Saudi Journal of Biological Sciences (2011) **18**, 73–77

King Saud University

Saudi Journal of Biological Sciences

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Consumption rate of some proteinic diets affecting hypopharyngeal glands development in honeybee workers

Ahmad AlKazim Al-Ghamdi *, Abeer M. Al-Khaibari, Mohamed O. Omar

Chair of Abdullah Baqshan Bee Research, Department of Plant Protection, College of Food Science and Agriculture, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia

Received 15 July 2010; revised 2 October 2010; accepted 2 October 2010
Available online 20 October 2010

KEYWORDS

Proteinic;
Hypopharyngeal;
Gland;
Honey bee;
Saudi Arabia;
Feed

Abstract The experiment was carried out under laboratory condition to study the consumption of some proteinic diets and their effect on hypopharyngeal glands (HPG) development during nursing period. The results showed that the bee bread and the pollen loads mixture with sugar (1:1) were more consumed by honeybee workers followed by Nectapol® and Yeast-Gluten mixture. The lowest consumption amount was recorded with traditional substitute.

Clear differences were found in HPG development under feeding with different diets. The maximum development degree was observed when fed with bee bread followed by pollen loads and mixture from Yeast, Gluten and sugar (1:1:2). The acinal surface of HPG showed clear difference under feeding with difference diets. The largest area was recorded when honeybee workers fed on bee bread followed by Yeast-Gluten-sugar mixture (diet,4) and pollen loads(diet,2).

1. Introduction

Hypopharyngeal glands (HPG) or brood food glands are located in worker honey bees' head and produce protein aqueous secretions (Jelly). These secretions are fed to the larvae and queens (Crailshem, 1991, 1992). The diameters of HPG are often used to describe the physiological status of worker honeybees. The normal course of development of these glands (size of acini) is well known (Maurizio, 1954; Simpson et al., 1968; Moritz and Crailsheim, 1987; Crailsheim and Stolberg, 1989).

At normal condition they are well developed when bees are nursed and they degenerate when bees become foragers. It depends on 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 degree (Crailsheim and Stolberg, 1989; Hrassnigg and Crailsheim, 1998).

Alqarni (2006) evaluated some proteinic diets for supplementary feeding of honeybee. The highest rate of food consumption was recorded with improved traditional substitute

* Corresponding author. Tel.: +966 505782019.
E-mail address: aalkhazim@hotmail.com (A.AlKazim Al-Ghamdi).

1319-562X © 2010 King Saud University. All rights reserved. Peer-review under responsibility of King Saud University.
doi:10.1016/j.sjbs.2010.10.001

Production and hosting by Elsevier

followed by mixture from date palm pollen and soybean flour. The bee bread or date palm pollen was the best sources for hypopharyngeal gland activation.

The aim of the present study was to determine the potentiality of using some proteinic diets in feeding honeybee workers under laboratory condition for developing the hypopharyngeal glands.

2. Materials and methods

The experimental work was carried in Bee Research Unit Apiary, College of Food Science and Agriculture, King Saud University, Riyadh, Saudi Arabia.

Carniolan honeybee hybrid *Apis mellifera carnica* was used in the present investigation. Five tested diets were compared to gather against the control as been explained in Table 1.

Mixed bee bread administrated for feeding was collected from combs during active season and stored under freezing condition until using. Mixed pollen loads were collected by pollen trap and stored under freezing conditions. The paste like diet from each variant mentioned above was prepared by using warm water to obtain a suitable patty.

3. Experimental cage

The experiment was carried out in cages using newly emerged honeybee workers (0–24 h). Experimental wooden cages $15 \times 15 \times 5$ cm dimensions covered with glass one side and other was covered with black muslin. Each cage was provided with a vial of tap water and other vial of sugar solution 1:1 (W/V) and a piece of wax comb was attached in one side of the cage.

Eight cages (each contains 100 workers) were used for every tested diet and divided in two groups:

- G_1: Used to determine the food consumption by the nursing bees (in mg/100 bee/3 days).
- G_2: Used to study the hypopharyngeal gland development degree and acini surface.

The diets were introduced for each cage in small plastic feeder. The diets were changed every 3 days. All cages were held in the dark in an incubator at 32 ± 1 °C and 65 ± 2% RH.

Hypopharyngeal gland was dissected to determine the HPG development degree and acini surface at 3, 6, 9, 12, 15, days old. HPG was put on a glass slide into a droplet of sodium chloride solution 0.85% (isotonic to the heamolymph). The glands were not covered by cover glass.

An arbitrary scale (I–IV) according to Maurizio (1954) was used to determine the HPG development degree (Grate, I represented undeveloped gland and gland IV represented complete development).

