IMPACT OF POLLEN SUPPLEMENTS AND VITAMINS ON THE DEVELOPMENT OF HYPOPHARYNGEAL GLANDS AND ON BROOD AREA IN HONEY BEES

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Summary

The test consisted of a study of pollen supplements and vitamins in the diet of honey bee (*Apis mellifera* L.) colonies in the drought seasons of 2001 and 2002 in northern Iraq. The impact of five different kinds of food on the hypopharyngeal gland of 8-day-old workers, and the area of brood in the nests was examined. The results showed that in 2001 bees fed with vitamin C had an acini of hypopharyngeal glands wider in comparison to bees given other feedings. The other feedings in 2001 were with multi-vitamins, soya bean flour and skim milk. Bees fed with vitamin C mixed with soya bean flour, and vitamin C mixed with skim milk had an acini of hypopharyngeal glands wider in comparison to other treatments in 2002. In bees fed with multi-vitamins and vitamin C the main ducts of the hypopharyngeal glands were longer than those fed with other supplemental diets in 2001. In 2002 there were no significant changes found on the length of the main duct in bees fed with supplemental diets. Colonies fed with vitamin C, soya bean flour and multi-vitamins produced more brood in the summer of 2001. Colonies fed with vitamin C mixed with soya bean flour and vitamin C mixed with skim milk showed a significant increase in workers' brood in 2002. It can be concluded that vitamin C mixed with soya bean flour can be used as the best choice of supplemental food during a drought seasons.

Keywords: supplemental diets, hypopharyngeal gland, brood, honey bee.

INTRODUCTION

Pollen is the primary source of protein, fats, vitamins, and minerals in the diet of the honey bee, *Apis mellifera* L. A shortage or poor quality of pollen results in stunted growth and weight gain of young bees, reduced longevity and incomplete development of hypopharyngeal glands. This leads to insufficient royal jelly production to support normal growth and development of larvae or egg production by the adult queen (Woyke 1976, Hays 1984, Standifer et al. 1987).

The proteins secreted with royal jelly are mainly derived from pollen. The pollen is ingested in large quantities by the nurse bees (Haydak 1970, Crailshein et al. 1992). Hrassnigg and Crailshein (1998) found that pollen consumption is positively correlated with hypopharyngeal gland development.

Hypopharyngeal glands are composed of a pair of ducts that are connected with more than 500 glandular acini (secretory cells). The glands are located underneath the pharynx in the head capsule. This gland plays an important role in rearing the queens and brood because it synthesizes and secretes royal jelly (Michener 1974).

Substantial amounts of protein are needed as a consequence of an increase in the protein content of the hypopharyngeal glands at the beginning of the adult stage, (Fluri and Bogdanov 1987).
Al-Naji (1980) and Graham (1993)

reported that the development of the hypopharyngeal glands in honey bee workers is a consequence of their diet. They reported that the best development of this gland was observed in bees fed with high levels of proteins. Fewell and Winston (1992) found that brood production increased significantly when colonies were given more extensive pollen stores. Honey bee colonies that were kept from pollen foraging by being placed under a wire mesh tent, reduced and finally stopped brood rearing. As a consequence, the number of workers became significantly lower in these colonies than in colonies which were allowed to forage freely. These results clearly indicate that honey bees respond to the availability of pollen by adjusting brood production (Imdore et al. 1988).

According to Deseyn and Billen (2005) the size of the hypopharyngeal glands is positively correlated with gland activity. The amount of secretion in the secretory cells is also positively correlated with the size of the acini. The amount of secretion produced by the workers hypopharyngeal glands depends on the needs of the colony. The gland has been reported to display a flexible secretory activity in relation to the needs for feeding brood (Free 1961). Sometimes, older workers seem to retain secretory activity (Ohashi et al. 2000), but the workers must always be stimulated by the brood pheromones. De Moraes and Bower (2000) pointed out that hypopharyngeal glands were well developed in young nursing bees producing protein for larval food. They were seen to regress naturally in foraging adult worker bees.

Pain (1956) demonstrated that vitamins added to the workers diet had no effect on the longevity of honey bees. Pantothenic acid, however, had a great influence on the development of the hypopharyngeal glands. The author concluded that vitamins exert a decisive effect on these glands in contrast to their minimal effect on longevity. Haydak and Dietz (1965) concluded that for the growth of emerging bees and the development of their hypopharyngeal glands only an appropriate protein source is necessary. They also concluded that for brood rearing activities vitamins are indispensable. They found that minerals were not essential in the diet of adult honey bees for the growth and development of their eggs. Standifer et al. (1970) did not find any diets producing maximum gland development.

