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REVIEW

The importance of feeding supplementation in *Apis mellifera* honeybee colonies

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Aditional Keywords

Carbohydrate.
Feed.
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Nutritional needs.
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SUMMARY

Food supplementation of Apis mellifera honeybee colonies is, together with management and genetic breeding, one of the bases for high productivity in beekeeping. However, the proper balance of nutrients that make up the diet is essential for supplementation to have productive benefits for colonies and, consequently, for the beekeeper's profits. Nutrients play a key role in a variety of physiological functions of individuals and the colony, including reproduction, growth, development, production and overwinter survival. Food is obtained through the collection of floral resources in nature, but the beekeeper can assist the colony by providing food supplementation during periods of scarcity or during productive activities with a high demand for energy and protein, such as the raising of the queen and the production of royal jelly. Furthermore, well-nourished individuals are more productive and resistant to pests and diseases. Thus, the objective of this review was to present the main nutrients in the honeybee diet and the importance of colony supplementation.

La importancia de la suplementación alimentaria en las colonias de abejas *Apis mellifera*

Palabras Clave

Carbohidrato.
Alimentar.
Suplementación alimentaria.
Proteína.

RESUMEN

La suplementación alimentaria de las colonias de abejas melíferas de Apis mellifera es, junto con el manejo y la cría genética, una de las bases para una alta productividad en la apicultura. Sin embargo, el equilibrio adecuado de nutrientes que componen la dieta es esencial para que la suplementación tenga beneficios productivos para las colonias y, en consecuencia, para las ganancias del apicultor. Los nutrientes juegan un papel clave en una variedad de funciones fisiológicas de los individuos y la colonia, incluyendo la reproducción, el crecimiento, el desarrollo, la producción y la supervivencia durante el invierno. Los alimentos se obtienen a través de la recolección de recursos florales en la naturaleza, pero el apicultor puede ayudar a la colonia proporcionando suplementos alimenticios durante períodos de escasez o durante actividades productivas con una alta demanda de energía y proteínas, como la crianza de la reina y la producción de jalea real. Además, los individuos bien alimentados son más productivos y resistentes a plagas y enfermedades. Por lo tanto, el objetivo de esta revisión fue presentar los principales nutrientes en la dieta de las abejas melíferas y la importancia de la suplementación con colonias.

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INTRODUCTION

Research in the area of honeybee nutrition is on the rise due to the loss of colonies caused by malnutrition and associated pathologies (Erler *et al.*, 2014; Degrandi-Hoffman & Chen, 2015; Maggi *et al.*, 2016). Furthermore, environmental stressors such as pesticide exposure (Battisti *et al.*, 2021), habitat depletion and infection by different pests and pathogens lead to nutritional stress that affects the strength and health of the colony, which contributes to the decrease in the number of colonies, i.e there is a synergistic interaction between factors (Naug, 2009; Klein *et al.*, 2017, Branchiccela *et al.*, 2019).

The nutrients honeybee colonies need for their survival and development are obtained through the floral resources available in nature (Haydak, 1970; Pasquale *et al.*, 2013). Nectar and pollen are the main resources of the colony, as they provide honeybees with carbohydrates, proteins, lipids, antioxidants and essential vitamins and minerals (Haydak, 1970; Degrandi-hoffman & Chen, 2015; Vaudo *et al.*, 2015; Wright *et al.*, 2018; Pudasaini *et al.*, 2020; Ricigliano, 2020).

Nutrition is essential to maintain the balance between the activities performed by honeybees and their immune system (Toth & Robinson, 2005) as healthy honeybees result from the collection of abundant resources, which provide adequate nutrition, leading to a more developed immune system and, consequently, increased resistance to stressors and diseases (Degrandi-Hoffman & Chen, 2015), for example *Varroa destructor* (Currie *et al.*, 2010). It is possible to remedy nutritional deficits, such as a lack of calories or low levels of stored nutrients such as fat, protein and glycogen, by increasing the quantity, quality and diversity of food or by providing food supplementation and thus increase host defence against invasion by pathogens and parasites (Dolezal & Toth, 2018). Nutritional deficiency, even for short periods in the larval and adult phases, can interfere with the queen's pheromone perception behaviour, both positively and negatively (Walton *et al*., 2018).

