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RESEARCH PAPER

The Effects of Sugar Syrup Enriched with Amino Acid Mixtures at Different Concentrations on Colony Population Dynamics and Development Characteristics of Bumble bee (*Bombus terrestris*)

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Abstract

In this study, we tested the effects of amino acid-supplemented diets on colony development performances in *Bombus terrestris*. A total of 75 queens artificially hibernated were randomly separated into three groups. These groups were fed with different diets: normal sugar syrups and normal pollen (Control), sugar syrup which contains recommended dose (for honey bees: 10 mL/L, Sucrose syrup) of amino acid supplement and normal pollen (10 mL/L), sugar syrup which contains twice higher recommended dose of amino acid supplement and normal pollen (20 mL/L). Some developmental traits of queens and their colonies were determined. According to our findings, there were no significant differences in any of the traits of colony development except the number of total individuals among the groups ($P<0.05$). Results showed that feeding with an amino acid-supplemented diet is not influencial on colony development traits in *B. terrestris.*

Introduction

Bumble bees are vital pollinators of wild flora and agricultural crops. *Bombus terrestris* is polylectic and the most commercially reared bumble bee species (Velthuis & van Doorn, 2006). In mass rearing of the *B. terrestris*, all life stages such as founding colonies from queens, rearing queens and males from colonies, enabling the virgin queens to mate, controlling the diapause period of mated queens, and ensuring that queens emerging from diapause are carried out under controlled conditions (Beekman & Stratum, 2000; Gosterit et al., 2009; Amin et al., 2010). Nevertheless, there are some losses in each of these stages in the rearing process, and these losses affect the colony founding success in suitable quality for pollination (Gosterit, 2011). Besides, even if all these stages are processed under the same conditions and in the same laboratory, there are many variations in colony development traits including the number of workers, queens, and males, colony initiation, colony production ratio, and competition and switch points. Genetic structure, environmental conditions, diseases and parasites, and food quality and food availability affect variations of these traits in mass rearing of *B. terrestris* (Riberio et al., 1996; Cnaani et al., 2000).

Food quality and availability are crucial for eusocial bees in terms of egg-laying of queens, improving brood rearing, obtaining more yield, and preventing diseases and stress, etc. (Herbert & Shimanuki, 1978; Brodschneider & Crailsheim, 2010; Gosterit & Cicek, 2017). In some harsh conditions in nature, honey bee colonies are fed artificially by using food supplements containing some ingredients such as vitamins and amino acids (Kumova, 2000). *B. terrestris* sometimes may face short-term food shortfalls just as honey bees. When harsh conditions occur, reduction of brood temperature and mobility in workers, increased brood developmental time, less and/or smaller individual production, and less sexual production may occur in bumble bees (Plowright & Pendrel 1977, Heinrich 1979, Sutcliffe & Plowright 1990, Schmid-Hempel & Schmid-Hempel 1998). Moreover, larvae ejection from the nest occurs in long-term food shortfalls (Plowright and Plowright 1999). To avoid this situation, queens and colonies of *B. terrestris* are fed *ad-libitum* with 50 Brix sugar syrup and honey bees-collected pollen in year-round rearing (Riberio et al., 1996; Gosterit, 2016).

Honey bee colonies are fed with different supplementary food to improve brood rearing, protect against disease, and increase yield. Investigation of the effects of this supplement on bumble bee colonies is also valuable for the sustainability of their rearing activities. This study aimed to determine the effects of amino acid-supplemented diets on colony development performances in *B. terrestris.*