Lehner (1983) found that colonies fed pollen raised more brood than those fed the test diets. Lehner found that pollen fed colonies on the average produced populations about twice as large as those fed the test diets. Stanger and Laidlaw (1975) found that colonies fed with supplemental pollen showed an increase in the brood area compared to less brood area in controls. According to Pokhrel and Thapa (2004) the supplemental diet which contained pollen was more suitable for higher comb building of *A. mellifera*. This higher comb building could facilitate brood production, strengthen colonies and contribute to honey production in flow seasons. Stephen and Pernal (2000) found that the development of hypopharyngeal glands and ovaries was strongly correlated with the amount of protein workers consumed. Pokhrel et al. (2006) found that three weeks of feeding with a pollen substitute resulted in a higher comb building, brood rearing, colony strength and hive storage of honey and pollen.

The aim of the present paper is to study the effect of different pollen supplements and vitamins on the development of the hypopharyngeal glands and consequently on the brood area in honey bee colonies. The reason for the study is to find better

supplemental diets that can be used during the drought seasons.

MATERIAL AND METHODS

Experimental Colonies

Fifteen experimental colonies of *Apis mellifera* L. *cyriaca* were selected randomly from the apiary of the Agriculture College of Dohuk University (northern Iraq). Before the start of the experiment, cupped brood combs without workers were exchanged between colonies. This meant that all experimental colonies included five combs with a similar number of workers and amount of brood. In two summer seasons we prepared five groups of three colonies fed with different food. The same colonies survived and were tested in both seasons.

Foods Tested

Five different feeding treatments were tested each year during the summer seasons of 2001 and 2002. Colonies were fed supplemental foods weekly. The feedings started the first week of May and lasted until the end of August in both of the 2001 and 2002 seasons. Each colony received 500 ml of supplemental food at the end of the day before sunset. The feeders were removed the next morning.

In 2001 the bees were fed with: a sugar solution in the control group (group Cn), a sugar solution with multi-vitamins (group MV), a sugar solution with vitamin C (group VC), a sugar solution with soya bean flour (group So), and a sugar solution with skim milk (group Mi).

In 2002 the bees were fed with: a sugar solution in the control group (Cn), a sugar solution with multi-vitamins mixed with soya bean flour (MVSo), a sugar solution with multi-vitamins mixed with skim milk (MVMi), a sugar solution with vitamin C mixed with soya bean flour (VCSo), and a sugar solution with vitamin C mixed with skim milk (VCMi).

Preparation of Supplemental Foods

In both years we used a sugar solution of 20% (w/v) to feed the control groups (Cn) and the same syrup with vitamins or/and pollen supplements to feed the experimental colonies. Group MV was given one 250 mg capsule of multi-vitamins. The multi-vitamins consisted of: Vit. A 400 IU., Vit. B1 1 mg., Vit. B2 1.2 mg., Vit. B6 2 mg., Vit. B12 2 mg., Vit. C 70 mg., Vit. D 400 IU. Folic acid 0.25 mg (Medico Labs. HOMS, Syria) was dissolved in 1.5 liters of the sugar solution. For group VC, one tablet of vitamin C 250 mg potency (Medico Labs. HOMS, Syria) was ground by a porcelain mortar and then mixed with 1.5 liters of sugar solution. For group So, 50 grams of soya bean flour with a content of: 47.38%, 6.29% of protein and lipid (Abdulla 1988) was mixed with 1.5 liters of sugar solution. For group Mi - 100 grams of skim milk powder (ingredients: skim milk, sucrose, maltodextrin, pakm olein, soya lecithin, vitamins, calcium citrate, potassium citrate, taurine, ferrous sulfate, zinc sulfate, copper sulfate, potassium iodide) was mixed with 1.5 liters of sugar solution.

In 2002 the supplemental foods were of the same ingredients as in 2001 but only half the concentration was used.

Sample Collection and Hypopharyngeal Gland Measurement

Newly emerged workers were marked on their thorax with a water proof marker and released into their colonies. Fifteen marked bees were collected after eight days and dissected.

