

Effect of proline as a nutrient on hypopharyngeal glands during development of *Apis mellifera* (Hymenoptera: Apidae)

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Abstract

Proline is known to be an energy source for protein synthesis and appears to have a major role in insect flying metabolism. Insects can detect proline in their food and use it as an energy substrate to start flight and other high energy consuming activities. Honey bee has a feeding preference for nectars with higher concentrations of this amino acid. In this research we present evidence that L-proline can be utilized as a phagostimulant for the honeybee worker (*Apis mellifera*). We reported the L-proline increase hypopharyngeal glands acini diameter and syrup consumption at the experimental cage. Honeybee workers fed on 1000 ppm treatment proline consumed 773.9 ± 31.8 μ l/bee after 18-days. It is obvious that the honeybee workers consumed 1000 ppm the more than other treatment. The feeding decreased when concentration of L-proline increased to 10000 ppm. The hypopharyngeal glands development increased gradually from honeybee workers emergence and started to decrease after 9 days old. The maximum acini diameter (0.1439 ± 0.001 mm) was recorded in the 9th day when newly emerged bees were fed on 1000 ppm proline syrup.

Keywords acini diameter, *Apis mellifera*, hypopharyngeal glands, L-proline.

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1 Introduction

Proline is known to be an energy source for protein synthesis in invertebrates (Osanai and Yonezawa, 1986). This amino acid appears to have a major role in insect flying metabolism (Sacktor and Childress, 1975; Bursell et al., 1974). Many insects contain a high concentration of proline in their haemolymph (Wyatt, 1961). The existence of relatively high amounts of proline has been reported in insect structural proteins isolated from cuticle, vitelline membranes (Andersen, 1979), spermatophore of some insect (Shinbo et al., 1987).

It is assumed that high concentration of L-proline in honey bee bread has phagostimulatory effect for honey bee diet (Degrandi-Hoffman et al. 2013). Insects can detect proline in their food and use it as an energy substrate to start flight and other high energy consuming activities (Brosemer and Veerabhadrapa, 1965;

Crabtree and Newsholme, 1970; Carter et al., 2006). Honey bee has a feeding preference for nectars with higher concentrations of this amino acid (Carter et al., 2006; Bertazzini et al., 2010).

The hypopharyngeal glands are age-dependent structures of the worker honey bee, *Apis mellifera*, acinous in form, are distributed in many loops and coils in the sides of the head especially in the region just behind the eyes. They are also the largest glands in the head cavity. The glands are composed of 1000 or more pear-shaped lobules attached to a long duct (Cheshire, 1886). Each lobule composed of groups of many unicellular glands. Royal jelly, which is fed to the larvae, queen, and drones, is secreted by the hypopharyngeal glands. Thus, the secretory activity and/or development of the hypopharyngeal glands directly affect the workers behavior (Free, 1961) and indirectly may affect the strength of the colony (Farrar, 1947; Moeller, 1962; Malone et al., 2004; Al-Ghamdi et al., 2011).

The diameters of hypopharyngeal glands are often used to describe the physiological status of worker honey bees. The normal course of development of these glands (size of acini) is well described (Maurizio, 1954; Simpson et al., 1968; Moritz and Crailsheim, 1987; Crailsheim and Stolberg, 1989; Al-Ghamdi et al., 2011). The hypopharyngeal glands are well developed when bees are nursed and degenerate when bees become foragers at normal condition which depends on the age of workers, the colony conditions and the time of the year. The effect of worker age on the hypopharyngeal glands development was examined by Huang and Otis (1989). Feeds containing protein additives had longer acini in the lobules of the hypopharyngeal glands. Also, pollen consumption is positively correlated with gland development (Crailsheim and Stolberg, 1989; Hrasnigg and Crailsheim, 1998).

Alqarni (2006) evaluated some protein rich diets for supplementary feeding of honeybee. The highest rate of food consumption was recorded with improved traditional substitute followed by mixture from date palm pollen and soybean flour. The bee bread or date palm pollen was the best sources for hypopharyngeal glands activation. The aim of the present study was to determine the potentiality of using L-proline in feeding honeybee workers under laboratory condition for developing the hypopharyngeal glands.

2 Materials and Methods

2.1 Bees

The experimental work was carried in Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran during the period June to September 2014. Local honey bees were used in the present study to investigate morphometrical characters of honey bee hypopharyngeal glands under different L-proline concentration feeding.

