USE OF PROTEIN DIETS AS A SUPPLEMENT FOR AFRICANIZED BEES *Apis mellifera*

PINTO, Mara Rúbia Romeu [1];
VELEIRINHO, Beatriz [2];
GONÇALVES, Vitória Sequeira [3];
MARASCHIN, Marcelo [3];
ALBUQUERQUE, Pedro Machado Medeiros de [3];
CAMPOS, Vinicius Farias [3];
CUNHA, Rodrigo Casquero [3];
LEITE, Fábio Pereira Leivas [3].

Received: 11/11/2017
Accepted: 07/04/2018

[1]Epagri, Secretaria de Estado da Agricultura e da Pesca, Florianópolis, SC, Brazil; [2]Universidade Federal de Santa Catarina, NanoBioMat, Florianópolis, SC, Brazil; [3]Universidade Federal de Pelotas, CDTec, Núcleo Biotecnologia, Pelotas, RS, Brazil.

ABSTRACT

At the nature, bees consume pollen and nectar to obtain its nutrients (proteins, lipids, carbohydrates, vitamins, and minerals). Changes in bee pastures have occurred as green areas and forests have been replaced by agricultural areas affecting colony health and productivity. Because of this, the efficiency of artificial protein diets for the Africanized honey bee (*Apis mellifera*, L.) was evaluated - considering the total protein content of the hemolymph, weight of the bees, and dietary consumption. The crude protein of the diets ranged from 12.2 to 24.4%. Newly emerged workers were caged and kept in an incubator with controlled temperature and humidity until six days old, receiving diets and water *ad libitum*. Hemolymph was collected from 0- and 6-day-old workers. Comparing the diets, significant differences in the protein content in the hemolymph, bee weight, and diet consumption were found among the treatments. A diet composed of 20% sugarcane yeast, 20% textured soy protein, and 60% sucrose was the most efficient according to measures of total protein in the hemolymph, bee weight and diet consumption. The use of protein sources as a supplement for hives can be a safe and viable alternative for beekeepers.

Keywords: Africanized honey bees. Food. Nutrition. Nutrition assessment.

INTRODUCTION

Bees need to consume proteins, carbohydrates, lipids, vitamins, minerals, and water. In nature, bees collect nectar as a source of carbohydrate, i.e., energy (BRODSCHNEIDER; CRAILSHEIM, 2010). Pollen is required for protein, mineral, lipid, and vitamin needs. Bees also use extra floral nectar as a source of energy, i.e., honeydew (SAMMATARO; AVITABILE, 2011; SKUBIDA et al., 2008). The nutritional composition is basically sucrose, fructose, glucose and some different sugars that vary depending on the species of the plant in which is collected. The concentration of each nutrient also varies with the species (CHALCOFF et al., 2006).

Larval food, produced by nurse bees, is secreted by hypopharyngeal and mandibular glands (HERBERT, 1992). Nurse bees present well-developed hypopharyngeal glands that show intensive activity around the third day of life (GIROU, 2003). They are responsible for secreting the royal jelly that feeds the queen throughout her life, as well as the drones and larvae in their first days (CRAILSHEIM, 1990). To ensure the development of glands and adipose tissue of young bees, a high-protein diet is required (GIROU, 2003).

Approximately 20 hours after emerging, bees start to consume pollen, reaching a maximum intake by five days of age (BRODSCHNEIDER; CRAILSHEIM, 2010; HAGEDORN; MOELLER, 1967). Protein production by the fat body increases between the 5^{th} and 15^{th} days of life of an adult bee, and a significant amount of vitellogenin accumulates in the hemolymph (ENGELS et al., 1990; GREGORY, 2006).