The length of the main duct and the length and width of ten randomly chosen acini were measured in each side of the gland. The measurements were performed two times per year, in the middle of June and July 2001 and 2002. Dimensions of the acini were calculated by multiplying the

length by the width and considered as a parameter for development of the acini.

Measurement of the Total Area of Brood

The total area of the brood was calculated two times per year in August 2001 and 2002 by using the standard Langstroth frame (Al-Naji and Mushael 1987). The standard Langstroth frame was divided by silk into squares of 1 inch^2. The area of brood was measured after the application of the standard Langstroth frame on the brood combs.

Statistical Analysis

ANOVA (one way) was applied to detect significant differences between groups and control. The Post-hoc test (Tukey test) was used to detect significant differences among groups at $p < 0.05$.

RESULTS

Hypopharyngeal Gland

The average dimensions of the acini in the Cn (control), MV (multi-vitamin), VC (vitamin C), So (soya bean flour) and Mi (skim milk) were 11726.2, 19150.33, 21800.8, 14395.6 and 13355.16 (μm^2) respectively in summer 2001 (Fig.1-A). Bees fed with vitamin C had an acini of hypopharyngeal glands wider in comparison to other treatments. They were followed by bees fed with multi-vitamins, soya bean flour and skim milk.

The average length of the the main duct in the Cn, MV, VC, So and Mi treatments was 8.94, 10.3, 9.75, 9.17 and 8.91 mm respectively (Fig.1-B). Bees fed with multi-vitamins and vitamin C had a longer main duct of their hypopharyngeal glands. Those fed with other supplemental diets, like soya bean flour and skim milk showed non significant effect.

The average dimensions of the acini for Cn (control), MVSo (multi-vitamin + soya bean flour), MVMi (multi-vitamin + skim milk), VCSo (vitamin C + soya bean flour), and VCMi (vitamin C + skim milk) treatments were: 12062.6, 15917.9, 16072.07, 18325.13 and 17408.6 μm^2 respectively in summer 2002 (Fig.2-A). Bees fed with vitamin C mixed with soya bean flour and vitamin C mixed with skim milk had an acini of hypopharyngeal glands wider in comparison to other treatments. These results confirmed the effect of vitamin C on the development of the acini of the hypopharyngeal glands.

The average length of the main duct of Cn, MVSo, MVMi, VCSo, and VCMi

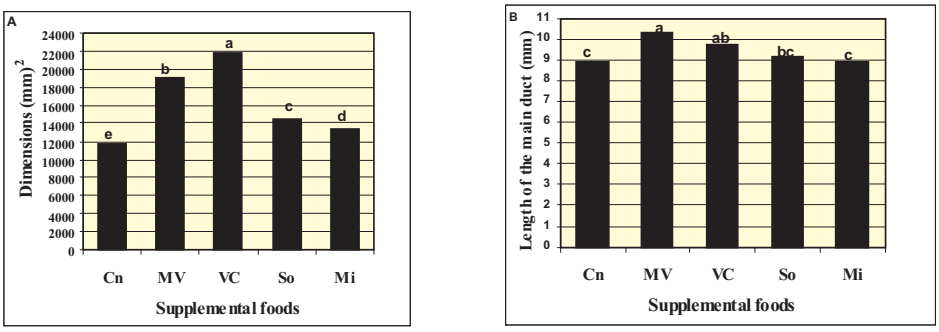

Fig. 1: Mean values of measurements of hypopharyngeal glands in 2001 (A-dimensions of the acini, B- length of the main duct) in bees fed with different supplemental diets. Cn stands for: sugar solution (control), MV: sugar solution with multi-vitamins; VC: sugar solution with vitamin C, So: sugar solution with soya bean flour, Mi: sugar solution with skim milk.
Differences significant at $p < 0.05$.
Note: Dimensions of the acini were calculated by multiplying the length by the width.