Table 1 Description of proteinic diets administrated to honeybee worker.

Variant	Constituents
Diet 1	Bee bread (comb – collected bee bread)
Diet 2	Pollen loads + Sugar power (1:1)
Diet 3	Nectapol®
Diet 4	Brewer's + Glutin + Sugar powder (1:1:2)
Diet 5	Tradional substitute Soybean meal, Skim milk Brewer's yeast sugar powder 3:1:1:5

The maximum length and width of ten acini were measured in mm for each worker under stereomicroscope using a micrometer eye piece.

The acinal surface was calculated according Maurizio's formula (1954).

$$\text{Acinal surface} = \pi \times \frac{a \times b}{2}$$

Where a = maximum length, b = maximum width, π = 3.14.

F-test was used for statistical analysis. Differences among means were determined by Duncans's multiple test SAS institute (1990).

4. Result and discussion

Data presented in Table 2 show the food consumption rate (mg/100 bees/3 days) of newly emerged bees from different proteinic diets against the control that feed with bee bread collected from combs of honeybee colonies during the active season. Honeybee workers fed on bee bread consumed 1.95 gm/100 bees/3 days during the first three days after emergence and 3.03 gm/100 bees/3 days during the 4th to 6th day, then a mount decreased to 1.38 gm/100 bees/3 days at the following periods.

It is obvious that the honeybee workers consumed the amount of any tested diet in a similar trend. The consumed diet decreased gradually from the 9 days toward the progressing period till the end of the observed periods. The least consumed amount during feeding period was recorded at 13–15 days for the most inspected diets.

The present result was in agreement with Zherebken results (1965) that showed that pollen is consumed by bees until they reach an age of 15–18 days. Similar results were obtained by Pain (1961). They reported that the peak of pollen consumption was obtained at 3–5 days of worker age and decreased to low level by time.

Hrassnigg and Crailsheim (1998) reported that the highest percentage of pollen consumption by honeybee workers was recorded at 3–10 days old. This percentage decreased sharply at 12 days old.

General means for food consumption by honeybee workers fed on pollen grains and bee bread (gm) insignificantly deferred. All the other compared diets are deviated in minus direction. The results indicated that the type of basic material used in diet greatly affected the food consumption. It can be concluded that honeybee nurse workers prefer to feed on diets supplied with pollen loads more than that without. Kleinschmidt and Kondos (1977); Hebert and Shumanuki (1980) stated that the food consumption is clearly stimulated by adding the pollen to the mixed diets for honeybee.

To ensure suitability of proteinic diets for developing hypopharyngeal glands during period of pollen scarcity, the glands development evaluated by measuring the degree of development and acinal surface after the honeybee workers had been fed on some proteinic diets.

Results presented in Table 3 indicated that the age of bees in which the development of hypopharyngeal glands could be influenced occurred during the nursing period. The considering increment in glands development has been obtained during this period. When newly emerged honey bee workers were fed on

Table 2 Amounts of proteinic diets consumed during nursing period of honeybee workers.

No.	Treatments	Means of proteinic diet amount consumed by honeybee workers (gm/100 bees/3 days)					General means	%
		3-days old	6-days old	9-days old	12-days old	15-days old		
1	Bee bread	1.9500 ± 0.3109c,b	3.0250 ± 0.3594a	1.3750 ± 0.1708f,e	1.3250 ± 0.3096g,f,e	0.6250 ± 0.1708i,h	1.6600 ± 0.8580A	
2	Pollen loads + sugar (1:1)	2.9650 ± 0.7411a	2.2250 ± 0.1258b	1.2750 ± 0.0957g,f,e	1.2000 ± 0.0817g,f,e	0.9000 ± 0,1633h,g	1.7150 ± 0.8493A	3.31%
3	Nectapol®	1.9250 ± 0.2872c,b	1.8750 ± 0.2872d,c,b	0.9500 ± 0.1291h,g,f	1.2500 ± 0.1291g,f,e	0.1750 ± 0.0957j	1.2350 ± 0.6877B	25.6%
4	Yeast, Gluten, Sugar (1:1:2)	1.5750 ± 0.3096 e,d,c	1.2500 ± 0.2887 g,f,e	0.9975 ± 0.6424h,g,f	1.1750 ± 0.1708g,f,e	0.3500 ± 0.1291j,i	1.0695 ± 0.5232B	35.57%
5	Traditional substitute	1.4750 ± 0.2500e,d	1.000 ± 0.1633 h,g,f	0.7000 ± 0.2160 i,h	0.3000 ± 0.0817j,i	0.1250 ± 0.0500j	0.7200 ± 0.5207C	56.63%

Means marked with different letters significantly differ at 5% level of probability.