The protein content of the diet must fulfill the bees' nutritional needs during the first days of adult life to allow hypopharyngeal and mandibular gland development. Also, to ensure the production of food for the queen and larvae maintaining strong, productive, and healthy bee colonies. Ideal levels of protein consumption contribute to an increase in the brood area, adult bee population, and productivity (CAPPELARI et al., 2009; HERBERT; SHIMANUKI, 1977). Additionally, it also allows the synthesis of several immunoproteins, such as lysozyme, apidexin, and phenoloxidase, as well as vitellogenin (ALAUX et al., 2010). An adequate diet has a positive effect on the prevention of diseases because it enables the synthesis of immunoproteins. Moreover, the protein level in a diet affects the quantity and the

percentage of granular hemocytes, as well as the phagocytic ability of defense cells (SZYMAS; JEDRUSZUK, 2003).

Studies have shown that pollen proteins can affect the physiological metabolism, hypopharyngeal gland development and immune functions (DI PASQUALE et al., 2013). The maintenance of the immune system can be costly, and deficient nutrition from a lack of dietary protein or pollen can increase the susceptibility of organisms to diseases (ALAUX et al., 2010) such as viruses (DEGRANDI-HOFFMAN et al., 2010), bacteria (RINDERER; ELLIOTT, 1977), and fungi (RINDERER et al., 1974), and may also affect the resistance threshold of bees to stress factors (NAUG, 2009).

Until a few decades ago, abundant flowering was capable of fulfilling the nutritional needs of bees, which are carbohydrates (nectar), amino acids (pollen), lipids (fatty acids, sterols), vitamins, minerals (salts), and water (BRODSCHNEIDER; CRAILSHEIM, 2010). However, in recent years, modifications in bee pastures have occurred as green areas and forests have been replaced by agricultural areas (GIROU, 2003). Some management conditions also alter the bee colony food inflow, and as landscapes have become increasingly characterized by intensive agricultural monocultures, bees' nutritional needs may not be provided properly (BRODSCHNEIDER; CRAILSHEIM, 2010; NAUG, 2009), which affects colony health and productivity (GIROU, 2003).

Beekeepers often use sugar syrup to increase the strength of the colonies when there are not enough flowers available (SAMMATARO; AVITABILE, 2011). Nevertheless, this increase occurs as a result of bodily resource consumption, e.g., lipids and proteins, which is debilitating to the bees and, consequently, decreases the productivity of the beehive and the longevity of the bees. Additionally, this practice also affects beehive health by causing starvation, cannibalism, increasing frequency of *Varroa destructor* infestation, and other diseases (SATTLER, 2001).

Thus, in this study we evaluated, as an alternative, the effect of protein diets prepared by using easily available and low-cost raw materials, on the hemolymph protein content, bee weight, and diet ingestion of the Africanized honey bee, *Apis mellifera.*

MATERIAL AND METHODS

Bees and caged bees

Apis mellifera were obtained from the "EPAGRI/Cidade das Abelhas" apiary in Florianópolis, SC (27°35'49" S, 48°32'58" E), Brazil. Combs containing emerging sealed worker broods were incubated at 32 ^{o}C with 70-80% relative humidity. Newly emerged bees (0-24 h old) were designated "day 0" bees. Bees from different brood combs were mixed and distributed in cages equipped with sliding glass sides and a screened roSCof (13x10×8 cm, 150 bees/cage). Cages were randomly distributed among the experimental groups (n=3 cages for each diet).

Composition of the diets

The following ingredients were used to prepare the seven diets (D1 to D7): commercial refined sugar from sugarcane (SC), texturized soy protein (TSP), inactivated sugarcane yeast (SCY), inactivated beer yeast (BY), and water. The total protein content of the dry matter diet samples was calculated as previously described (SILVA; QUEIROZ, 2006). Water and food were given *ad libitum* to all groups. Diet composition and total protein content are summarized in Table 1.

Table 1 - Composition and total protein content of the various diets (%).