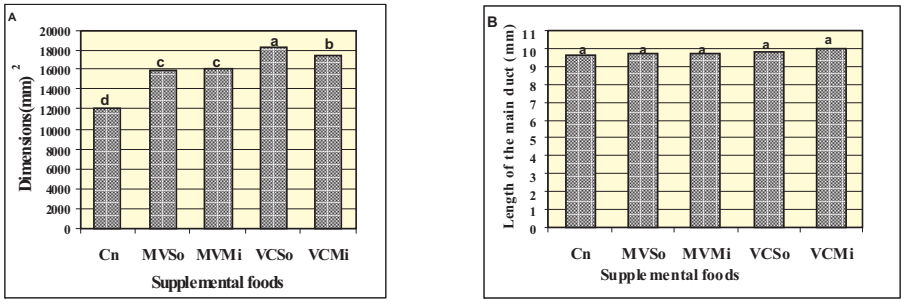

Fig. 2: Mean values of measurements of hypopharyngeal glands in 2002 (A-dimensions of the acini, B-length of the main duct) in bees fed different supplemental diets. Cn stands for: sugar solution (control), MVSo: sugar solution with multi-vitamins mixed with soya bean flour, MVMi: sugar solution with multi- vitamins mixed with skim milk, VCSo: sugar solution with vitamin C mixed with soya bean flour, VCMi: sugar solution with vitamin C mixed with skim milk.
Significant at $p<0.05$.

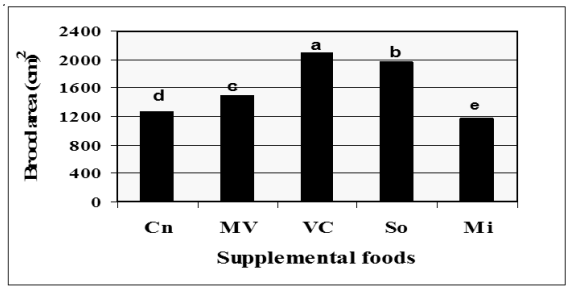

Fig. 3: 2001 - mean values of brood area in colonies fed different supplemental diets. Cn stands for: sugar solution as a control, MV: sugar solution with multi-vitamins, VC: sugar solution with vitamin C, So: sugar solution with soya bean flour, Mi: sugar solution with skim milk.
Significant at $p<0.05$.

treatments were: 9.64, 9.72, 9.68, 9.82 and 10.06 mm respectively in summer 2002 (Fig.2-B). All the supplemental diets had no significant effect on the length of the main duct of the hypopharyngeal glands. This can be attributed to the reduced concentrations of the diets used in 2002.

Brood Area

Statistical analysis showed significantly more workers' brood area in the colonies fed with vitamin C, soya bean flour and multi-vitamins which were 2095.5, 1968.5 and 1485.9 cm^2/colony respectively in summer 2001 (Fig.3). In the summer of 2002 colonies fed with vitamin C mixed with soya bean flour showed significantly more workers' brood area (4005.0 cm^2/colony) followed by colonies fed with vitamin C mixed with skim milk (3335.9 cm^2/colony) (Fig.4). No significant effect was seen in colonies fed with multi-vitamins mixed with soya flour and multi-vitamins mixed with skim milk.

DISCUSSION

Bees fed with vitamin C have an acini of hypopharyngeal glands wider than bees fed with other treatments. These results agree with those found by Herbert et al. (1984). We assume that the size of the acini is a better indicator for the development and activity of the hypopharyngeal glands than the main ducts. This is because soya bean flour revealed a significant effect on the

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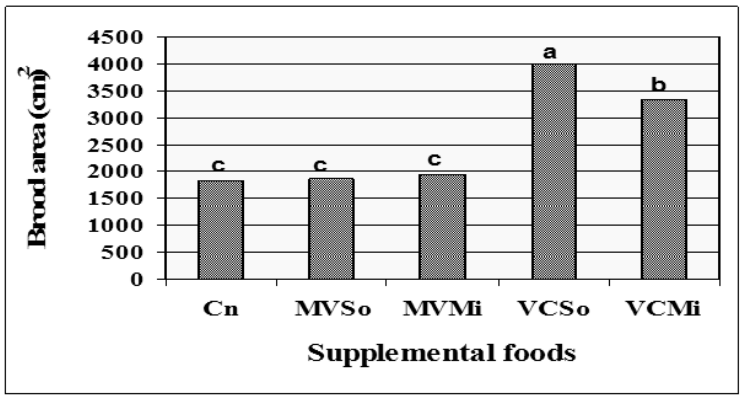

Fig. 4: 2002- mean values of brood area in colonies fed different supplemental diets. Cn stands for: sugar solution (control),
MVSo: sugar solution with multi-vitamins mixed with soya bean flour, MVMi: sugar solution with multi- vitamins mixed with skim milk,
VCSo: sugar solution with vitamin C vitamin C mixed with soya bean flour,
VCMi: sugar solution with mixed with skim milk.
Significant at $p<0.05$.

size of the acini in both 2001 and 2002, while it had no effect on the development of the main duct.