The growth of a colony is mainly related to the quality and quantity of food available (Škerl & Gregorc, 2014). The main colony members involved in providing nutrition to all of the larvae as well as the queen are the nursing honeybees. As nursing honeybees, they offer two types of food, royal jelly, which is of animal origin, and pollen and nectar, which are of plant origin (Tautz, 2008). The development of these bees is essential for the larvae to receive adequate nutrition and also to increase the colony's resistance to toxic natural or synthetic metabolites (Lucchetti *et al*., 2018), such as the pesticides present in pollen (Böhme *et al.*, 2018). The glands responsible for producing royal jelly in worker honeybees increase in size as nursing honeybees age (Dietz, 1975; Zaytoon *et al*., 1988). However, gland development begins around the seventh day of life and continues until around the 21st day and can vary according to the population size of the colony (Cruz-Landim, 2009).

For this reason, nutritional management practices carried out by the beekeeper, such as protein and energy supplementation, can interfere in the nutrition of bees, and supplementation can thus potentiate the gains by increasing the production of drones, royal jelly, honey and wax; producing queens of better quality; developing colony immunity and, potentially, by helping to maintain colonies in the off-season (Toledo *et al*., 2012; Rousseau & Giovenazzo, 2016; Njeru *et al*., 2017; Oliveira *et al*., 2020). On this basis, the objective of this review is to present the main nutrients in the diet and the importance of supplementation for *Apis mellifera* honeybee colonies.

LITERATURE REVIEW

Carbohydrates

Nectar is the main source of carbohydrates and is collected by honeybees in floral and extrafloral nectaries (Herbert & Hill, 2015) or from excretions of mealybugs that feed on the sap of plants to produce honeydew (Brasil, 2000). The transformation of nectar into honey begins when foraging honeybees find a source of nectar, which, as we can see in **Figure 1**, is a sugary substance with around 80% water, 18% sugars and amino acids, acids, proteins, lipids, minerals and other components (Nicolson & Thornburg, 2007; Roy *et al*., 2017).

The nutritional value of nectar depends on the sugars present in its composition, normally formed by molecules of the disaccharide sucrose and the monosaccharides fructose and glucose (Wright *et al*., 2018). In addition to the main sugars, there are the secondary sugars found in lesser quantities, such as α-maltose, erlose, maltotriose, isomaltose and kojibiose (Moreira & Maria, 2001).

Sugars such as galactose, lactose, raffinose, stachyose, glucuronic acid, galacturonic acid and polygalacin, found in pollen substitute products, are toxic to honeybees (Barker, 1977). In a comparison of galactose and lactose, lactose was associated with high mortality in an accelerated way when used at a concentration of 10% in syrups supplied to honeybees (Sylvester, 1979). In addition, the use of lemon juice in the production of syrups for energy supplementation is not recommended, due to the production hydroxymethylfurfural (HMF), a toxic compound produced by the degradation of reducing sugars and amino acids, added to heating and the acid medium in which the syrup is found (Frizzera *et al*., 2020).Although HMF occurs naturally in the fermentation of honey, its use in the feeding of bees is not desirable because it causes dysentery and ulcers in the gastrointestinal tract, leading to their death (Shapla *et al*., 2018).

Bee colonies need carbohydrates in the form of honey for their maintenance in the amount of approximately 70 kg/year (Herbert & Hill, 2015) up to 120 kg/year (Seeley, 1995). In preference tests, honeybees preferred solutions with 30%–50% sugar concentration (Waller, 1972), which is remarkably like those values found in nectar collected by forager honeybees. Analysis of individuals shows that worker honeybees have different demands during their stay in the colony, in which young honeybees consume a greater amount of protein-rich food to support glandular development, while forage honeybees need greater amounts of carbohydrates (Haydak, 1970; Paoli *et al*., 2014). Metabolically, worker honeybees spend more glucose per meter flown and per gram of body weight during the flight compared to other individuals (Gmeinbauer & Crailsheim, 1993).