Diet	Composition	Total protein (%)
D1*	50% SC, 50% water (v/v)	0.00
D2**	SCY, TSP, vitamin, and mineral mixture	24.4
D3	35% SCY, 65% SC (m/m)	17.0
D4	35% BY, 65% SC (m/m)	14.6
D5	20% SCY, 20% TSP, 60% SC (m/m/m)	20.9
D6	40% SCY, 60% SC (m/m)	18.5
D7	25% SCY, 75% SC (m/m)	12.2

* Negative control, free of protein. **Commercial bee feed (positive control), (SC) sugar from sugarcane, (TSP) texturized soy protein, (SCY) sugarcane yeast, (BY) beer yeast.

Diets were prepared by adding water to the other ingredients until a pasty consistency was achieved. The D1 diet was given as syrup, and used as a protein-free control. A commercial diet (D2) made of SCY, TSP, vitamins, and minerals were tested as a positive control.

Hemolymph extraction and sample preparation

Hemolymph was collect from the bees on day 0 and day 6. Briefly, a small incision was made with fine-pointed scissors at the level of the 3rd dorsal tergite, and the hemolymph was collected using a micropipette (Digipet®, 0.5-10.0 µL). Each hemolymph sample consisted of a pool collected from 10 bees. A total of 99 µL of stock solution (0.1% phenylthiourea (Sigma-Aldrich®, P-7629, Grade I, 98%) and 0.005% phenylmethanesulfonyl fluoride (Sigma-Aldrich®, P-78830, 99%) in phosphate buffered saline (PBS) pH 7.1 was added to 1 µL of the hemolymph sample to prevent hemolymph degradation reactions, and this dilution was kept at -18 ^{o}C prior to the total protein content analysis.

Total protein content of the hemolymph (TPCH)

The TPCH from day 0 and day 6 bees was measured to evaluate the efficiency of the diets (CREMONEZ et al., 1998). The TPCH was determined according to a methodology that had been previously established (BRADFORD, 1976). A standard curve was developed using a series of bovine serum albumin concentrations (25-175 µg/mL, y=0.002×, r^2=0.997). The absorbance was read spectrophotometrically using a microplate reader (TP-Reader, Thermoplate).

Weight and diet consumption

The average weight of bees was calculated by weighing 20 bees from each cage (at day 0 and day 6) on an analytical balance (Adventurer Toledo, SP, Brazil). The total consumption of each diet was determined by the difference between the quantity of diet supplied and the amount remaining (dried mass) (SILVA; QUEIROZ, 2006).

Statistical analysis

Statistical analysis was performed using the Statistix 8.0 package for Windows. The results were compared using the Kruskal-Wallis ANOVA of ranks; $p<0.05$ was considered statistically significant. Consumption and TPCH were compared using one-way ANOVA, followed by a Tukey test for multiple comparisons. Pearson correlation was used to test consumption and TPCH association. Significance was considered at p-value <0.05 in all analyses. All data were expressed as mean ± SEM.

RESULTS AND DISCUSSION

Table 2 summarizes the total protein content of the hemolymph for the tested diets. The diets D4 and D5 showed the highest levels of total protein content of the hemolymph (TPCH), and these levels were significantly superior to all other treatments. The TPCH of D4 was significantly higher than D1, and D2. The D3 treatment showed TPCH levels similar to control positive diet (D2). Although, D6 had more protein than D3 and D4 in its composition, the hemolymph levels were significant lower. D7, being the diet with lower protein content in its composition, showed the lowest TPCH levels, which did not differ significantly from those levels in D1 (negative control).

Table 2 - Total protein content in the hemolymph, bee weight, and diet consumption at Day 6 according to diet, as the mean ± standard deviation.