2.2 Experimental cage

The experiment was carried out in cages using newly emerged honeybee workers (0–24 h). Experimental wooden cages (9*18*14 cm dimensions) were used in the experiments that had three apertures for water, syrup and food. Each cage was provided with a vial of water and another vial for sugar solution (1 M) in addition to a piece of wax comb attached in the top of the cage. Thirty cages (each contains 100±20 workers) were used for determining the feeding and studying the hypopharyngeal glands acini diameter. Different concentrations (0, 10, 100, 1000 and 10000) were introduced for each cage in small vial of sugar solution. The syrups were changed every 3 days. All cages were held in the darkness in an incubator at 32±2 C° and 65±5 % RH.

2.3 Hypopharyngeal glands dissection

Hypopharyngeal glands were dissected to determine acini diameter after 3, 6, 9, 12, 15 and 18 days old. The glands were dissected from the head capsule and placed into a drop of saline (0.25 mol/l NaCl) and the mean length of acini were measured in mm for each worker under binocular with the help of DinoCapture eye and using DinoCapture 2.0 Software (Electronics Corporation).

2.4 Statistical analysis

Statistical differences between hypopharyngeal mean glands (acinal diameter) were compared using ANOVA (analysis of variance) and Duncan's multiple-range test (DMRT) when significant differences were found at $P = 0.05$ using the SPSS 22.0 statistical package.

3 Results

3.1 Syrup consumption

Data presented in Table 1 show the cumulative syrup consumption ($\mu\text{l}/\text{bee}$) of honey bees from different L-proline with the control during the cage experiment. Honey bee workers fed on 1000 ppm prolin treatment consumed $773.9 \pm 31.8 \mu\text{l}/\text{bee}$ after 18 days. As shown in Table 1, it is obvious that the honeybee workers consumed 1000 ppm more than other treatments. The feeding decreased when concentration of L-proline increased to 10000 ppm.

Table 1 Amounts of L-proline Treatments consumed during nursing periods of honeybee workers.

NO.	L-proline Treatments	Cumulative feeding ($\mu\text{l}/\text{bee}$) during development of honeybee the cage experiment					General means
		6-days old	9-days old	12-days old	15-days old	18-days old	
1	Control (sugar (1M))	144.6 ± 21.1	223.4 ± 33.3	285.6 ± 42.5	373.3 ± 50.1	593.3 ± 69.1	324.0 ± 43.2
2	10 ppm + sugar (1M)	144.3 ± 13.1	247.5 ± 27.6	305.3 ± 31.2	398.6 ± 54.4	657.6 ± 62.2	356.6 ± 37.7
3	100 ppm + sugar (1M)	167.6 ± 29.4	288.8 ± 33.2	323.4 ± 42.1	428.3 ± 61.8	647.3 ± 66.6	371.0 ± 46.6
4	1000 ppm + sugar (1M)	267.3 ± 33.1	413.9 ± 34.1	558.0 ± 40.1	612.3 ± 62.7	773.9 ± 31.8	525.0 ± 40.3
5	10000 ppm + sugar (1M)	188.3 ± 27.7	256.3 ± 46.1	313.3 ± 49.9	417.3 ± 62.1	668.7 ± 61.8	368.7 ± 49.5

3.2 Hypopharyngeal gland development

The differences of hypopharyngeal glands in honeybee workers reared under different treatment in experimental cage conditions were studied. Diameter of glands acini in worker bees depended on the honey bee's age and sizes of the glands increased from 1st day, remained at a high level until the 9th day and decreased afterwards (Fig. 1).

