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Effects of Supplemental Feeding of Queen Rearing Colonies and Larval Age on the Acceptance of Grafted Larvae and Queen Traits

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Abstract: The research was carried out at the apiary of the Dept. of Animal Science, Faculty of Agriculture, Ankara University. Twelve rearing colonies were assigned to 4 feeding groups of syrup, syrup with pollen and syrup with vitamin mixture and control group on natural sources. Another colony was prepared as breeder to take grafting material. The effects of supplemental feeding and the age of larva on the acceptance rate and queen qualities were measured. In addition, the reproductive performances of light and heavy weight resultant queens were observed. It was found that supplemental feeding of rearing colonies improves the acceptance rate of grafted larvae ($p<0.05$). Larval age did not affect the acceptance rate. On the other hand, 2-day-old larvae were more readily accepted ($82.3\pm2.16\%$) than 1-day-old larvae ($73.4\pm4.56\%$). The live weights of queens after emergence were not influenced of feeding, whereas the queens reared from 1-day-old larvae (166.6 ± 1.74 mg) were significantly ($p<0.01$) heavier than those from 2-day-old larvae (160.8 ± 1.22 mg). The length and volume of queen cells were measured after emergence and found affected by feeding ($p<0.01$ and $p<0.05$) and larval age ($p<0.05$ and $p<0.01$), respectively. The reproductive performances of light and heavy weight queens were observed in colonies throughout the production season. The heavy weight queens produced more brood area (945 ± 114.0 cm^2) than the light weight queens (709 ± 93.1 cm^2). The difference between the averages of queen weight groups was significant ($p<0.05$). The heavy weight queens also built up larger colonies. The periodic changes both in brood areas and the numbers of combs covered with bees were significant ($p<0.01$).

Key words: honey bee, supplemental feeding, queen, grafting, brood area

Introduction

Honey bee colony traits, such as honey production, swarming, aggressiveness, etc. are the reflection of queen and her offspring. The queen, reproductive female, is the source of variation in the colony since she lays 1500-2000 eggs a day. The primary requisite for being successful in beekeeping is that the colonies should be headed by the young queens with high qualitative traits. No matter how well a colony is managed; it is not anticipated that the performance of the colony with older or inferior queen will be high. Besides, it is not only sufficient that the queen has superior heritable traits, but the queen also has to bear high egg laying performance to reflect her outstanding traits in her colony (Ruttner, 1988). Since the productivity and the stability of honey bee colonies largely depend on queens, this brings the obligation to control every stage of queen rearing instead of using queens reared haphazardly (Fyratly, 1988).

The quality of a queen is determined by her parents and by her rearing conditions. The criteria related to the queen quality are the traits such as weight at emergence, ovarian weight, number of ovarioles and the diameter and the volume of spermatheca. The relations among these traits and the relations of them with brood production, population increase as well as honey production were determined by several investigators (Abdellatif, 1967; Avetisyan *et al*., 1967; Zhadanova, 1967; Woyke, 1967, 1971; Fyratly, 1982; Nelson and Gary, 1983; Harbo, 1986). According to Weiss (1983), age of grafted larva, queen cell as a mechanical factor, nutritional factor and the external factors such as microclimate, supply of food, weather, climate and season are the rearing conditions affecting the quality of a queen.

One of the most important factors affecting queen quality is the age of larva in controlled queen rearing. Queens can be reared from worker larvae up to 3 days of age by grafting into artificial queen cell cups (Weiss, 1983). Although larvae at any age up to the end of the third day have the potential of being reproductive queens, the quality of queen decreases as the age of larva increases. According to Woyke (1967, 1971), each increase of 1 day in the age of larva grafted decreased the body weight, the diameter and the volume of spermatheca and the number of ovarioles of virgin queens. Rawash *et al*. (1983) found that of those reared virgin queens by grafting different aged larvae, the heaviest and the lightest queens were those from 1-day and 3-day old ones.