Diet	TPCH (µg/µL)	Bee weight (mg)	Consumption (g)
D1	9.90 (± 0.66)^{e}	86.20 (± 1.80)^{a}	8.50 (± 0.40)^{a}
D2	27.86 (± 1.37)^{bc}	100.00 (± 1.80)^{b}	6.45 (± 0.20)^{b}
D3	28.88 (± 2.18)^{bc}	105.00 (± 2.60)^{b}	6.60 (± 0.20)^{b}
D4	36.14 (± 1.80)^{ab}	108.00 (± 2.10)^{b}	6.95 (± 0.20)^{b}
D5	37.81 (± 1.15)^{a}	110.00 (± 1.60)^{c}	5.60 (± 0.10)^{c}
D6	15.68 (± 2.80)^{cd}	105.00 (± 3.40)^{b}	6.38 (± 0.10)^{b}
D7	14.17 (± 1.55)^{de}	92.10 (± 1.50)^{a}	6.33 (± 0.40)^{b}

Different letters indicate significant differences among treatments ($p<0.05$). TPCH - total protein content in the hemolymph.

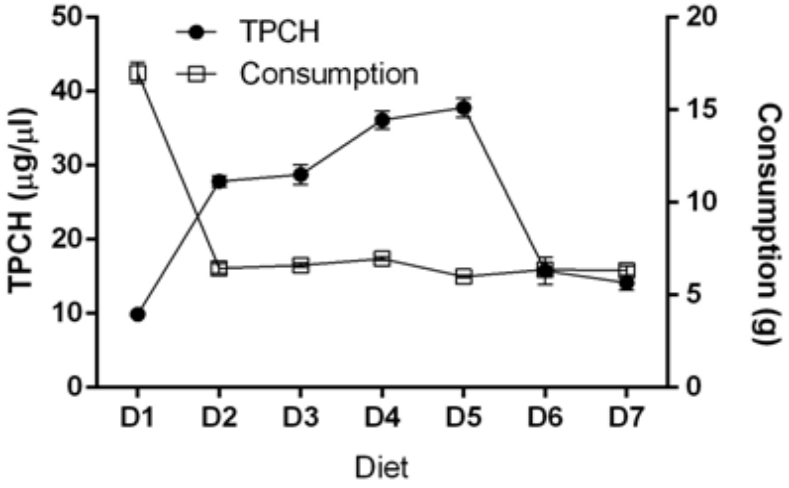

Figure 1 - Correlation between total protein content in the hemolymph (TPCH) and diet consumption among the different diets.

The bees that were fed with the D5 diet had a significantly higher weight than the bees that fed the other diets. The bees fed with diets D3, D4, and D6 showed similar weight values; however, they were superior to the positive control (D2), and D7 bees' weight. The weight values for D7 bees were not significant different from those fed with D1.

The lowest consumption was found for diet D5, while the highest consumption was found in D1 ($p<0.05$), even considering that this was a liquid diet. Among D2, D3, D4, D6 and D7 groups, no significant differences in consumption were detected.

Food supplementation can ensure the continuous development of bee colonies even when the supply of natural food is scarce. Supplementary feeding results in benefits when provided approximately six weeks before the expected nectar flow, increasing the colony population growth, optimizing the utilization of the nectar flow, and resulting in an increase in honey production (WHEELER; ROBINSON, 2014).

Frequently, beekeepers use sugar syrup to obtain population increase. However, the use of sugar is inadequate when there is not an appropriate quantity of pollen and/or protein, in the environment (MORSE, 1976). Traditionally, the main protein substitutes used in bee feed supplements are soy flour, yeast, and milk substitutes (FREITAS; ECHAZARRETA, 2001). The use of commercial pollen also occurs, but its cost and the possibility of transmitting pathogens limit its use as a protein source (DE JONG, 1977).

The preparation of diets through the manipulation of essential amino acids and other ingredients in their pure forms is possible, but extremely expensive, making their use economically unviable. Many protein sources have been used for feeding bees, and availability and costs are determining factors when choosing raw materials (CREMONEZ et al., 1998).

The diets in our study were formulated with commercial sugar cane, textured soybean protein, yeast from beer brewery, and yeast from processed sugarcane, since these materials are inexpensive and easy to obtain.