Materials and Methods

In this study, 75 hibernated *Bombus terrestris* queens, purchased from commercial bumble bee supplier (Bio Group Antalya, Türkiye) were used. Queens and their colonies were reared under standard laboratory conditions (28 ± 1°C, 50 ± 5% R.HAs a supplemental food material, BeeTonic was added to sugar syrup according to its recommended dose (for honey bees: 10 ml/L Sucrose syrup). BeeTonic is a food supplement used for honey bee feeding and contains 20 different amino acids (choline chloride 2500 mg, glycine 34900 mg, methionine 2400 mg, histidine 1340 mg, lysine 5650 mg, hydroxylysine 2250 mg, inositol 2500 mg, hydroxyproline 18300 mg, leucine 5000 mg, phenylalanine 2390 mg, isoleucine 3740 mg, proline 20530 mg, alanine 12600 mg, serine 5090 mg, arginine 11500 mg, threonine 2830 mg, aspartic acid 6700 mg, tyrosine 890 mg, glutamic acid 15000 mg, and valine 3740 mg). These queens were randomly assigned into three groups containing 25 queens feeding with standard sugar syrup and pollen (Control), feeding with sugar syrup containing recommended dose of the amino acid supplement and normal pollen (10 mL/L), feeding with sugar syrup containing twice higher recommended dose of amino acid supplement and normal pollen (20 mL/L). Supplemental food material was mixed with 50 Brix sucrose syrup, and queens and their colonies were fed with their assigned diets *ad-libitum*. Queens were transferred to starting boxes (8x8x6 cm) allowed to begin the colony founding process. After the first workers emerged, colonies were moved into larger rearing boxes (26x23x14 cm). Colony developmental characteristics were observed twice a week periodically. Egg-laying ratio, colony production ratio and marketable colony production ratio of queens, colony initiation (time of the first egg-lay) time, the timing of first worker emergence, the timing of the young male and queen production, the timing of switch point and competition point, and the total number of individuals were recorded. Queens that produced more than 10 workers were considered to produce colonies, and colonies that had 50 or more workers were deemed marketable (Gosterit & Cicek, 2017).

Descriptive statistics of parameters were analyzed in Minitab Statistical Software. Parameters were tested for normality. One-Way ANOVA analyses were run to determine the effects of amino acid-supplemented diets on development characteristics. Two proportion z-tests were used to compare the percentages of the queens that laid eggs and produced 10 and 50 workers.

Results and Discussion

The ratio of egg-laying, colony founding, and marketable colony have a wide range of variations reported in previous studies in *Bombus terrestris* (Velthuis & van Doorn, 2006; Baloglu & Gurel, 2015; Gosterit & Gurel, 2016). The effects of feeding with amino acid-supplemented diets on egg-laying ratio, colony founding ratio, and marketable colony ratio were given in Table 1. According to our results, there were no significant differences among experiment groups for each characteristic. The highest egg-laying ratio and colony founding ratio were determined in the control group (96% and 88%, respectively), while the highest marketable colony production ratio was in 20 mL/L (76%).

Table 1. Ratios of egg-laying, colony founding, and marketable colony production (%)

Groups	N	Egg-laying	Colony founding	Marketable colony
Control	25	96.00	88.00	64.00
10 mL/L	25	84.00	72.00	64.00
20 mL/L	25	92.00	84.00	76.00

There are three crucial stages for colonies of *B. terrestris*: colony initiation, switch point, and competition point. In the first stage, the queen (founder queen) lays eggs, and the first workers emerge from these eggs (beginning of social phase). Switch point, the second stage, is that the queen changes her reproductive strategies and starts laying haploid eggs (males) instead of diploid eggs (females). Egg-robbing and conflict between workers and the founder queen are seen in the competition point, the last stage (Duchateau & Velthuis, 1988; Gurel et al., 2008). Switch and competition points indicate that the end of colony life is approaching. When these stages are observed in detail, the production time of individuals and marketable colonies are also important for sustainable mass rearing success. In this study, the findings belonging to mentioned developmental characteristics were given in Table 2. The results showed that no significant differences were found among the groups in terms of colony developmental characteristics.