This experiment was conducted to test the effects of different diets of rearing colonies and the age of larvae grafted into artificial queen cell cups on the acceptance of grafted larvae and on the queen quality and performance before and after natural mating.

Materials and Methods

This experiment was carried out from April through October at the apiary of Dept. of Animal Science. Anatolian honey bee (*A. m. anatoliaca*) colonies with queens of 1 year old and the same strength were selected. Each colony occupied a brood chamber and one full-depth super was prepared as a starter-finisher colony as follows: the queen was removed and the whole colony was congested in the brood chamber. Two combs with eggs and younger larvae were picked out of brood chamber to provide empty space for grafting frame and for division board feeder.

The colonies were randomly assigned to one control and three experimental feeding groups of three colonies each: (A) one litre syrup (sugar: water, 1:1) each colony per day; (B) one litre syrup (sugar: water, 1:1) with 3 g vitamin mixture (vitamin A, 50000 IU; vitamin E, 30 mg; vitamin B_1, 150 mg; vitamin B_2, 100 mg; vitamin B_{12}, 1 mg; vitamin C, 3.270 mg; vitamin K_1, 50 mg; pantothenic acid, 400 mg; nicotineamid, 800 mg; folic acid, 100 mg; biotin, 2 mg; K_2HPO_4, 2.500 mg; additives, 92 mg) each colony per day; (C) one litre syrup

(sugar: water, 1:1) with 10 g. fresh pollen each colony per day; (D) the control group only on natural sources. The colonies were started feeding in division board feeders 3 days ahead of grafting until the queen cells were sealed.

An Anatolian breeder colony was selected from breeder stock in the apiary by evaluating colony records. The queen of breeder colony was confined on to 2 drawn empty combs by perpendicular queen excluders to provide larvae of known age for grafting. The successive replacement of combs at 1-day interval provided the necessary larvae of 1 and 2 days of age on the grafting day (Laidlaw, 1985).

A grafting frame of two bars carrying 16 artificial queen cell cups each was prepared for each starter-finisher colony. From each combs with 1-day and 2-day-old larvae, 16 larvae aged 1 day and 16 larvae aged 2 days were grafted into queen cell cups wetted with a drop of water: royal jelly (1:1) solution. The effect of location within starter-finisher colony (Visscher, 1986) was eliminated by distributing the queen cells containing larvae of different ages reciprocally as 8 cells on each side of the grafting bars.

One day before emergence of virgin queens, mature queen cells in each starter-finisher colony were transferred to the banking frames with individual chambers, where they emerged. The queens were weighed on the second day of emergence and the empty cells were kept to measure length and volume. Statistical analysis was applied to data of queen weights after emergence and 6 light and 6 heavy queens were selected randomly from those between 1 and 2 standard deviations above and below the mean. The selected queens were introduced to newly established 12 colonies with 2 combs covered with bees. After mating and egg laying of selected queens, brood areas of colonies headed by light and heavy weight queens were measured at 21 days intervals during the summer season until October to test their reproductive performances.

The data of acceptance rate, weight of virgin queens after emergence and the lengths and the volumes of empty queen cells in groups were analysed by ANOVA. As for reproductive performances of light and heavy weight queens, repeated measurements design was applied to data.

Results and Discussion

Acceptance rates of grafted larvae: The acceptance rates of grafted larvae by colonies are recorded in Table 1. Total of 384 larvae of 1 day and 2 days of age were grafted in 12 colonies assigned to 4 experimental groups. The acceptance percentages of larvae grafted were $72.9 \pm 5.98\%$, $85.4 \pm 1.33\%$, $83.3 \pm 4.48\%$ and $69.8 \pm 5.91\%$ in experimental groups A, B, C and D, respectively. The differences in acceptance rates in groups were statistically significant ($p < 0.05$).