When all diets were compared to the protein-free control diet (D1), those with higher protein content afforded a higher content of TPCH (D2, D3, D4, D5, D6) (Table 2) as

expected. However, TPCH of D7, which in its composition has ~12% of protein, did not differ significantly of the D1, which did not have any protein in its formulation. Similar results were observed when other authors compared protein diets containing bee bread, soy flour, yeast, pollen with sugar syrup, demonstrating that protein-free diets do not give adequate levels of total protein in the hemolymph (CAPPELARI et al., 2009; MORAIS et al., 2013). They also observed, as expected, that the proteins identified in the hemolymph of bees that fed on sucrose alone differed from those fed with diets contained different protein source (DE JONG et al., 2009; GREGORY, 2006; MORAIS et al., 2013).

In comparisons between the protein results based on the TPCH, the D2 and D3 treatments were not significantly different. These observations suggest that the use of yeast from sugar cane at 35% as protein source provides enough protein as commercial bee feed. Considering that the crude protein content of yeast ranges from 50-60% on a dry weight basis (ZANETI, 1984), this might suggest that ~17 $mg.g^{-1}$ of feed is enough to supply the bee protein necessities. In contrast, the D6 and D7 diets gave lower levels of TPCH, differing significantly from the other protein diets. These results were unexpected, if considering that the difference between diets D3 and D6 was by an increase of 5% in yeast concentration, one should expect an increase or at least the same results in the hemolymph protein content. Comparing the group D7, in which the yeast content was reduced approximately 10% in comparison to D3, one might expect a reduced protein in the hemolymph as we observed. This might suggests that the results in bees fed with the diet D7 are probably due to the low crude protein content of that diet (12.2%), since did not differ from that of bees fed the negative control diet (D1 protein-free diet). According to previous studies, the ideal content of protein for pollen substitutes is between 20 and 23% (HERBERT, 1992). However, we may speculate that the proportions among the diet components might have an effect in the bee metabolism resulting in different protein concentration in the hemolymph.

Also, we observed a difference in bees' weight when comparing the group D7 with groups D3 and D6, in which the group D7 showed a significant reduced weight (Table 2). Nevertheless, no difference in feed consumption among the groups D3, D6 and D7 were observed. The sugar concentration may play a role, since the sugar concentration was 65, 60

and 70% in the groups D3, D6 and D7 respectively. Thus, this might suggest that the ratio between yeast and sugar might play a role, in the absorbance or metabolism, of the protein present in the hemolymph. However, we did not study this hypothesis; since this was not the objective of study.

When we examined the consumption parameter (Table 2), we noted that the highest consumption occurred with the D1 diet (protein-free control). Although consumption was similar to that for most of the protein diets, the bees' weight and the TPCH levels of the bees fed with D7 did not differ from those of the control group D1 or the 0-day-old bees, supporting the previous observation that diet consumption, by itself, does not constitute an adequate method for evaluating diets. The D5 was the most efficient diet, and although it did not differ significantly in the protein present in the hemolymph levels from the D4 diet, it showed greater bees' weight and lower consumption, which means better feed conversion. Considering the difference in crude protein between the D4 (14.6%) and D5 (20.9%), and the similarity in their protein present in the hemolymph levels, the utilization of the D4 can be advantageous because of its simplicity of composition and cost. Additionally, the D4 provided significantly more protein than the positive control diet (D2), with 24.4% crude protein. According the results obtained with the protein concentration in the hemolymph, weight and consumption of the bees could be possible to use a more secure alternative to supplement the diets of the bees. In conclusion, by measures protein in the hemolymph, weight and bee diet consumption, we were able to design a diet that was efficient to supply bees' necessities before the expected nectar flow. Nutrient storage and mobilization are coupled to hormonal signals that include insulin and adipokinetic hormone to fulfill ongoing physiological bee demands (ARRESE; SOULAGES, 2010). D4 and D5 showed the highest correlation between TPCH and diet consumption (Figure 1), thus suggesting that the diet proportion composition might play a role in the bees' metabolism diet. These observations corroborate with Lehner (1983) that report similar diet substitutes effect on honey bee *Apis mellifera*.