Table 2. Some developmental characteristics of queens and colonies fed with amino acids supplements

Characteristics (days)	Groups	N	$\bar{x} \pm s.d$	*P* value
Colony initiation time	**Control**	24	10.54 ± 4.12	0.650
	10 mL/L	21	10.38 ± 4.78	
	20 mL/L	23	9.48 ± 0.76	
Timing of first workers emergence	**Control**	22	31.86 ± 4.29	0.870
	10 mL/L	18	31.89 ± 5.04	
	20 mL/L	22	31.23 ± 4.60	
Timing of first male emergence	**Control**	17	72.06 ± 4.70	0.769
	10 mL/L	16	70.76 ± 6.58	
	20 mL/L	18	72.00 ± 6.07	
Timing of young queen emergence	**Control**	16	43.29 ± 5.98	0.549
	10 mL/L	17	45.00 ± 5.83	
	20 mL/L	19	45.21 ± 5.07	
Competition point	**Control**	17	29.53 ± 4.96	0.725
	10 mL/L	16	28.94 ± 4.80	
	20 mL/L	18	30.22 ± 4.22	
Switch point	**Control**	16	16.06 ± 4.28	0.537
	10 mL/L	17	13.88 ± 7.46	
	20 mL/L	16	15.31 ± 4.63	
Timing of marketable colony production	**Control**	16	58.19 ± 3.25	0.587
	10 mL/L	16	59.56 ± 9.00	
	20 mL/L	19	57.42 ± 4.83	

According to the findings of previous studies, founder queens are affected by various factors such as food quality and quantity, worker/larva ratios, etc. (Duchateau et al., 2004; Holland et al., 2013). Gosterit and Cicek (2017) investigated the effects of pollen and syrup which include vitamin supplements on colony developmental characteristics in bumble bees. According to their reporting, it was determined that feeding with a diet supplemented with vitamins did not have a positive effect on the colony developmental characteristics of *B. terrestris*, but there was a significant difference in the number of young queens. In this study, the number of egg cells in the first brood, the number of workers in the first brood, and the total number of workers, males, young queens, and the total number of individuals were determined (Table 3). Our results showed that there were no significant differences among the experimental groups except in the total number of individuals ($P<0.05$).

Table 3. Some characteristics in the colonies fed with amino acids supplements

Characteristics	Groups	N	$\bar{x} \pm s.d$	*P* value
Numbers of egg cells in first brood	**Control**	24	3.625 ± 0.275	0.377
	10 mL/L	21	3.429 ± 1.207	
	20 mL/L	23	3.957 ± 1.224	
Number of workers in first brood	**Control**	22	8.500 ± 2.988	0.158
	10 mL/L	18	7.778 ± 3.209	
	20 mL/L	22	6.727 ± 2.914	
Total number of workers	**Control**	22	156.40 ± 73.50	0.192
	10 mL/L	18	183.60 ± 49.40	
	20 mL/L	21	189.50 ± 60.30	
Total number of males	**Control**	16	89.80 ± 44.90	0.050
	10 mL/L	17	128.50 ± 50.80	
	20 mL/L	18	125.00 ± 48.80	
Total number of young queens	**Control**	17	90.41 ± 35.42	0.089
	10 mL/L	16	100.10 ± 48.80	
	20 mL/L	19	128.30 ± 66.50	
Total number of individuals	**Control**	22	291.60 ± 154.60 b	0.020
	10 mL/L	18	393.80 ± 125.30 ab	
	20 mL/L	21	412.80 ± 155.50 a	

For each characteristic, means followed by different letters (a, b) in the same column are different for each characteristic ($P<0.05$)

Data on sex (queen and male) production strategies in colonies produced in the study groups were given in Figure 1. Gosterit (2011) and Gosterit and Baskar (2016) categorized the reproductive strategies of bumble bees into four groups: colonies that produce only males, produce only queens, produce both queens and males, and produce neither queen nor male bees in their studies. According to our results, all reproductive strategies belonging to bumble bees were observed in this study.