Table 3 Means of hypopharyngeal gland development degree under feeding with different proteinic diets during nursing period of honeybee workers.

No.	Treatments	Means of proteinic diet amount consumed by honeybee workers (gm/100 bees/3 days)					General means
		3-days old	6-days old	9-days old	12-days old	15-days old	
1	Bee bread	3.5500 ± 0.3681c	3.7500 ± 0.1987b	3.9750 ± 0.0770a	3.8625 ± 0.1716b,a	3.8000 ± 0.2513b,a	3.7875 ± 0.2692A
2	Pollen loads + sugar (1:1)	3.2625 ± 0.2625g,f,e	3.4250 ± 0.3354e,d,c	3.4750 ± 0.3131d,c	2.9500 ± 0.3403h	2.9250 ± 0.2936h	3.2075 ± 0.3828B
3	Nectapol®	2.7625 ± 0.3292i,h	2.2000 ± 0.2513l	2.0625 ± 0.2910l	1.7875 ± 0.3170m	1.7125 ± 0.3065n,m	2.1050 ± 0.4770D
4	Yeast, Gluten, Sugar (1:1:2)	3.1875 ± 0.3616g	3.3375 ± 0.2333g,f,e,d	3.4000 ± 0.3183f,e,d,c	3.2875 ± 0.2470g,f,e,d	2.6500 ± 0.3752j,i	3.1725 ± 0.4093B
5	Traditional substitute	3.2125 ± 0.4236g,f	2.4750 ± 0.2913k,j	2.8000 ± 0.2379i,h	2.4250 ± 0.3152k	2.0875 ± 0.2842l	2.6000 ± 0.4924C
6	Sugar syrup (1:1)	2.5000 ± 0.2433k,j	2.2125 ± o.3065l	2.0375 ± 0.2900l	1.6125 ± 0.2625n,m	1.5750 ± 0.1832n	1.9875 ± 0.4386C

Means marked with different letters significantly differ at 5% level of probability.

Table 4 Means of hypopharyngeal gland acini surface under feeding with different protein proteinic diets during nursing period of honeybee workers.

No.	Treatments	Means of proteinic diet amount consumed by honeybee workers (gm/100 bees/3 days)					General means
		3-days old	6-days old	9-days old	12-days old	15-days old	
1	Bee bread	0.2057 ± 0.0669d	0.2432 ± 0.0482c	0.3102 ± 0.0671b	0.3114 ± 0.049b	0.3688 ± 0.0704a	0.2878 ± 0.0830A
2	Pollen loads + sugar (1:1)	0.1878 ± 0.0444e,d	0.1593 ± 0.0287h,g,f,e	0.2035 ± 0.0472d	0.1310 ± 0.0332j,i,h	0.1440 ± 0.0403h,g	0.1651 ± 0.0471B
3	Nectapol®	0.1406 ± 0.0307 h	0.0881 ± 0.0206m,l,k	0.1051 ± 0.0439k,j,i	0.0875 ± 0.0194m,l,k	0.0899 ± 0.0246m,l,k	0.1022 ± 0.0351D
4	Yeast, Gluten, Sugar (1:1:2)	0.1631 ± 0.0382 h,g,f,e	0.1880 ± 0.0532e,d	0.1560 ± 0.0544h,g,f,e	0.2374 ± 0.0924c	0.1412 ± 0.0320h	0.1771 ± 0.0661B
5	Traditional substitute	0.2827 ± 0.0725f,e,d	0.1344 ± 0.0243i,h	0.1017 ± 0.0427m,l,k,j	0.1758 ± 0.0443g,f,e,d	0.1059 ± 0.0234k,j,i	0.1400 ± 0.0559C
6	Sugar syrup (1:1)	0.1029 ± 0.0210 l,k,j	0.1520 ± 0.0321h,g,f	0.0702 ± 0.0269m	0.0887 ± 0.0260m,l,k	0.0719 ± 0.0224m,l	0.0971 ± 0.0394D

Means marked with different letters significantly differ at 5% level of probability.

bee bread, the grades of HPG development degree increased from 3.55 at 3 days-old to 3.97 at 9 days-old. This result reflected a high development degree among the compared diets. The glands of honey bee workers fed on diet no.4 which was based on yeast and Gluten had 3.40 degrees at 9 days-old. It is the best treatment after feeding bees on bread or pollen loads for HPG activation.