CONCLUSION

In summary, the use of protein sources as a supplement for hives can be a safe and viable alternative for beekeepers, considering the use of easily available and low-cost raw material, reducing then the losses during the shortage or absence of natural pollen. As well, the most important conclusion from our findings is that feeding the right supplementary diet, principally considering the use of easily available and low-cost raw materials for commercial beekeepers, can reduce the losses during the shortage or absence of natural pollen.

UTILIZAÇÃO DE DIETAS PROTEICAS COMO SUPLEMENTO PARA ABELHAS AFRICANIZADAS A*pis mellifera*

RESUMO

Na natureza, as abelhas consomem pólen e néctar para obter seus nutrientes (proteínas, lipídeos, carboidratos, vitaminas e minerais). As frequentes mudanças no ambiente devido à expansão de áreas agricultáveis e os reflorestamentos de monoculturas de árvores, vêm afetando alimentação das abelhas e a saúde e a produtividade das colônias. Devido a isso, a eficiência de dietas proteicas artificiais para abelhas africanizadas (*Apis mellifera, L.*) foi avaliada considerando o teor total de proteína da hemolinfa, peso das abelhas e consumo dietético. A proteína bruta das dietas variou de 12,2 a 24,4%. Trabalhadoras recém-emergentes foram mantidas em uma incubadora com temperatura e umidade controladas, até seis dias de idade, recebendo dietas e água *ad libitum*. A hemolinfa foi coletada de trabalhadoras de 0 e 6 dias de idade. Comparando as dietas, foram encontradas diferenças significativas no teor de proteína na hemolinfa, peso da abelha e consumo de alimentos entre os tratamentos. Uma dieta composta por 20% de levedura de cana-de-açúcar, 20% de proteína de soja texturizada e 60% de sacarose foi a mais eficiente de acordo com as medidas da proteína total na hemolinfa, peso das abelhas e consumo. O uso de fontes de proteína como suplemento para colmeias é uma alternativa segura e viável para os apicultores.

Palavras-chave: Abelhas. Proteína. Dieta. Hemolinfa.

UTILIZACIÓN DE DIETAS PROTEICAS COMO SUPLEMENTO PARA ABEJAS AFRICANIZADAS *Apis mellifera*

RESUMEN

En la naturaleza, las abejas consumen polen y néctar para obtener sus nutrientes (proteínas, lípidos, carbohidratos, vitaminas y minerales). Los cambios en el ambiente debido a la expansión de las áreas agrícolas, a la reforestación de cultivos de árboles monocultivo han afectado la salud de las colonias y la productividad. En este sentido, se evaluó la eficiencia de las dietas proteicas artificiales para la abeja africanizada *(Apis mellifera*, L.), considerando el contenido total de proteínas de la hemolinfa, el peso de las abejas y el consumo dietético. La proteína bruta de las dietas varió de 12,2 a 24,4%. Las abejas obreras recién emergentes fueron enjauladas y mantenidas en una incubadora con temperatura y humedad controladas hasta seis días, recibiendo dietas y agua *ad libitum*. Se recogió hemolinfa de obreras de 0 y 6 días de edad. Comparando las dietas, se encontraron diferencias significativas en el contenido de proteínas en la hemolinfa, peso de las abejas y consumo de la dieta entre los tratamientos. Una dieta compuesta de 20% de levadura de caña de azúcar, 20% de proteína de soja texturada y 60% de sacarosa fue la más eficiente según las medidas de proteína total en la hemolinfa, peso de las abejas y consumo de la dieta. El uso de fuentes de proteínas como suplemento para las colmenas es una alternativa segura y viable para los apicultores.

Palabras clave: Abejas africanizadas. Comida. Nutrición. Evaluación nutricional.