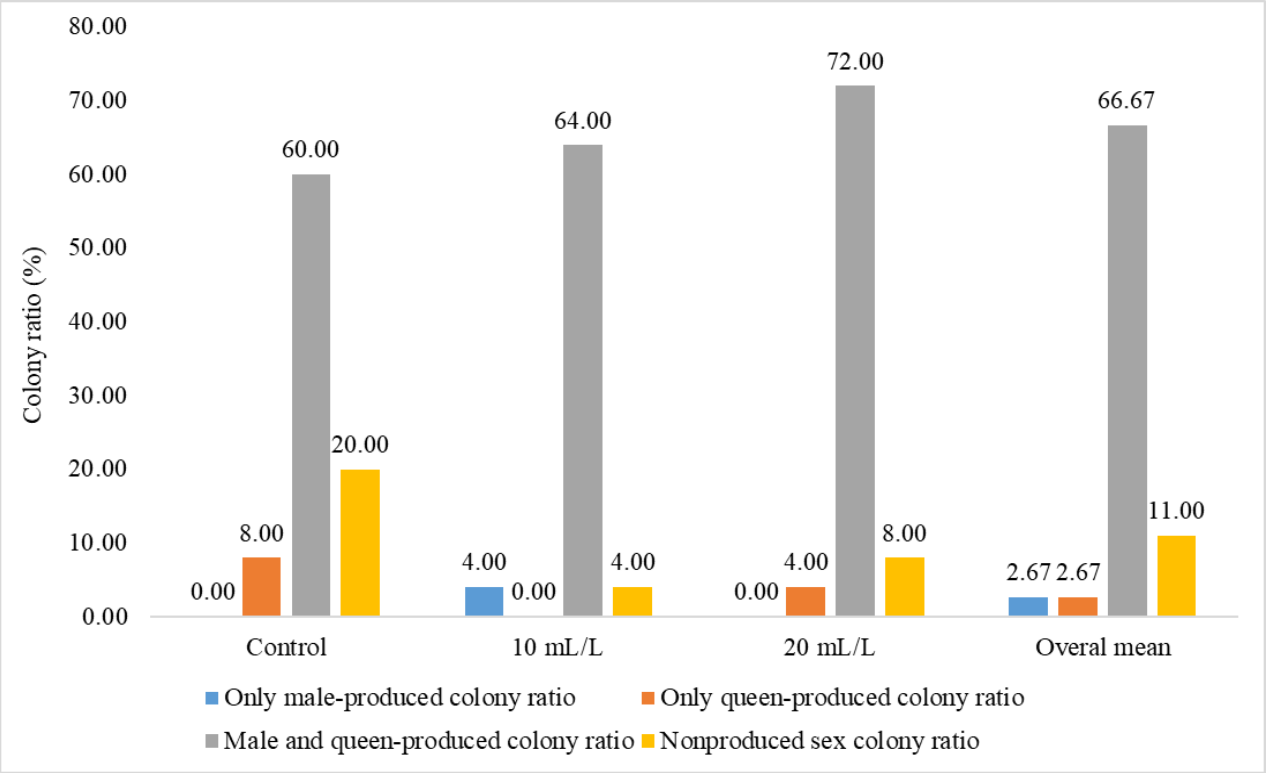

Figure 1. Sex production strategies in colonies

Conclusion

Our results have shown that feeding with the amino acid mix is not effective for bumble bees. When twice the recommended dose of amino acid supplement (BeeTonic) was added to the sugar syrup and pollen, a margin of 20% was observed in the total number of males and queens in comparison with the control groups. While this margin is not statistically significant, it should not be overlooked considering it affects the colony productivity ratio and efficient use of resources for sustainable Bombus terrestris mass rearing. For this reason, it becomes important to investigate even the smallest factor that may affect the success of the mass rearing process of bumble bees.

Ethical Statement

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Author 1: Investigation, Writing – review & editing,
Author 2: Investigation, Writing – review & editing; Supervision, Formal Analysis
Author 3: Methodology, Writing – review & editing; Supervision, Formal Analysis

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