The supplemental feeding of starter-finisher colonies affected the acceptance rate of larvae. It can be suggested that supplemental feeding of queen rearing colonies with vitamin mixture or pollen added syrup will increase the queen rearing activities. According to Stace and White (1994), the acceptance of queen cells after grafting and cell production per colony increased when queen rearing colonies were fed 4 g iso-leucine per week in sugar syrup.

The acceptance rates of 1-day and 2-day-old larvae were $73.4 \pm 4.56\%$ and $82.3 \pm 2.16\%$, respectively. The acceptance rate was not affected by larval age and the difference between them was not found statistically significant. However, it was clear that the 2-day-old larvae were more readily accepted than 1-day-old larvae.

Weights of virgin queens after emergence: Table 2 shows the weights of queens after emergence reared from 1-day and 2-day-old larvae. As seen in Table 2, the heaviest average weight of queens was obtained from 1-day-old larvae in group D (170.4 ± 4.71 mg) and the lightest from 2-day-old larvae in group C (157.0 ± 2.11 mg).

The weight of queens after emergence was influenced of larval age. The queens reared from 1-day-old larvae (166.6 ± 1.74 mg) were significantly ($p < 0.01$) heavier than the queens from 2-day-old larvae (160.8 ± 1.22 mg). On the other hand, supplemental feeding did not affect the weight of queens. The comparison of experimental groups with respect to queen weight showed that the differences were nonsignificant.

Krol *et al*. (1992) concluded that the queens reared in colonies receiving sugar syrup supplemented with vitamin B_1 one week prior to grafting had 11% greater body weight and 6% more ovarioles. Our results did not confirm this conclusion. The abundance of pollen sources in the experimental area at the grafting period may have concealed the impact of supplements in sugar syrup. On the other hand, the weight of queen was affected by age of larva. The queens reared from 1-day-old larvae were heavier than those reared from 2-day-old larvae.

The lengths and the volumes of queen cells: After emergence, the lengths and the volumes of empty queen cells were measured (Table 3, 4). The length of queen cells was affected both by larval age ($p < 0.05$) and by supplemental feeding ($p < 0.01$). Among experimental groups, group C had the longest cell (2.48 ± 0.030). As to the larval age, the cells of the queens from 1-day-old larvae (2.42 ± 0.036) were longer than that of queens from 2-day-old ones (2.34 ± 0.021). Nevertheless, the expectancy that the heavier queens emerge from the longer queen cells did not realise in this experiment and any correlation between queen weight and the length of queen cell was not found.

Reproductive performances of light and heavy weight queens: To determine the reproductive performances of queens, 6 heavy and 6 light weight queens, total 12 queens were selected and introduced to the newly established 12 nucleus colonies with 2 frames of worker bees. Although the heavy weight queens were randomly chosen regardless of feeding and age factors, the heavy weight queens came from 1-day-old larvae and the light weight queens from 2-day-old larvae. In groups with light and heavy weight queens, the number of combs covered with bees and brood areas of the colonies were determined during the summer season. The nucleus colonies were not fed during this period and built up through natural pollen and nectar sources. The numbers of combs covered with bees and brood areas were measured 9 times at 11 days intervals and 5 times at 21 days intervals from May 5 to September 9, 1998.

The light weight group was evaluated with missing data due to the queen loss in 1 colony just before the end of the term. Table 5 shows the numbers of combs covered with bees in groups with light and heavy weight queens at 9 measurement periods. There were statistically significant differences both between groups ($p < 0.05$) and between the periods ($p < 0.01$, $p < 0.05$, Table 6). The means of numbers of combs with bees of group A and group B were 2.4 ± 0.07 and 2.7 ± 0.08, respectively. The highest mean values (2.8 ± 0.17 and 3.5 ± 0.13) were reached at the 6^{th} period in group A and group B.