The present results are in agreement with many authors. They described that Soybean flour; dried yeast and skim milk were the most suitable substance used for supplementary feeding of honey bee colonies after pollen (Standifer et al., 1973). Symas and Przyble (1995,1996) found that supplementary feeding with pollen substitute contains soybean flour and yeast activated the hypopharyngeal gland as bee bread.

Means of acinal surface of hypopharyngeal gland after feeding on some proteinic diets are presented in Table 4. The acinal surface increased gradually with the age and reached to the maximum at 12–15 days from emergence. These results agreed with those obtained by Atallah et al. (1995). They compared the effect of some diets with bee bread.

In the present results, it is clear that the greatest acinal area was observed when bees were fed on bee bread, diet no.4 and mixed pollen loads. The activation effect of feeding bees with diet 3.5 was less than other diets. The lowest surface was recorded in honey bee workers fed sugar syrup only. It can be concluded that mixture diets based on yeast and Gluten (diet no.4) was superior over the other diets. Hrassnigg and Crailsheim (1998) reported that there was a positive relationship between the pollen consumption and worker head weight, diameter of HPG and protein synthesis during the nursing period of honeybee worker.

References

Alqarni, A.S., 2006. Influence of some protein diets on the longevity and some physiological conditions of honeybee *Apis mellifera* L. worker. J. Bio. sci. 6 (4), 734–737.

Atallah, M.A., Mofttah, E.A., Eahbah, H.M., Mohamed, A.A., Eyssa, N.A., 1995. Effect of protein feeding on the development of hypopharyngeal gland of two races of honeybee and the chemical composition of royal jelly. 6th Nat. Conf. of Pest. & Dis. Of Veg. & Fruits in Egypt, 88–100.

Crailsheim, K., 1991. Interadult Ceding of jelly in honeybee (*Apis mellifera* L.) colonies. J. Comp. Physiol. B 161, 55–60.

Crailsheim, K., 1992. The flow of jelly within a honeybee colony. J. Comp. Physiol., B. 162, 681–689.

Crailsheim, K., Stolberg, E., 1989. Influence of diet, age and colony condition upon intestinal proteolytic activity and size of the hyopharyngeal glands in the honeybee (*Apis mellifera* L.). Journal of Insecta Physiology 35 (8), 595–602.

Hebert Jr., E.W., Shumanuki, H., 1980. An evaluation of seven potential pollen substitutes for honeybees. Amer. Bee J. 120 (5), 349–350.

Hrassnigg, N., and Crailsheim, K., 1998. The influence of brood on the pollen consumption of worker bees (*Apis mellifera* L.). Journal of Insect Physiology 44, 393–404.

Huang, Z.Y., and Otis, G.W. 1989. Factors determining hypopharyngeal gland activity of worker honey bees (*Apis mellifera* L.). Insectes Sociaux 36, 264–276.

Kleinschmidt, G.J., Kondos, A.C., 1977. The influence of crude protein level on colony performance. The Australian Beekeeper 79, 357–361.

Maurizio, A., 1954. Pollen nutrition and vital processes in the honey bee. Landwirtschaftliche Jahrbuch der Schweiz 62, 115–182.

Moritz, B., Crailsheim, K., 1987. Physiology of protein digestion in the midgut of the honeybee (*Apis mellifera* L.). Journal of Insect Physiology 33 (12), 923–931.

Pain, J., 1961. Sur quelques facteures alimentaires, accelerateurs du developmental des oeuf dans les ovaries des ouvrieres d' beill (*Apis mellifera* L.). Insectes Sociaux 8, 31–93.

SAS Institute, 1990. SAS/STAT.user's Guide:Release 6.04.SAS Institute Inc.,Cary, N.C.

Simpson, J., Riedel, I.B.M., Wilding, N., 1968. Invertase in the hypopharyngeal glands of the honeybee. Journal of Apicultural Research 7 (1), 29–36.

Standifer, L.N., Owens, C.D., Haydak, M.H., Mills, J.P., Levin, M.D., 1973. Supplementary feeding of honeybee colonies in Arizona. Amer. Bee J. 113 (8), 298–301.

Szymaś, B., Przyby, A., 1995. Zastosowanie biaka ziemniaka w żywieniu pszczoy miodnej (*Apis mellifera* L.). Pszczeln. Zesz. Nauk. 39, 49–53.

Szymaś, B., Przyby, A., 1996. Physiological condition of worker bees *Apis mellifera* L. after consumption of pollen substitute. Pszczeln. Zesz. Nauk. 40 (2), 109–117.

Zherebkin, M.V., 1965. Digestion in bees from weak and strong colonies. Pchelovodstvo 42, 25–27. (A.A. 254/66).