut pollen, PPLP; poppy-labdanum pollen, LPCP; labdanum-chestnut pollen

Colony initiation rate (%): The proportion of queen bees placed in the starter boxes that lay eggs and produce at least 10 worker bees.

Viability (%): The proportion of queen bees placed in the starter boxes that were alive at the end of the first brood.

Worker bee emergence time (days): Time from the placement of queen bees in the starter boxes to the first worker bee emergence.

Number of worker bees (number): Number of worker bees that become adults from the eggs laid by the queen bees until the beginning of the second brood.

Amount of pollen consumed (g): Total amount of pollen consumed by the colony at the end of the experiment.

Worker bee weight (g): Weight of worker bees produced in the first brood.

Statistical analysis

The normality assumption of the data was examined with the Kolmogorov-Smirnov test and it was determined that the data met the normality assumption ($P>0.05$). Since the data was suitable for analysis of variance, statistical analyses were performed according to one-way analysis of variance (One-way ANOVA). Differences between groups were determined by Duncan's multiple comparison test. Whether viability, egg-laying status, and colony formation were dependent on the applications were analyzed using the chi-square test. The relationships between some variables examined were examined with Pearson correlation analysis.

RESULTS

There was no statistical difference among the groups in terms of the weight of queen and worker bees, and first egg-laying time ($P>0.05$, Table 2). Queen bees that died before laying eggs (PP:3, CP:6, LP:7, PPCP:2, PPLP:4, LPCP:3) were excluded from the study (Table 2, Table 3).

Pollen consumption varied among the groups, with PPCP and CP bee colonies consuming the highest and lowest amounts of pollen, respectively ($P<0.01$, Table 1). In addition, the worker bee emergence time in the first brood significantly differed among the groups, with the earliest and latest emergence of worker bees in LP and PP groups, respectively ($P<0.05$, Table 2). Also, there was a significant difference among groups regarding the

Table 2. Effects of monofloral and multifloral feeding of labdanum, poppy, and chestnut pollens on colony initiation success

Group	Queen bee weight		Pollen consumption		First egg-laying time		Worker bee emergence time		Number of worker bees		Worker bee weight	
	n	G	n	g	n	Days	n	days	n	Number	n	g
PP	20	0.83	17	10.37^{a}	12	8.75	12	19.67^{a}	12	4.75^{a}	12	0.27
CP	20	0.77	14	11.48^{bc}	9	11.67	9	24.67^{bc}	9	5.33^{a}	9	0.26
LP	20	0.82	13	11.48^{bc}	12	11.08	12	26.25^{c}	12	5.42^{a}	12	0.27
PPCP	20	0.80	18	12.09^{c}	14	9.64	14	19.93^{ab}	14	7.07^{b}	14	0.26
PPLP	20	0.82	16	11.31^{abc}	9	9.33	9	23.33^{abc}	9	4.78^{a}	9	0.23
LPCP	20	0.80	17	10.96^{ab}	13	10.00	13	23.15^{abc}	13	4.54^{a}	13	0.26
SEM		0.011		0.145		0.419		0.676		0.189		0.004
P value		0.720		0.010^{**}		0.393		0.018^{*}		0.00^{**}		0.292

Abbreviations: PP; poppy pollen, CP; chestnut pollen, LP; labdanum pollen, PPCP; poppy-chestnut pollen, PPLP; poppy-labdanum pollen, LPCP; labdanum-chestnut pollen, SEM; Standard error of mean, n; number of observations. *; means with different superscripts in the same column are significant (P<0.05).

**; means with different superscripts in the same column are significant (P<0.01)

number of worker bees in the first brood, the number being higher in PPCP colonies (P<0.01, Table 2) compared to the remaining groups, whose values were statistically similar.

Table 3 indicated that there was no difference among groups regarding egg-laying rate ($\chi2$ =5.584; P=0.349) and colony formation ($\chi2$ =4.849; P=0.435).

Table 4 shows the survival rate findings. It was identified that the survival rate did not differ among the groups ($\chi2$ =5.709 P=0.435).

Table 5 indicated that there was a negative relationship between the first egg-laying time and the weight of queen bees (r= -0.325; P<0.01).