Brood areas of colonies were measured by using Puchta

Table 1: Grafting results by larval age in experimental groups

Groups	1-day-old larvae				2-day-old larvae				Total			
	n	AC	RC	AR (%)	n	AC	RC	AR (%)	N	AC	RC	AR (%)
A	3	30	18	64.5 ± 8.33	3	38	10	81.2 ± 6.27	6	68	28	72.9 ± 5.98[ef]
B	3	31	17	83.3 ± 2.10	3	38	10	87.5 ± 0.00	6	69	27	85.4 ± 1.33[e]
C	3	23	25	87.5 ± 7.22	3	37	11	79.1 ± 5.52	6	60	36	83.3 ± 4.48[e]
D	3	25	23	58.3 ± 5.50	3	38	10	81.2 ± 3.60	6	63	33	69.8 ± 5.91[f]
Total	12	109	83	73.4 ± 4.56	12	151	41	82.3 ± 2.16	24	260	124	77.8 ± 2.64

e, f; $p<0.05$; AC, no. of accepted cells in groups; RC, no. of rejected cells in groups; AR, acceptance rate (%) in groups

Table 2: The weights of virgin queens reared from 1-day and 2-day-old larvae after emergence (mg)

Groups	1-day-old larvae			2-day-old larvae			Total		
	$\bar{x}$	S $\bar{x}$	n	$\bar{x}$	S $\bar{x}$	n	$\bar{x}$	S $\bar{x}$	n
A	168.7	2.30	23	160.9	2.50	26	164.6	1.78	49
B	165.1	2.95	22	162.7	2.35	29	163.7	1.83	51
C	160.9	3.35	17	157.0	2.11	29	158.5	1.81	46
D	170.4	4.71	22	163.0	2.86	24	166.5	2.73	46
Total	166.6[e]	1.74	84	160.8[f]	1.22	108	163.3	1.04	192

e, f; $p<0.01$

Table 3: The lengths of empty queen cell cups from which virgin queens emerged in experimental groups (cm)

Groups	1-day-old larvae			2-day-old larvae			Total		
	$\bar{x}$	S $\bar{x}$	n	$\bar{x}$	S $\bar{x}$	n	$\bar{x}$	S $\bar{x}$	n
A	2.37	0.043	15	2.21	0.035	17	2.28[e]	0.030	32
B	2.22	0.143	11	2.33	0.042	19	2.29[e]	0.058	30
C	2.56	0.042	13	2.42	0.036	20	2.48[f]	0.030	33
D	2.49	0.041	15	2.42	0.030	12	2.46[f]	0.027	27
Total	2.42[E]	0.036	54	2.34[F]	0.021	68	2.38	0.020	122

e, f; $p<0.01$; E, F; $p<0.05$

Table 4: The volumes of empty queen cell cups from which virgin queens emerged in experimental groups (ml)

Groups	1-day-old larvae			2-day-old larvae			Total		
	$\bar{x}$	S $\bar{x}$	n	$\bar{x}$	S $\bar{x}$	n	$\bar{x}$	S $\bar{x}$	n
A	1.19	0.016	15	1.10	0.020	17	1.14[F]	0.015	32
B	1.24	0.018	11	1.18	0.019	19	1.20[E]	0.014	30
C	1.28	0.023	13	1.17	0.025	20	1.22[E]	0.020	33
D	1.26	0.172	15	1.17	0.019	12	1.22[E]	0.024	27
Total	1.24[e]	0.014	54	1.16[f]	0.011	68	1.193	9.678	122

e, f; $p<0.01$; E, F; $p<0.05$

Table 5: Descriptive statistics of the numbers of combs covered with bees in colonies headed by light and heavy weight queens