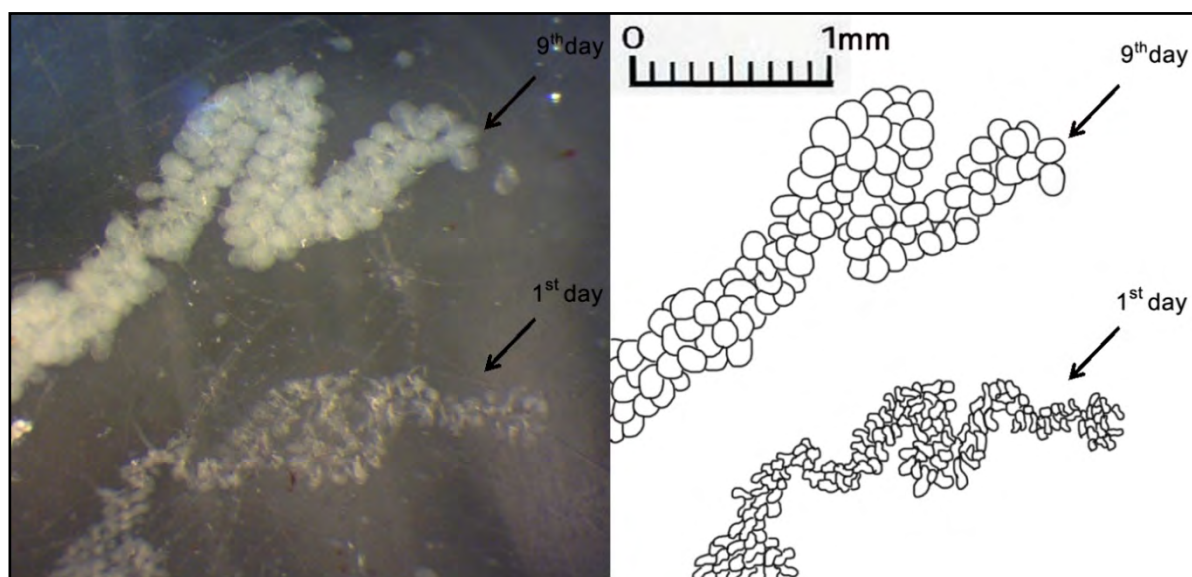


Fig. 1 Hypopharyngeal glands development of honeybee workers in the 1st and 9th days old.

Data represented in Table 2 indicates that the acini diameter of hypopharyngeal glands was influenced with increasing age. The hypopharyngeal glands acini diameter increased gradually from honeybee workers emergence and started to decrease after 9 days old. The maximum acini diameter (0.1439 ± 0.001 mm) was recorded in the 9th day when newly emerged bees were fed on 1000 ppm proline syrup.

Table 2 Development of hypopharyngeal glands acini diameter of honeybee workers of different age.

NO.	L-proline Treatments	Means of hypopharyngeal gland acini diameter (mm) under feeding with different treatment during nursing period of honeybee workers.					General means
		3-days old	6-days old	9-days old	12-days old	15-days old	
1	Control (sugar (1M))	0.0743±0.006	0.1043±0.003	0.1332±0.002	0.0981±0.005	0.0748±0.002	123.33±2.11
2	10 ppm + sugar (1M)	0.0815±0.005	0.0993±0.002	0.1399±0.001	0.1111±0.004	0.0837±0.004	123.33±2.11
3	100 ppm + sugar (1M)	0.0849±0.007	0.1217±0.002	0.1439±0.001	0.1234±0.004	0.0982±0.002	123.33±2.11
4	1000 ppm + sugar (1M)	0.0865±0.008	0.1318±0.003	0.1576±0.003	0.1335±0.007	0.0976±0.003	123.33±2.11
5	10000 ppm + sugar (1M)	0.0798±0.005	0.0978±0.004	0.1116±0.002	0.1097±0.002	0.0887±0.007	123.33±2.11

The diameters of the glands acini in worker bees depended on the bees age (Fig. 2). In this experiment the glands were already well developed when bees were 6 and 9 days.