REFERENCES

ALAUX, C.; DUCLOZ, F.; CRAUSER, D.; et al. Diet effects on honeybee immunocompetence. **Biology Letters**, v. 6, n. 4, p. 562-565, 2010. doi: 10.1098/rsbl.2009.0986

ARRESE, E. L.; SOULAGES, J. L. Insect fat body: energy, metabolism, and regulation. **Annual Review of Entomology**, v. 55, p. 207–225, 2010. doi: 10.1146/annurev-ento-112408-085356

BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, v. 72, p. 248-254, 1976. doi: 10.1016/0003-2697 (76)90527-3

BRODSCHNEIDER, R.; CRAILSHEIM, K. Nutrition and health in honey bees. **Apidologie**, v. 41, n. 3, p. 278-294, 2010. doi: 10.1051/apido/2010012

CAPPELARI, F. A.; TURCATTO, A. P.; MORAIS, M. M.; et al. Africanized honey bees more efficiently convert protein diets into hemolymph protein than do Carniolan bees (*Apis*

mellifera carnica). **Genetics and Molecular Research**, v. 8, n. 4, p. 1245-1249, 2009. doi: 10.4238/vol8-4gmr628

CHALCOFF, V. R.; AIZEN, M. A.; GALETTO, L. Nectar concentration and composition of 26 species from the temperate forest of South America. **Annals of Botany**, v. 97, n. 3, p. 413–421, 2006.

CRAILSHEIM, K. The protein balance of the honey bee worker. **Apidologie**, v. 21, n. 5, p. 417-429, 1990. doi: 10.1051/apido:19900504

CREMONEZ, T. M.; DE JONG, D.; BITONDI, M. G. Quantification of hemolymph proteins as a fast method for testing protein diets for honey bees (*Hymenoptera: Apidae*). **Journal of Economic Entomology**, v. 91, p. 1284-1289, 1998.

DE JONG, D. Experimental enhancement of chalk brood infections. **Bee World**, v. 57, p. 114-115, 1977.

DE JONG, D.; SILVA, E. J.; KEVAN, P. G.; et al. Pollen substitutes increase honey bee haemolymph protein levels as much as or more than does pollen. **Journal of Apicultural Research**, v. 48, n. 1, p. 34-37, 2009. doi: 10.3896/IBRA.1.48.1.08

DEGRANDI-HOFFMAN, G.; CHEN, Y.; HUANG, E.; et al. The effect of diet on protein concentration, hypopharyngeal gland development and virus load in worker honey bees (*Apis mellifera* L.). **Journal of Insect Physiology**, v. 56, n. 9, p. 1184-1191, 2010. doi: 10.1016/j.jinsphys.2010.03.017

DI PASQUALE, G.; SALIGNON, M.; LE CONTE, Y.; et al. Influence of pollen nutrition on Honey Bee health: do pollen quality and diversity matter? **PloS One**, v. 8, n. 8, p. 1-13, 2013. doi: 10.1371/journal.pone.0072016

ENGELS, W.; KAATZ, H.; ZILLIKENS, A.; et al. Honey bee reproduction: vitellogenin and caste-specific regulation of fertility. In: INTERNATIONAL CONGRESS OF INVERTEBRATE REPRODUCTION - ADVANCES IN INVERTEBRATE REPRODUCTION, 5, 1990, Nagoya. **PROCEEDINGS**. Nagoya, 1990. P. 495-502.

FREITAS, J. C. A.; ECHAZARRETA, C. Fontes de proteína para suplementos de las abejas. In: SEMINÁRIO AMERICANO DE APICULTURA, 15, 2001, Tepic. **ANAIS**. Tepic, p. 48-53, 2001.

GIROU, N. G. **Fundamentos de la producción apícola moderna**. Bahia Blanca: Editorial Encestando SRL. 2003.