DISCUSSION

For the commercial raising of *Bombus terrestris* bees in the desired quality and quantity, factors such as queen bee quality, mating success of the queen bee, environmental conditions in which the colonies are raised, as well as the quality of the food consumed are of great importance (Vanderplanck et al., 2014; Genissel et al., 2002). Bumblebees deliver the nu-

Table 3. Effect of different pollen sources on laying rate of queen bees and colony development (%)

Group	n	Laying status				Colony development status			
		Laying queens		Non-laying queens		Established colonies		Non-established colonies	
		Number	%	Number	%	Number	%	Number	%
PP	17	12	70.6	5	29.4	12	100.0	0	0.0
CP	14	9	64.3	5	35.7	8	88.9	1	11.1
LP	13	12	92.3	1	7.7	11	91.7	1	8.3
PPCP	18	14	77.8	4	22.2	14	100.0	0	0.0
PPLP	16	9	56.3	7	43.7	9	100.0	0	0.0
LPCP	17	13	76.5	4	23.5	13	100.0	0	0.0
χ^2		5.584				4.849			
P		0.349				0.435			

Abbreviations: PP; poppy pollen, CP; chestnut pollen, LP; labdanum pollen, PPCP; poppy-chestnut pollen, PPLP; poppy-labdanum pollen, LPCP; labdanum-chestnut pollen, n; number of observations, χ^2; Chi-square value, P; significant level

Table 4. Effect of different pollen sources on survival rate of queen bees (%)

Group	Survival rate				χ^2	P
	Alive		Dead			
	n	%	n	%		
PP	17	85.0	3	15.0	5.709	0.336
CP	14	70.0	6	30.0		
LP	13	65.0	7	35.0		
PPCP	18	90.0	2	10.0		
PPLP	16	80.0	4	20.0		
LPCP	17	85.0	3	15.0		

Abbreviations: PP; poppy pollen, CP; chestnut pollen, LP; labdanum pollen, PPCP; poppy-chestnut pollen, PPLP; poppy-labdanum pollen, LPCP; labdanum-chestnut pollen, n; number of observations, χ^2; Chi-square, P; significant level.

trients they obtain in nature to the larvae without any modification. Therefore, compared to honey bees, the food they consume becomes much more important (Pereboom, 2000). It is known that there is a negative relationship between the protein ratio, which is shown as the most important quality criterion, and the amount of pollen consumption (Vaudo et al., 2016; Baloğlu and Gürel, 2015). However, Tasei and Aupinel (2008) and Genissel et al. (2002) found that nutritional content did not affect pollen consumption in their studies conducted in microcolonies containing only worker bees. In this study, pollen consumption was lowest in the PP group (22.2%), which had the highest protein content among the nu-

Table 5. Correlations between the examined features

		Weight of queen bee (g)	Pollen consumption (g)	First laying time (day)	Worker bee emergence time (day)	Number of worker bees (number)	Weight of worker bees (g)
Weight of queen bee (g)	r	1					
	P						
	n	69					
Pollen consumption (g)	r	0,121	1				
	P	0,321					
	n	69	69				
First laying time (day)	r	-0.325**	0,167	1			
	P	0,006	0,170				
	n	69	69	69			
Worker bee emergence time (day)	r	0,124	-0,030	0,099	1		
	P	0,312	0,810	0,418			
	n	69	69	69	69		
Number of worker bees (number)	r	0,118	0,031	-0,096	-0,138	1	
	P	0,335	0,798	0,432	0,258		
	n	69	69	69	69	69	
Weight of worker bees (g)	r	0,112	0,015	0,071	-0,037	-0,228	1
	P	0,361	0,900	0,560	0,766	0,060	
	n	69	69	69	69	69	69

Abbreviations: r; Pearson correlation coefficient, P; significant level, **; the relationship between two features is significant at P<0.01, *; the relationship between the two features is significant at P<0.05.

trition groups. In this regard, the results of this study are compatible with the findings of Vaudo et al., (2016) and Baloğlu and Gürel, (2015). It is thought that the fact that the average number of workers in the group is significantly higher than the other feeding groups may have played a role in the fact that PPCP, another feeding group with a high protein content, was the second most consumed pollen by bumblebees (21.2%).

In addition, although there was no statistically significant change, it is noteworthy that PPCP and CP groups experienced numerically less queen loss in parallel with their protein ratios.

The time to first oviposition is related to the development rate of female eggs and hormone synthesis (Behmer and Nes, 2003; Nation, 2002). In addition, it is known that pollens rich in amino acids accelerate the above processes (Ren et al., 2023). Although there was no statistical difference between the pollens used in the study, the group fed with poppy pollen, which is among the richest pollens in terms of essential amino acid content, showed the shortest time (8.75 days) in terms of first egg laying time. In addition, it was found remarkable that in other groups (PPCP, PPLP) where poppy pollen was used, the transition to oviposition occurred numerically earlier. The strong negative relationship between first egg-laying time and the weight of queen bees indicates that queen bee weight is an important quality criterion (Aktürk et al., 2023; Güneşdoğdu and Şekeroğlu, 2020).