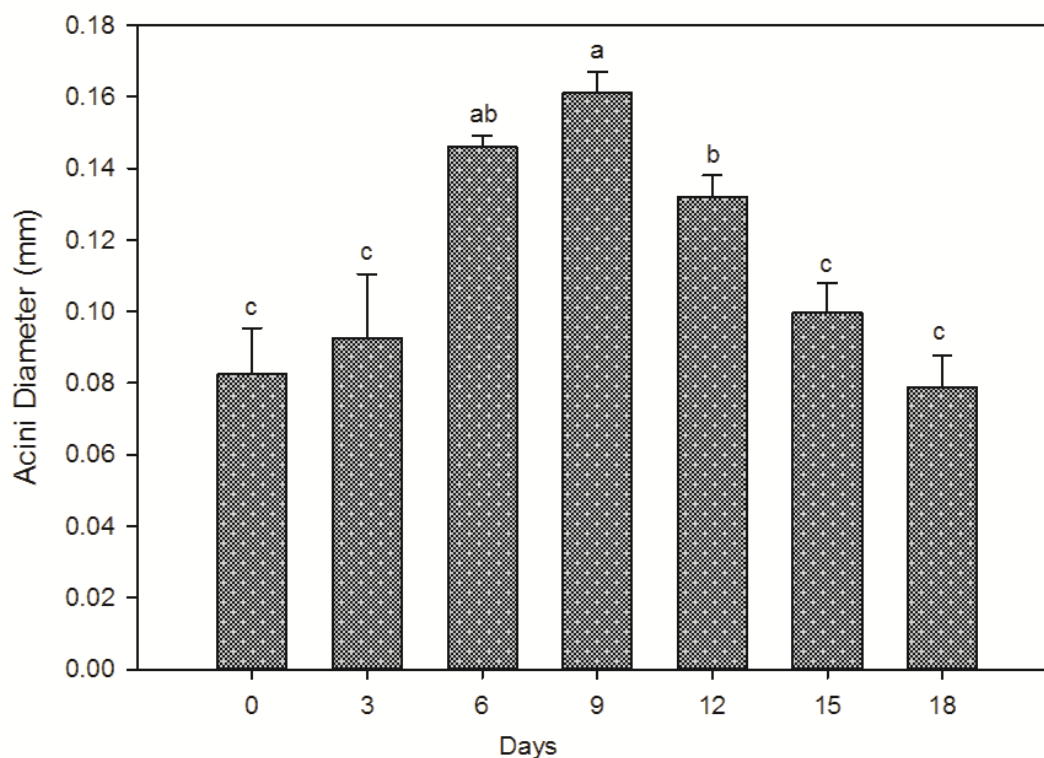


Fig. 2 Development of hypopharyngeal glands acini diameter of honeybee workers of different age (Different letters over columns indicate significant differences according to Duncan's multiple-range test at $\alpha = 0.05$; Columns with the same letter are not significantly different; Vertical bars indicate standard error (\pm)).

4 Discussion

In this research we have presented evidence that L-proline can be utilized as a phagostimulant for the honeybee (*Apis mellifera*). Consistently with the information given in the introduction, we reported the L-proline can increase hypopharyngeal glands acini diameter and syrup consumption. In some insects, proline is the most abundant free amino acid in hemolymph (Henn et al., 1998; Uchida et al., 1990; Goldstrohm et al., 2003) and we investigated whether proline plays a phagostimulating role in honeybees. Amino acids have both phagostimulatory and inhibitory effect on feeding in insects (Robbins et al., 1965; Shimada, 1978; Lanza, 1988). Whether they are inhibitory or exciting their feeding effects depends on their concentration (Carter et al., 2006) and on which sensory neurons they excite. For example, in flesh flies and blow flies phenylalanine stimulates sugar-sensing neurons, whereas proline excites salt-sensing neurons in flies (Shiraishi and Kuwabara, 1970; Goldrich, 1973). Degrandi-Hoffman et al., (2013) compared bee bread made by Africanized and European honey bees and its effects on hemolymph protein titers. Their result showed Africanized bee bread (ABB) is more consumed than European bee bread (EBB). One reason that ABB was consumed more than EBB might be because of the different concentrations of phagostimulatory compounds between the two types of bee bread. One such compound might be proline. There was about 20 % more proline in ABB than EBB.

Nutrient balance sometimes requires that animals integrate information about nutritional state with decisions about what foods to consume (Schwartz et al., 2000; Perveen et al., 2012; Simpson and Raubenheimer, 2012; Ahsaei et al., 2014). L-proline is an endogenous substrate believed to serve many metabolic functions. It also shares a number of properties with recognized neurotransmitters. In insects, proline is considered a major fuel source utilized to generate energy through the Krebs's cycle during flight (Kowaloff et al., 1977). For instance, in response to light in photoreceptor neurons of honey bees, L-proline is rapidly oxidized by proline oxidase to L-glutamate, which subsequently fuels the Krebs's cycle in the form of α -ketoglutarate (Tsacopoulos et al., 1994).

In conclusion, this study has revealed that L-proline at 1000 ppm concentration of diet can have a positive impact on the hypopharyngeal glands development of honey bees since L-proline has a phagostimulant role of syrup consumption in honey bees. However, the threshold response shown in this study strongly suggests that diets must be containing less than 1000 ppm.

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