Honeybees, when storing food energy as honey, use this reserve to maintain the colony for long periods without a flow of carbohydrates in the environment (Brodschneider & Crailsheim, 2010). In some subspecies of honeybees in their places of origin, this period can last up to 6 months (Brandorf & Rodrigues, 2020). Stored honey is subsequently used as the main energy source for heat production, feeding larvae and flight activity (Seeley, 1995; Pudasaini *et al*., 2020).

Proteins

Pollen provides a large proportion of nutrients to honeybees (Keller *et al*., 2005; Pasquale *et al*., 2013), so it is the main protein source (Singh & Singh, 1996; Brodschneider & Crailsheim, 2010). Pollen contains the 10 amino acids essential for colony development, namely arginine, histidine, lysine, tryptophan, phenylalanine, methionine, threonine, leucine, isoleucine and valine (De Groot, 1953).

The proteins present in pollen are essential for the larval development of all individuals in the colony. The food provided in the early days of the larval stage is rich in proteins (Haydak, 1970), and for the proper hypopharyngeal and mandibular gland development

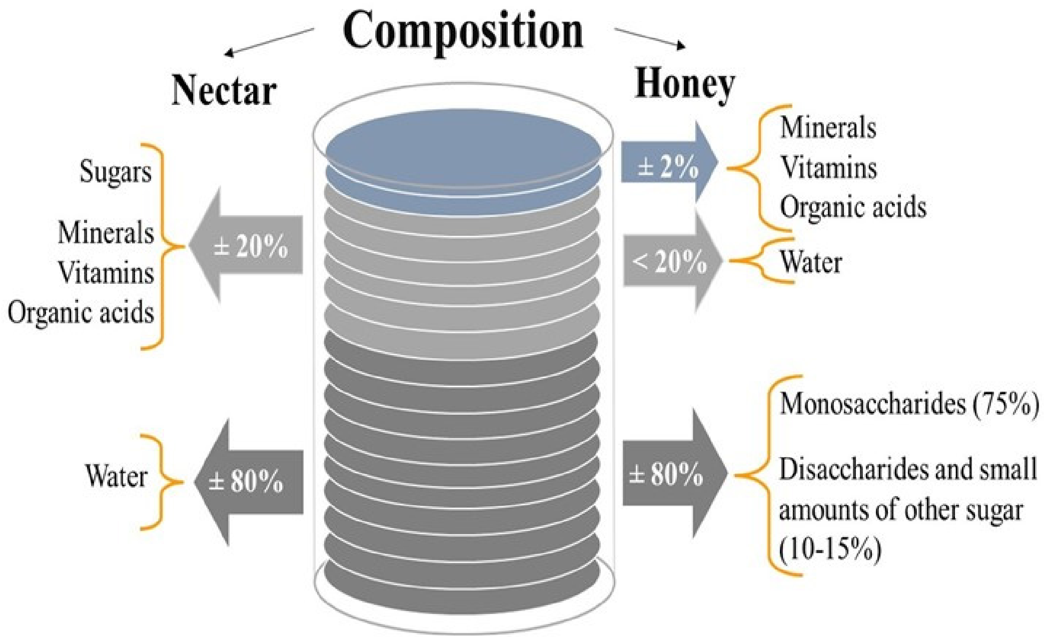

Figure 1. Composition of nectar and honey used by honeybee as an energy source (Crane, 1985) (Composición del néctar y la miel utilizados por las abejas melíferas como fuente de energía).

Camili *et al*. (2020) found that the ideal crude protein content for the development of the mandibular glands is 22.2%. Also, pollen is fundamental for increasing the longevity of newly emerged workers (Wang et al., 2014). Omar et al. (2017) observed that pollen intake lasts until the eighth day of life, with higher consumption occurring 3 days after the workers emerge.

When pollen reserves are low in the colony, the next generation may experience developmental difficulties in development due to a lack of proteins (Brodschneider & Crailsheim, 2010); moreover, colony vulnerability to abiotic and biotic stresses may be increased (Archer *et al*., 2014). The pollen requirement of a colony varies, but for medium sized colonies, consumption is approximately 15–55 kg/year (Seeley, 1995; Tautz, 2008). Regarding pollen consumption, honeybees prefer pollen that has been freshly collected, within approximately one day, rather than stored for longer periods, even without nutritional differences (Carrol *et al*., 2017).

