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# RESEARCH PAPER

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# Influence of vitamin C in sugar syrup on brood area, colony population, body weight and protein in honey bees

M. Amiri Andi<sup>1\*</sup>, A. Ahmadi<sup>1</sup>

Department of Animal Sciences, Faculty of Agriculture, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran

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#### **Abstract**

In this investigation effects of different levels of vitamin C in sugar syrup on the rate of queen laying, colony population and body weight and protein in honey bee (*Apismellifera* L.) were studied. Experimental colonies had the same age queens and the same population and fed with sugar syrup (50 percent sugar) in three levels 2000, 4000, 6000 mg/L syrup—soluble vitamin C while the control group fed only with sugar syrup (treatment 1 control, treatment 2, 3 and 4, respectively 2000, 4000 and 6000 mg/L vitamin C) were compared. In this experiment feeding colonies for 60 days in May and June (the first 45 days of feeding every second day and the other without feeding-period of 15 days) were done. The highest average brood area was in treatment 2 with 2000 mg/L vitamin C (9049 cm²) while the lowest one was in treatment 1 (control) (4848 cm²), respectively (p<0.05). Mean colony population in treatment 4 was higher than control (10.41 vs. 8.38 comb, respectively) (p<0.05). The highest mean percent of protein was in treatment 2 (17.5 percent) while the lowest was in treatment 3 (14.18 percent) (p<0.05). Worker bees in treatment 4 had the greater mean body weight than other groups (p<0.05). The results indicated that supplementing the level of vitamin C to spring nutrition (1:1sugar syrup) to the colonies, increase the brood area, colony population and the workers body weight and protein.

<sup>\*</sup>Corresponding Author: M. Amiri Andi 🖂 m-amiriandi@iausdj.ac.ir

#### Introduction

The primary source of nutrients specially vitamins, in the feed of the honey bee, *Apismellifera* L. is the pollen. Insufficient or poor quality of pollen leads in stunted growth and gain of young bees and reduced longevity (Hays, 1984; Standifer *et al.* 1977). For this reason, supplementary feeding of honey bees with protein feeds (Al-Naji, 1980; Graham, 1993; Stephen and Pernal, 2000; Zahra and Talal, 2008) and vitamin-mixed feeds (Heydak and Dietz, 1965; Pain, 1956) is recommended.

Honey bees (or their symbiotic microorganisms) are assumed to be able to synthesize vitamin C (Herbert et al., 1985). Pain (1956) indicated that vitamins supplemented to the workers honey bee feed had no effect on the longevity of honey bees. Heydak and Dietz (1965) resulted that for brood rearing activity vitamins are not essential. But Zahra and Talal (2008) found that multi vitamins and especially vitamin C affect brood area and hypopharyngeal glands development in honey bees. Vitamin C is need for protein synthesis. On the other hand, Fluri and Bogdanov (1987) and Imdore et al. (1988) indicated the importance of body protein in honey bee. In addition, Otis et al., (2004) documented that storage body proteins affect wintering of honey bees and life span. In spite of above explanations, there is no research about the effects of supplemental vitamin C on body protein of worker honey bees.

The objectives of this investigation were to evaluate the effects of supplemental vitamin C in sugar syrup on brood area, colony population, body weight and protein in honey bees (*Apismellifera* L.).

#### Material and methods

Experimental Colonies

Twenty eight experimental colonies of *Apismellifera* L. were selected randomly from a private apiary in a mountainous area in Alborz province, Iran. Capped brood combs without workers were exchanged between colonies, before the initial of the experiment. This meant that all experimental colonies included

seven combs (20\*40 cm) with a similar number of workers and amount of brood.

### Experimental Treatments

Four levels of vitamin C (0, 2000, 4000 and 6000 mg/L) in sugar syrup 100% (w/v) were tested during spring and summer seasons. Pure vitamin C prepared from CanaVit® (Canadian Feed Additives Inc). The feeding started 30 April 2011 and lasted 15. Colonies were fed graded levels of vitamin C in syrup skip 2 day. Each colony fed 500 ml of supplemented syrup at the end of the day before sunset. Feeders were removed the next morning.

