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EFFECTS OF VITAMIN ADDITIVE DIETS ON COLONY FOUNDATION SUCCESS IN BUMBLEBEE, *Bombus terrestris*

Ayhan GOSTERIT, Gizem OYTUN CICEK

Department of Animal Science, Faculty of Agriculture, Süleyman Demirel University, 32260, Isparta, Turkey

Corresponding author e-mail: ayhangosterit@sdu.edu.tr

Abstract

The effects of vitamin additive diets on colony foundation success in Bombus terrestris were investigated in this experiment. A total of 120 artificially hibernated queens were used. Queens were randomly divided to four groups (30 queens for each group). Queens and their colonies were fed with different diets: standard sugar syrup and normal pollen (group 1), vitamin additive sugar syrup and normal pollen (group 2), standard sugar syrup and vitamin additive pollen (group 3) vitamin additive sugar syrup and vitamin additive pollen (group 4). No significant differences were found in egg laying and colony foundation ratio of queens among the experimental groups. However, vitamin addition to pollen or sugar syrup negatively affected the marketable colony production ratio. Marketable colony production ratios of queens were found 60.00%, 26.66%, 53.33%and 45.00% in four groups, respectively. While feeding with vitamin additive diet affected colony initiation time, other traits such as timing of first worker emergence, timing of gyne (young queen) production, timing of switch point and timing of competition point was not affected. Total numbers of individuals produced in colonies were also determined. Significant differences were found only in terms of total number of young queens (gynes) among the groups, but not total number of workers and males. Results showed that feeding with vitamin additive diet has not positive effect on colony development traits in B. terrestris.

Key words*: Bombus terrestris, colony development, feeding, vitamin.*

INTRODUCTION

Bumble bees which have economic and ecological importance also play a crucial role in the pollination of a wide variety of field, forage and fruit crops, particularly greenhouse crops. Bumblebee pollination reduces pollination labor costs and improves the quality and quantity of crops (Velthuis and van Doorn, 2006). There are about 250 species of bumble bees (Williams, 1998). Currently five species of bumble bees are reared commercially. The main commercially reared species is *Bombus terrestris*. The large-scale laboratory rearing of *B. terrestris* has been promoted around the world since 1985. Annually, more than one million *B. terrestris* colonies are commercially produced by over 30 producers worldwide (Gosterit and Gurel, 2016). The mass rearing of bumblebees includes some stages such as colony initiation, queen and male rearing, mating, and breaking of diapause. These stages are realized in controlled conditions for sustainable rearing. Knowledge and technological possibilities are needed for provide the required conditions and achieve mass rearing (Beekman and van Stratum, 2000; Kwon et al., 2003; Amin et al., 2007).

B. terrestris colonies show much variation in the number of workers, males, and gynes (young queens) produced (Duchateau and Velthuis, 1988). Significant variations are also seen in the colony production ratio and colony initiation time. These traits are important criteria in laboratory rearing of *B. terrestris.* Colony development characteristics are affected by different factors such as the genetic structure, environmental conditions, diseasesandparasites, and food quality (Riberio et al., 1996; Cnaani et al., 2000).

B. terrestris queens and their colonies are fed ad libitum with freshly thawed pollen which collected by honeybees and sugar solutions (50 Brix) in mass rearing (Riberio et al., 1996; Gosterit, 2016).

It is known that pollen quality affects the colony development. Pollen which containing high content of protein, amino acid and vitamin is preferred for successful rearing (Genissel et al., 2002; Baloğlu and Gurel, 2015).