Another quality criterion in commercial beekeeping is the time it takes for worker bees to emerge from the first brood. The fact that worker bees emerged significantly later in colonies fed LP than PP was associated with poor protein content. In this respect, the current study results are consistent with the findings of Tasei and Aupinel (2008) and Ren et al. (2023). When the study findings are examined, the PP (22.2%) and PPCP (21.2%) groups, which had the highest protein ratio, had the lowest averages for worker bee emergence time (19.67 d and 19.93 d), respectively, while the LP (11.8%) group, which had the lowest protein ratio, had an average of 26.25 d. Here, the significant difference among the groups regarding worker bee emergence time shows how important the protein ratio is in the commercial rearing of bees.

The number and hatching weight of *Bombus terrestris* worker bees, especially in the first brood, affect the future of the colony and further increase their importance for commercial rearing (Gösterit and Çiçek, 2017). Sperms in the queen spermatheca can maintain their viability with the spermatheca gland secretion (Pascini and Martins, 2017), and the quality of this secretion decreases due to malnutrition (Wynants et al., 2022). As a result, the number and weight of emerged worker bees directly or indirectly are also negatively affected (Wynants et al., 2022). Additionally, there are many studies in which the relationship between the food used in raising bees, larval mortality rate, and body size has been determined (Vanderplanck et al., 2014; Brodschneider and Crailsheim, 2010). In the present study, it was thought that the PPCP group's statistical prominence in terms of the number of worker bees may have been influenced by the fact that it is the group that consumed the most pollen, as well as being one of the groups with the highest protein content, in parallel with previous studies. In this respect, it is compatible with the results of Schmid-Hempel and Schmid-Hempel (1998) that feeding colonies with a limited amount of pollen causes the emergence of lower numbers of worker bees. In addition to the amount of pollen consumption, another reason why more worker bees on average emerged from the PPCP group than the PP group, which had a high protein content, is that the colonies were fed a multi-floral pollen diet. Baloğlu and Gürel (2015) and Alaux et al. (2010), in their studies conducted with mono-floral and multi-floral pollens, reported that the protein level alone would not be sufficient to determine the nutritional value of pollen and that the high variation in the components (fatty acids, vitamins, etc.) that make up multi-floral pollens could stand out even when compared to mono-floral pollens with similar protein ratios.

The weight of worker bees, which is one of the first indicators of the effectiveness of the food consumed in studies and affects the future of the colony just like the number of workers (Tasei and Aupinel, 2008; Roulston and Cane, 2002), varies greatly within the colony, especially among worker bees (Goulson, 2003). Studies have generally shown that larger worker bees are more successful in tasks such as colony feeding, field farming, etc (Kerr et al., 2020; Kerr et al., 2019; Goulson et al., 2002; Ings, 2007), while a few studies have stated that the size and diversity of worker bees do not make a difference (Jandt and Dornhaus 2014). In the present study, it was determined that the size of worker bees was at a medium level in all group averages, and feeding with different pollens did not cause any change in the weight of worker bees in the first brood. Although deviant values were obtained in terms of weight in the groups, it was predicted that the ad-libitum feeding of the experimental units may have been effective in the lack of difference between the averages. When previous studies were examined, it was reported that the body sizes of bees were affected in the absence of pollen (Macháčková et al., 2019; Czekońska et al. 2015; Sutcliffe and Plowright, 1990; Sutcliffe and Plowright 1988).

Some of the queen bees that are taken to the egg-laying stage to form a colony, die during the egg-laying stage. While some of the remaining queen bees lay eggs, others do not, and only some of those that lay eggs can form a colony (Sağlam, 2015). Previous feeding studies, similar to this study, show that the effect of pollen on egg laying and colony formation rates is limited (Gösterit and Çiçek, 2017; Gürel et al., 2012). In other studies where changes were observed between groups in terms of egg laying and colony formation, it was found remarkable that animal products such as milk powder, which can have a toxic effect on insects (Sağlam, 2015), or products with high biological value, such as royal jelly, which are not in the nature of bumble bees (Gürel and Gösterit, 2008), were used.

CONCLUSIONS

This study demonstrated that feeding queen bees with diets supplemented with polyfloral poppy and chestnut pollens has the potential to ensure a better colony initiation. However, studies involving poppy-chestnut pollen mixtures in different percentages are warranted to expand further this particular study direction.

CONFLICT OF INTEREST

The authors have no competing interests to declare that are relevant to the content of this article.

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