Preparing the sugar syrup solution with vitamin C In control group no vitamin C added to the sugar syrup (o mg/L), then 3.5 L sugar syrup 100% distributed equally among 7 colonies (o.5 L per each colony). In second treatment 7000 mg (7 g) pure vitamin C dissolved in 3.5 L sugar syrup and this solution fed to the colonies of this group (o.5 L per each colony). The third solution had 14000 mg (14 g) vitamin C in 3.5 L sugar syrup for feeding of the colonies of this treatment (o.5 L per each colony). The vitamin C content in the fourth experimental sugar solution was 21 g per 3.5 L that distributed among 7 colonies of this group.

# Measurements and samples collection

Measurements and samples collection was done in 4 times, in dates of April 30, May 31, June 15 and July 1 2011. The brood area was calculated using the standard Langstroth frame divided by silk into squares of 1 inch<sup>2</sup>. The Langstroth frame was divided by silk into squares of 1 inch<sup>2</sup>. Colony population was estimated by number of combs. For measuring of body weight and 50 to 60 workers were randomly collected in a nylon bag and freeze. These bees were weighed by a scale with sensitivity of 0.001 g. After dehydration of freezing bees in an oven with temperature of 90 °C, they grind. Crude protein measured by Kjeldahl method (as nitrogen×6.25).

# Statistical analysis

All data were analyzed using the One Way ANOVA

procedure of SAS (SAS Institute Inc, 1996) for analysis of variance. Significant differences among treatments were identified at  $\alpha$  = 0.05 level by Duncan's multiple range tests.

# Results

Brood Area

Effect of vitamin C in sugar syrup on brood area (cm<sup>2</sup>) in honey bees were indicated in Table 1. The highest average brood area was in treatment 2 with 2000 mg/L vitamin C (9049 cm<sup>2</sup>) while the lowest one was in treatment 1 (control) (4848 cm<sup>2</sup>), respectively (p<0.05).

Table 1. Effect of vitamin C in sugar syrup on brood area (cm<sup>2</sup>) in honey bees.

Contents of vitamin C		Average numb	average		
(mg/L) in sugar syrup	April 30	May 31	June 15	July 1	
0	4393	4982ª	4982ª	5036	4848 <sup>a</sup>
2000	4286	10375 <sup>b</sup>	14786 <sup>b</sup>	6750	9049 <sup>b</sup>
4000	4714	$7553^{ m ab}$	6321 <sup>a</sup>	6214	6200 <sup>a</sup>
6000	4392	7017 <sup>ab</sup>	8357 <sup>a</sup>	6589	6589ª
SEM	487	1327	1264	1226	832

a-b: Means in each column with different superscript are significant different (p≤0.05).

#### Colony Population

Data of colony population were shown in Table 2. Mean colony population in treatment 4 was higher than control (10.41 vs. 8.38 comb, respectively) (p<0.05).

#### **Body Protein**

In Table 3 the observations of body protein of workers' honey bee were indicated. The highest mean percent of protein was in treatment 2 (17.5 percent) while the lowest was in treatment 3 (14.18 percent) (p<0.05).

**Table 2.** Effect of vitamin C in sugar syrup on colony population (comb) in honey bees.

Contents of vitamin C Average number of combs					- OVOTO GO
(mg/L) in sugar syrup	April 30	May 31	June 15	July 1	average
0	7.00	8.29 <sup>a</sup>	9.36ª	8.86a	8.38a
2000	7.00	8.64 <sup>b</sup>	10.21 <sup>ab</sup>	10.29 <sup>ab</sup>	9.04 <sup>ab</sup>
4000	7.00	10.29 <sup>ab</sup>	10.50 <sup>ab</sup>	10.79 <sup>ab</sup>	9.64 <sup>ab</sup>
6000	7.00	$10.93^{\rm b}$	11.71 <sup>b</sup>	$12.00^{\rm b}$	10.41 <sup>b</sup>
SEM	0.31	0.73	0.72	0.92	0.62

a-b: Means in each column with different superscript are significant different (p≤0.05).