Groups	Group A with light queens			Group B with heavy queens			Total		
	$\bar{x}$	S $\bar{x}$	n	$\bar{x}$	S $\bar{x}$	n	$\bar{x}$	S $\bar{x}$	n
05.07.98 (1)	2.0	0.00	6	2.0	0.00	6	2.00	0.00	12
16.07.98 (2)	2.0	0.00	6	2.3	0.17	6	2.22	0.09	12
27.07.98 (3)	2.6	0.15	6	2.7	0.11	6	2.63	0.09	12
07.08.98 (4)	2.8	0.17	6	3.1	0.08	6	2.96	0.10	12
18.08.98 (5)	2.6	0.33	6	3.3	0.11	6	2.92	0.19	12
28.08.98 (6)	2.8	0.28	6	3.5	0.13	6	3.12	0.19	12
08.09.98 (7)	2.3	0.17	6	2.8	0.17	6	2.58	0.14	12
18.09.98 (8)	2.0	0.16	5	2.4	0.15	6	2.23	0.12	11
29.09.98 (9)	2.0	0.16	5	2.5	0.37	6	2.27	0.22	11
Total	2.4[e]	0.07	52	2.7[f]	0.08	54	2.54	0.06	106

e, f; $p<0.05$

Table 6: The result of Duncan's multiple range test for the comparison of periods in respect of the numbers of combs covered with bees

Periods	1-2	1-3	1-4	1-5	1-6	1-7	1-8	1-9	2-3	2-4	2-5	2-6	2-7	2-8	2-9	3-4	3-5	3-6
Differences		**	**	**	**	**			**	**	**	**	**			*		*
Periods	3-7	3-8	3-9	4-5	4-6	4-7	4-8	4-9	5-6	5-7	5-8	5-9	6-7	6-8	6-9	7-8	7-9	8-9
Differences		*	**				**	**			**	**	*	**	**	*	*	

*, $p<0.05$; **, $p<0.01$

Table 7: Descriptive statistics for brood area (cm^2) in groups A and B

Groups	Group A with light queens			Group B with heavy queens			Total		
	$\bar{x}$	S $\bar{x}$	n	$\bar{x}$	S $\bar{x}$	n	$\bar{x}$	S $\bar{x}$	n
16.07.98(1)	1212	99.4	6	1630	208.0	6	1421	127.0	12
0708.98 (2)	1107	200.0	6	1562	155.0	6	1334	139.0	12
28.08.98 (3)	493	91.1	6	743	89.5	6	618	71.5	12
18.09.98 (4)	322	89.5	5	439	40.8	6	386	47.4	11
10.10.98 (5)	275	102.0	5	351	40.5	6	317	50.0	11
Total	709^E	93.1	28	945^F	114.0	30	831	75.2	58

E, F; $p<0.05$

Table 8: The result of Duncan's multiple range test for the comparison of periods in respect of brood area

Periods	1-2	1-3	1-4	1-5	2-3	2-4	2-5	3-4	3-5	4-5
Differences		* *	* *	* *	* *	* *	* *	*	*	

*, $p<0.05$; **, $p<0.01$

method (Frasnaye and Lensky, 1961). First measurements were taken 2 weeks after the queens started laying. Table 7 shows the brood areas in groups with light and heavy weight queens at 5 measurement periods. The mean values of group A and group B were determined 709 ± 93.1 and 945 ± 114.0 cm^2, respectively and the difference between the group means was statistically significant ($p<0.05$) as well as the means of periods ($p<0.01$, $p<0.05$; Table 8). During pollen and nectar flow, brood areas of the colonies with heavy weight queens were larger than those with light weight queens. With decreasing pollen and nectar sources in nature, the queens of colonies also started to slow down egg laying activity.

In light queens, the onset of oviposition was reported to be 17 days after emergence and in heavier queens 10 days (Taranov, 1973). In this research, the exact onset of oviposition date of queens after emergence were not recorded, but it was observed that the queens in the light weight group started laying eggs later than the heavy weight queens. Furthermore, the light weight queens ceased laying eggs earlier than the heavy weight queens at the end of the season whereas the heavy weight queens laid more eggs and longer period than light weight queens.

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