Bees fed with a diet containing vitamin C reared more workers' brood in both years: 2001 and 2002. These results agree with those of Haydak (1945), Ewies and Ali (1977), Hertel et al. (1984).

The effect of skim milk on brood area in our experiment was negative which agrees with the results of Chalmers (1980).

REFERENCES

Abdulla, M.A (1988) – Effect of diet with high protein content on the activity of honey bee *Apis mellifera* (L). M.Sc. Thesis, Mosul University, pp. 155 (In Arabic).

Al-Naji, K.L. (1980) – Rearing of honey bee and silk moth SIMA France.1st .ed. pp. 489.

Al-Naji, K.L., Mushael S.Z. (1987) – Effect of different over-wintering methods on the activity and production of honey bees. *J. Agric*. 29(3): 11-21.

Chalmers, W.T. (1980) – Fish meal as pollen protein substitutes for honey bees. *Bee World*, 61: 89-96.

Crailsheim. K., Schneider, L.H.W., Hrassnigg, N., Buhlmann, G., Brosch, U., Gmeinbauer, R., Schoffmann, B. (1992) – Pollen consumption and utilization in worker honey bees (*Apis mellifera carnica*): Dependence on individual age and function. *J. Insect Physiol*. 38: 409- 419.

DeMoraes, S.R.L., Bower, I.D. (2000) – Modes of cell death in the hypopharyngeal gland of the honey bee (*Apis mellifera* L.). *Cell Biol. Int*. 24(10): 737-743.

Deseyn, J., Billen J. (2005) – Age-dependent morphology and ultra structure of the hypopharyngeal gland of *Apis mellifera* workers (Hymenoptera, Apidae). *Apidologie*. 36: 49-57.

Ewies, M.A., Ali, M.C. (1977) – Test of a new protein supplement on honey bee activity. *Bull. Fac. Agric*., Cairo Univ. 28: 11-23.

Fewell, J.H., Winston, M.L. (1992) – Colony state and regulation of pollen foraging in the honey bee, *Apis mellifera* L. *Behav. Ecol. Sociobiol*. 30: 387-393.

Fluri, P., Bogdanov, S. (1987) – Age dependence of fat body protein in summer and winter bees (*Apis mellifera*) In Ender, J; Rembold, H. (eds) *Chemistry and biology of social insects.* Verlag J. Peperny; Munic, Germany; pp. 170-171.

Free, J, B. (1961) – Hypopharyngeal gland development and division of labour in honey bee (*Apis mellifera* L.) colonies. *Res. Entomol. Soc*. 36: 5-8.

Graham, J.M. (1993) – The hive and the honey bee. Dadant and Sons.Hamilton. Illions.2nd ed. pp. 1324.

Haydak, M.H. (1945) – Value of pollen substitutes for brood rearing of honeybees. *J. Econ. Entomol*. 38: 484-487.

Haydak, M.H. (1970) – Honey bee nutrition. *Ann. Rev. Entomol*. 15: 143-156.

Haydak, M.H., Deitz, A. (1965) – Influence of the diet on the development and brood rearing of the honey bee. *International Beekeeping Congress,* Bucharest. 20: 158-162.

Hays, G.W.J. (1984) – Supplemental feeding of honey bees. *Am. Bee J.* 124: 35-37.

Herbert, E.W., Vanderslice, J.T., Higgs, D.J. (1984) – Vitamin C enhancement of brood rearing by caged honey bees fed a chemically defined diet. *Arch. Insect Biochem. Physiol*. 2(1): 29-37.

Hrassnigg, N., Crailshein, K. (1998) – Adaptation of hypopharyngeal gland development to the brood status of honey bee (*Apis mellifera L.*) colonies. *J. Inst. Physiol*. 44(10): 929-939.

Imdore, F.A., Rickle, M., Kilchinmann, V., Bogdanov, S., Wille, H. (1988) – Nitrogen and mineral constitutes of honey bee worker brood during pollen shortage. *Apidologie.* 29: 315-325.

Lehner, Y. (1983) – Nutritional considerations in choosing protein and carbohydrate sources for use in pollen substitutes for the honey bee *Apis mellifera*. *J. Agric. Res*. 22(4): 242-248.