# Body Weight

The effects of vitamin C in sugar syrup on body weight (mg) in honey bees were indicated in Table 4. Worker bees in treatment 4 had the greater mean body weight than other groups (p<0.05).

### Discussion and conclusion

Supplementary feeding of honey bees with protein feeds (Al-Naji, 1980; Graham, 1993; Stephen and Pernal, 2000; Zahra and Talal, 2008), vitamin-mixed

feeds (Herbert *et al.* 1985; Haydak and Dietz, 1965; Pain, 1956) and carbohydrate source (Lehner, 1983) is recommended.

Based on many literatures it seems that vitamin C has a positive impact on brood rearing in honey bees (Herbert *et al.* 1985; Zahra and Talal, 2008). Zahra and Talal (2008) demonstrated that vitamin C (250 mg/1.5 L sugar syrup) had advantages for brood rearing and hypopharyngeal glands' development. In

our experiment vitamin C in the level of 2000 mg/L had greater effect on brood rearing area which in agree with those found by Ewies and Ali (1977),

Herbert *et al.* (1985), Haydak (1945) and Zahra and Talal (2008). In parallel of these findings, vitamin C had positive effects on colony population.

**Table 3.** Effect of vitamin C in sugar syrup on body protein (% body) in honey bees.

Contents of vitamin C (mg/L)	Average protein content (%)				average
in sugar syrup	April 30	May 31	June 15	July 1	
0	12.66	13.02 <sup>ab</sup>	14.49 <sup>a</sup>	19.95	15.03 <sup>ab</sup>
2000	12.66	14.53 <sup>b</sup>	21.41 <sup>b</sup>	20.00	17.15°
4000	12.66	12.55 <sup>ab</sup>	13.01 <sup>a</sup>	18.51	14.18 <sup>a</sup>
6000	12.66	12.11 <sup>a</sup>	18.80 <sup>b</sup>	20.19	15.94 <sup>bc</sup>
SEM	0.45	0.68	1.07	1.25	0.49

<sup>&</sup>lt;sup>a-c:</sup> Means in each column with different superscript are significant different (p≤0.05).

Fluri and Bogdanov (1987) and Imdore *et al.* (1988) indicated the importance of body protein in honey bee. In addition, Otis *et al.* (2004) documented that storage body proteins affect wintering of honey bees and life span. In spite of above explanations, there is no research about the effects of supplemental vitamin

C on body protein and weight of worker honey bees. Vitamin C is need for protein synthesis, therefore it can help body content of protein and body weight. In our investigation the positive effects of vitamin C on body weight and protein were indicated.

Table 4. Effect of vitamin C in sugar syrup on body weight (mg) in honey bees.

Contents of vitamin C (mg/L) in		Average body weight (mg)			average
sugar syrup	April 30	May 31	June 15	July 1	_
0	100.00	106.14	99.86ª	97.00 <sup>a</sup>	100.75 <sup>a</sup>
2000	100.00	113.86	104.00 <sup>ab</sup>	93.57 <sup>a</sup>	102.86 <sup>ab</sup>
4000	100.00	105.43	103.71 <sup>ab</sup>	88.00 <sup>a</sup>	99.29 <sup>a</sup>
6000	100.00	112.14	115.87 <sup>b</sup>	112.00 <sup>b</sup>	110.00 <sup>b</sup>
SEM	1.95	5.92	4.04	3.83	2.21

a-b: Means in each column with different superscript are significant different (p≤0.05).

The results indicated that supplementing the level of vitamin C to spring nutrition (1:1suger syrup) to the colonies, increase the brood area, colony population and the workers body weight and protein.

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