Taha (2015) and Pasquale *et al*. (2016), when collecting pollen in different seasons, observed different levels of protein, which varied according to the floral origin. Thus, the protein content in the pollen ranges from 6% to 61% (Roulston *et al*., 2000; Salazar-González & Díaz-Moreno, 2016) and is mainly linked to the botanical diversity of ecosystems (Nogueira *et al*., 2012). However, when honeybees are infected with the pathogen *Nosema ceranae*, pollen diversity can improve colony resistance (Pasquale *et* 147 *al*., 2013), or the infection can alter the behaviour for higher quality pollen collection (Fergudon *et al*., 2019).

Honeybees prefer food diversity and seek resources to complete their diet in the best way possible (Hendriksma & Shafir, 2016). In this way, honeybees are classified as polyletic, with generalist behaviour due to their ability to collect resources from several plant species (Requier *et al*., 2015). Also, honeybees can differentiate pollen using multisensory information, chemical compounds and olfactory and visual cells (Ruedenauer *et al*., 2018).

Somerville (2005) classified the minimum level of protein in the pollen that honeybees need. Pollen with more than 25% protein was of excellent quality, and a protein content below 20% constituted pollen of low quality. Costa *et al*. (2007) stated that mean pollen protein values of 24.51% during the year favoured good colony development.

Among the various uses of pollen by honeybees, the main one is in the formation of bee bread, a product originating from the handling of pollen by workers and subsequently fermented by microorganisms (Salazar-González & Díaz-Moreno, 2016). The foragers when collecting pollen from flowers take it to the colony; during this process, they deposit glandular secretions and enzymes on the pollen before storage (Barene *et al*., 2015).

The pollen fermentation process is carried out by microorganisms responsible for producing lactic acid, making bee bread more digestible and enriched with new nutrients (Fao, 2009). From the collection of pollen to obtaining the final product, microorganisms are inoculated that carry out fermentation, reduce the pH, and help to preserve the food from other harmful microorganisms (Ellis & Hayes, 2009). In the transformation of bee bread, the pollen pH is reduced from 4.8 to 4.1 (Herbert & Shimanuki, 1978a). The microorganisms responsible for fermentation belong to the genera Lactobacillus and Bifidobacterium (Vásquez & Olofsson, 2009). When collecting pollen, honeybees start the fermentation process as soon as they touch it to place it in the corbicula; that moment marks the beginning of their biochemical changes (Gilliam, 1979). After fermentation, bees consume bee bread, secrete royal jelly and mix it with honey to feed the larvae. At the colony level, bee bread is the main source of nutrients and essential for the development and maintenance of productivity (Tomáz *et al*., 2017).

Lipids

Lipid contents vary according to the pollen source (Nascimentos *et al*., 2019); in particular, long-chain fatty acids such as linoleic acid are found in greater amounts in Brassica pollen (Szczêsna, 2006). Honeybees, like any other insects, need lipids for energy maintenance, growth and reproduction (Arrese & Soulages, 2010). Another important point is the presence of 0.5% sterols in pollen, which are essential for cholesterol metabolism in honeybees (Winston, 1991) and all insects (Canavoso *et al*., 2001; Cohen, 2015). Fuenmayor *et al*. (2014) found a higher amount of alpha-linolenic, palmitic and linoleic acids in pollen samples.

Essential lipids present in the pollen include alpha-linoleic acid (1.61%–53.07%), linoleic acid (4.88%–34.65%) and oleic acid (1.95%–27.81%), known as omega 3, 6 and 9, respectively (Salazar-gonzález & Díaz-Moreno, 2016). The same authors identified that the concentration of lipids in pollen ranged from 0.15% to 20.00%. For brood production, the ideal amount of lipids in the diet is 8% with an omega 6:3 ratio of 0.75; values above this limit decrease brood production and increase the mortality rate (Arien *et al*., 2020).

In supplements formulated from corn flour, soybean meal and yeast using 2%–4% levels of alpha linoleic acid, there was better reproductive performance, digestion and nutrient absorption by honeybees during the spring period (Ma *et al*., 2015). Sereia *et al*. (2010) recommended increasing lipids such as palm oil and linseed oil in supplements formulated for honeybees, having observed a higher life expectancy. Toledo *et al*. (2012) recommended the use of 5% 8% sunflower oil in the composition of supplements to produce royal jelly.