In honeybees, *Apis mellifera* colonies are fed with supplementary food containing vitamin premix to stimulate the egg laying of queens, improve the brood rearing, obtain more yield, and prevent the diseases and stress (Herbert and Shimanuki, 1978; Kumova, 2000). This study was carried out to determine the effects of feeding with vitamin additive diets on colony foundation success in *B. terrestris.*

MATERIALS AND METHODS

A total of 120 laboratory reared which mated and hibernated *B. terrestris* queens were used in the study. Four experimental groups of queens (30 queens for each group) were established and queens of each group were fed ad libitum with different diets: standard sugar syrup and normal pollen (group 1), vitamin additive sugar syrup and normal pollen (group 2), standard sugar syrup and vitamin additive pollen (group 3) vitamin additive sugar syrup and vitamin additive pollen (group 4). Vitamin premix which used for supplementary feeding for honeybees (containing 500.000 IU Vitamin A, 50.000 IU Vitamin D3, 500 mg Vitamin E, 1.000 mg Vitamin C, 200 mg Vitamin B1, 250 mg Vitamin B2, 100 mg Vitamin B6, 0,5 mg Vitamin B12, 500 mg, 150 mg Vitamin K for its 100 g) was added to sugar syrup and pollen cake. The proportion of vitamin premix was 5‰ in pollen cake and sugar syrup.

Standard rearing procedure was followed for rear colonies (Gosterit and Gurel, 2016). All queens were placed in starting boxes and allowed to found colonies in a climate-controlled room (27–28 °C and 50 % RH). One callow *B. terrestris* worker was placed next to each queen to stimulate their egg laying. The nests were checked every day and the syrup and pollen were replaced or added when necessary. After the first worker emergence (beginning of social phase), the nests were transferred to the larger rearing boxes and colony development was controlled by daily observation.

Egg laying ratio, colony production ratio and marketable colony production ratio of queens were calculated. Queens that produced more than 10 workers were considered to produce colony, and colonies that reached 50 or more workers were considered to marketable. Developmental traits such as colony initiation time, timing of first worker emergence, timing of gyne (young queen) production, timing of switch point, timing of competition point, and total number of individuals were also determined.

Descriptive statistics of colony development traits were calculated. Data were square-root transformed and tested for normality before analysis. One-way analyses of variance were run to determine the effects of vitamin additive diets on colony development traits (Minitab Statistical Software, Version 16.2.4). Two-proportion *z*-tests were used to compare the percentages of the queens that laid eggs and produced 10 and 50 workers.

RESULTS AND DISCUSSIONS

In *B. terrestris*, egg laying and colony production ratios of queens are varied within a wide range (Velthuis and van Doorn, 2006; Baloglu and Gurel, 2015). In the present study, effects of feeding with pollen and sugar syrup containing vitamin premix on egg laying ratio, colony production ratio and marketable colony production ratio are shown in Table 1.

Table 1. Egg laying, colony production and marketable colony production ratios of queens (%) (a, b: P<0.01)

Experimental groups	N	Egg laying ratio	Colony production ratio	Marketable colony production ratio
Group 1	30	93.33	73.33	60.00[b]
Group 2	30	96.66	66.66	26.66[a]
Group 3	30	96.66	73.33	53.33[b]
Group 4	30	86.66	66.66	45.00[ab]

Feeding of queens with vitamin additive pollen and sugar syrup affected their marketable colony production ratio (P<0.01) but not egg laying and colony production ratios. In the study, 28 of 30 queens in group 1, 29 of 30 queens in group 2 and group 3, and 26 of 30

queens in group 4 laid eggs. While the queens fed with normal sugar syrup and normal pollen founded more marketable colony (60.00%), queens fed with vitamin additive sugar syrup and normal pollen founded less marketable colony (26.66%). On the other hand, marketable colony production ratio of queens fed with vitamin additive sugar syrup and vitamin additive pollen was found as 45.00%.

In *B. terrestris*, there are three main stage of colony development: colony initiation, switch point and competition point. The hibernated queen lays diploid eggs and produces the first workers in the first stage. The second stage is when the queen switches to laying haploid eggs concurrently with diploid eggs. The third stage (competition point) is characterized by oophagy by the founder queen, egg-robbing and attacks on the founder queen by workers (Duchateau and Velthuis, 1988). Egg-laying by workers also takes place in the third phase (Cnaani et al., 2000). Switch and competition points are determinative for colony life cycle. In the present experiment, no significant differences were determined between the groups in term of production time of sexuals (males and young queens), switch point and competition point (Table 2). Feeding with vitamin additive diets only affected the colony initiation time ($P<0.01$).