GREGORY, P. G. Protein diets and their effects on worker weight, longevity, consumption, and hemolymph protein levels of *Apis mellifera*. In: AMERICAN BEE RESEARCH CONFERENCE, 20, 2006, Louisiana. **PROCEEDINGS**. Louisiana, 2006. Available in:

<http://www.uoguelph.ca/canpolin/Publications/Gregory%202006%20Protein%20diets%20on%20Apis%20mellifera.pdf>

HAGEDORN, H. H.; MOELLER, F. E. The rate of pollen consumption by newly emerged honey bees. **Journal of Apicultural Research**, v. 4, p. 85-88, 1967.

HERBERT, E. W. Honey bee nutrition. In: GRAHAM, J. M.; AMBROSE, J.; LANGSTROTH, L. (Eds.). **The Hive and the Honey Bee**. Dadant & Sons, 1992.

HERBERT, E. W.; SHIMANUKI, H. Brood-rearing capacity of caged honeybees fed synthetic diets. **Journal of Apicultural Research**, v. 16, p. 150-153, 1977.

LEHNER, Y. Nutritional considerations in choosing protein and carbohydrate sources for use in pollen substitutes for the honey bee *Apis mellifera*. **Journal of Agriculture Research**, v. 22, p. 242-248, 1983.

MORAIS, M. M.; TURCATTO, A. P.; FRANCOY, T. M.; et al. Evaluation of inexpensive pollen substitute diets through quantification of haemolymph proteins. **Journal of Apicultural Research**, v. 52. n. 3, p. 119-121, 2013. doi: 10.3896/IBRA.1.52.3.01

MORSE, R. A. **Bees and Beekeeping**. 2. ed. Ithaca: Cornell University Press. 1976.

NAUG, D. Nutritional stress due to habitat loss may explain recent honeybee colony collapses. **Biological Conservation**, v. 142, n. 10, p. 2369-2372, 2009. doi: 10.1016/j.biocon.2009.04.007

RINDERER, T. E.; ELLIOTT, K. D. Worker honey bee response to infection with *Nosema apis*. **Journal of Economic Entomology**, v. 70, p. 431-433, 1977.

RINDERER, T. E.; ROTHENBUHLER, W. C.; GOCHNAUER, T. A. The influence of pollen on the susceptibility of honey-bee larvae to *bacillus larvae*. **Journal of Invertebrate Pathology**, v. 23, n. 3, p. 347-350, 1974. doi: 10.1016/0022-2011(74)90100-1

SAMMATARO, D.; AVITABILE, A. **The Beekeeper's Handbook**, 4. ed. Ithaca: Cornell University Press, 2011.

SATTLER, A. Doenças das abelhas. In: SEMINÁRIO ESTADUAL DE APICULTURA, 6, 2001, Santa Cruz do Sul. **ANAIS**. Santa Cruz do Sul, 2001. P. 37.

SILVA, D. J.; QUEIROZ, A. C. **Análises de alimentos: métodos químicos e biológicos**. 3. ed. Viçosa: Editora UFV, 2006.

SKUBIDA, P.; SEMKIW, P.; POHORECKA, K. Stimulative feeding of bees as one factor in preparing colonies for early nectar flows. **Journal of Apicultural Science**, v. 52, n. 1, p. 65-72, 2008.

SZYMAS, B.; JEDRUSZUK, A. The influence of different diets on haemocytes of adult worker honey bees, *Apis mellifera*. **Apidologie**, v. 34, n. 2, p. 97-102, 2003. doi: 10.1051/apido:2003012

WHEELER, M. M.; ROBINSON, G. E. Diet-dependent gene expression in honey bees: honey vs. sucrose or high fructose corn syrup. **Scientific Reports**, v. 4, p. 5726, 2014. doi: 10.1038/srep05726

ZANETI, R. J. Breathing new life into single-cell protein. **Chemical Engineering**, v. 91, n. 3, p. 18-21, 1984.

Corresponding author:
Fábio Pereira Leivas Leite.
Campus Capão do Leão, Caixa Postal 354, 96010-900, Pelotas – RS, Brazil.
fabio@leivasleite.com.br
fabio_leite@ufpel.edu.br