Michener, C.D. (1974) – The social behavior of the bees, Harvard University press, Cambridge, Massachusetts. pp. 242.

Ohashi, K., Sasaki, M., Sasagawa, H., Nakamura, J., Natori, S., Kubo, T. (2000) – Functional flexibility of the honey bee hypopharyngeal gland in a dequeened colony. *Zool. Sci*. 17: 1089-1094.

Pain, J. (1956) – Vitamin and ovarian development of worker bees. *Sciete de Biologie*, Paris. 145: 1505-1507.

Pokhrel, S., Thapa, R. B. (2004) – Supplemental diets in relation to comb building of cross breed honey bee. *J. Inst. Agric. Anim. Sci*. 25: 71-74.

Pokhrel, S., Thapa, R. B., Neupane, F.P., Shrestha, S.M. (2006) – Absconding behavior and management of *Apis cerana* F. honey bee in Chitwan, Nepal. *J. Inst Agric. Anim. Sci.* 27: 77-86.

Standifer, L.M., Mac Donald, R.H., Levin, M.D. (1970) – Influence of the quality of protein in pollen and of a pollen substitutes on the development of the hypopharyngeal glands of honey bees. *Ann. Entomol. Soc. Amer*. 63: 909- 910.

Standifer, L.N., Moeller, F.E., Kauffeld, N.M., Herbert, E.W.J., Shimanuki, H. (1977) – Supplemental feeding of honey bee colonies. *Ann. Entomol. Soc. Amer.* 70: 691-693.

Stanger, W., Laidlaw, H.H. (1974) – Supplemental feeding of honey bees (*Apis mellifera* L.). *Am. Bee J.* 114 (4): 138-141.

Stephen, F., Pernal, R.W. (2000) – Pollen quality of fresh and 1-year-old single pollen diets for worker honey bees (*Apis mellifera* L.) *Apidologie*.31: 387-409.

Woyke, J. (1976) – Brood rearing efficiency and absconding in Indian honey bees. *J. Apic. Res*. 15(3/4): 133-143.

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WPŁYW DODATKU PYŁKU I WITAMIN NA ROZWÓJ GRUCZOŁÓW GARDZIELOWYCH ORAZ POWIERZCHNIĘ CZERWIU U PSZCZOŁY MIODNEJ

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S t r e s z c z e n i e

Eksperyment dotyczył dodatków pyłku i witamin w diecie rodzin pszczelich (*Apis mellifera* L.) w porach suszy w latach 2001 i 2002 w północnym Iraku. Zbadano wpływ pięciu różnych diet na rozwój gruczołu gardzielowego 8-dniowych robotnic oraz na powierchnię czerwiu
w gniazdach. Wyniki badań wykazały, że w roku 2001 pszczoły karmione dietą z dodatkiem witaminy C wykazywały poszerzone gronka gruczołów gardzielowych w porównaniu do pszczół karmionych pozostałymi dietami. W badaniach z roku 2001 diety te zawierały mieszankę multiwitaminową, mąkę sojową i odtłuszczone mleko. Pszczoły karmione mieszanką witaminy C i mąki sojowej oraz pszczoły otrzymujące mieszankę witaminy C z odtłuszczonym mlekiem również charakteryzowały się poszerzonymi gronkami gruczołów gardzielowych w porównaniu do pszczół karmionych pozostałymi dietami w roku 2002. Z kolei, u pszczół karmionych mieszanką multiwitaminową i witaminą C, główne kanaliki gruczołów gardzielowych były dłuższe niż u pszczół karmionych dietami z pozostałymi dodatkami w roku 2001. W badaniach z roku 2002 nie obserwowano żadnych znaczących zmian w długości głównych kanalików u pszczół karmionych wzbogacanymi dietami. Rodziny karmione dietami z dodatkiem witaminy C, mąki sojowej i mieszanki multiwitaminowej charakteryzowały się większą powierzchnią czerwiu notowaną latem 2001. Rodziny karmione dietami z dodatkiem mieszanki witaminy C i mąki sojowej oraz mieszanki witaminy C z odtłuszczonym mlekiem wykazywały znaczący wzrost w powierchni czerwiu pszczelego w roku 2002. Można zatem uznać, że mieszanka witaminy C i mąki sojowej jest najlepszym suplementem diety w czasie okresów suszy.

Słowa kluczowe: suplementy diety, gruczoł gardzielowy, czerw, pszczoła miodna.