Table 2. Developmental characteristics of colonies founded by queens fed with vitamin additive diets (a, b: $P<0.01$)

Characteristics	Experimental groups	N	$\bar{x}$ ±s.e	min.	max.
Colonyinitiation time (days)	Group 1	28	12.64 ± 0.63 [b]	10	24
	Group 2	29	16.31 ± 1.17 [a]	10	37
	Group 3	29	12.44 ± 0.62 [b]	10	24
	Group 4	26	12.81 ± 0.44 [b]	10	17
First workeremergence (days)	Group 1	23	35.26 ± 1.04	24	48
	Group 2	21	36.86 ± 0.92	31	48
	Group 3	23	36.10 ± 0.81	31	45
	Group 4	21	36.47 ± 0.73	31	45
Timing of gyneproduction (days)	Group 1	16	12.78 ± 1.99	-5	22
	Group 2	6	8.67 ± 3.19	-5	19
	Group 3	13	11.46 ± 2.15	-5	22
	Group 4	12	11.50 ± 1.36	2	22
Switch point (days)	Group 1	14	0.00 ± 4.24	-32	21
	Group 2	10	-3.30 ± 3.79	-22	11
	Group 3	14	-8.07 ± 3.88	-36	21
	Group 4	15	-3.00 ± 2.94	-17	21
Competitionpoint (days)	Group 1	22	26.55 ± 1.12	18	42
	Group 2	13	27.54 ± 1.58	21	35
	Group 3	21	27.10 ± 1.10	18	35
	Group 4	20	27.40 ± 1.04	22	39

Table 3. Total number of individual produced in colonies founded by queens fed with vitamin additive diets

Diets	Colony traits	N	$\bar{x}$ ±s.e	min.	max.
Standard sugar syrup and normal pollen	Total number of workers	22	108.73 ± 6.51	58	160
	Total number of males	16	34.94 ± 7.57	2	110
	Total number of gynes	18	29.44 ± 4.55	3	60
Vitamin additive sugar syrup and normal pollen	Total number of workers	20	74.90 ± 11.10	13	183
	Total number of males	13	22.23 ± 5.34	6	70
	Total number of gynes	9	30.89 ± 5.95	8	60
Standard sugar syrup and vitamin additive pollen	Total number of workers	23	96.80 ± 11.10	8	230
	Total number of males	16	30.13 ± 6.24	3	87
	Total number of gynes	14	63.50 ± 9.59	11	126
Vitamin additive sugar syrup and vitamin additive pollen	Total number of workers	20	100.00 ± 13.00	30	225
	Total number of males	19	40.58 ± 7.17	3	111
	Total number of gynes	12	49.60 ± 11.00	2	123

Different factors such as split sex ratios, worker/larva ratios, food quality and quantity, and the diapause history of the founder queen affect the individual production (Duchateau and Velthuis, 1988; Duchateau et al., 2004; Gosterit and Gurel, 2009; Holland et al., 2013). The high number of workers, males and young queens (gynes) were produced in group 1 (108.73 ± 6.51), group 4 (40.58 ± 7.17) and group 3 (63.50 ± 9.59), respectively (Table 3).

CONCLUSIONS

Balanced nutrition is the most important dietary factor for effective growth in insects. Previous studies have shown that the origin of the pollen diet might influence the development and the reproductive capacities bumblebee colonies. Pollen which contains the some amino-acids, lipids, or vitamins affects the reproductive capacities of *B. terrestris* queens. It is known that supplementary feeding of honeybees with diets containing vitamin premix increases the some physiological properties. However, our results showed that standard diet is more effective than vitamin additive diets for colony development of *B. terrestris.*

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