Vitamins

In general, honeybees' vitamin needs are met by pollen consumption, as they find both types of vitamins, water-soluble and fat-soluble, in pollen (Herbert & Hill, 2015). A lack of vitamins in the colony can cease the production of offspring and decrease the survival rate if the deficiency is not corrected in time (Brodschneider & Crailsheim, 2010). Salazar-González & Díaz-Moreno (2016) stated that the most important vitamins are those of the B complex. Herbert & Shimanuki (1978b) fed honeybees with diets deficient in the B vitamins thiamine and riboflavin and observed less development of hypopharyngeal glands in newly emerged honeybees, lower nitrogen concentrations and shorter honeybee longevity, when compared to pollen and casein diets containing the vitamins. Rodriguez et al. (2018) concluded that pollen is an important source of B vitamins and can be exploited to the maximum by the colony. Fratini et al. (2016) in an extensive review obtained data only on the presence of vitamins from the water-soluble group (B and C), mainly from the B5 group in royal jelly. These authors state that the vitamin content varies, mainly according to the diversity of pollen that the honeybees collected.

Minerals

Honeybees use the minerals found in three sources, nectar, water and the main one, pollen. The levels of minerals found in pollen can reach rates of up to 8.3%, with the presence of more than 20 elements (Dietz, 1975). Bonoan *et al*. (2017) found that honeybees prefer water containing minerals over deionized water, indicating that honeybees seek out minerals in water that are not present in the floral diet.

In the royal jelly supplied to worker and queen larvae, there was a difference in mineral contents: the royal jelly supplied to the queen presented higher concentrations of zinc, copper, sodium, potassium and magnesium, which can contribute to the formation of larger individuals (Wang *et al*., 2016). The minerals found in greater quantities in pollen are potassium (K), phosphorus (P), sulphur (S), calcium (Ca), magnesium (Mg) and sodium (Na) (Day *et al*., 1990). In general, minerals improve the physical structure and support physiological functions such as nerve impulses and muscle contraction (Cohen, 2015).

Water

Honeybees collect water from various sources, including lakes, rivers, drinking fountains and dew, and transport it to the colony. The distance travelled to collect water varies according to its availability. What defines the distance travelled is the amount of sugar stored for the flight to the source, as the returning flight is performed using body reserves (Visscher *et al*., 1996). In the implementation of apiaries, the natural existence of water sources close to the colonies or provided by the beekeeper is essential. Dietz (1975) and Seeley (1995) reported that water consumption in temperate conditions can reach 200 g in a day and an estimated 20 kg of water/year/colony. In tropical regions, with an increase in temperature, there is an increase in the amount of forage, which requires a water source in the proximity of the apiary (Seeley, 1995).

Water is used in the colony to maintain the temperature and metabolic pathways of nursing bees in the preparation of larval food (Seeley, 2020). Ostwald *et al*. (2016) observed that honeybees can keep a small amount of water stored for evaporative cooling until the moment that the collection begins, and the foragers bring with them the first loads of water. The need for water collection is regulated according to the increase or decrease in the availability of receiving honeybees in the colony (Kühnholz & Seeley, 1997). The same authors also reported that the behaviour of the receiving honeybees is to distribute the water on the wall of the operculated alveoli brood or to pass it on to other receiving honeybees in the colony, so they concluded that the water is distributed in small puddles in the depressions of the capped alveoli; the other form of use is by exposing a drop of water on the proboscis, generating a larger contact surface for water evaporation.

Food Supplementation

Dolezal & Toth (2018) reported that, in times of food scarcity in nature, it is essential to provide food supplementation, keeping colonies healthy and productive. Morais *et al*. (2013) presented two supplements that can be provided to honeybees in times of scarcity of floral resources, as they present the potential for maintaining the colony (**Table I**). However, the entire supplement must be supplied continuously, and its quantity increased progressively according to the colony's demand, in times of scarcity or in production processes that require a large number of nutrients (Souza, 2019).

There are several ways to provide supplements for honeybee colonies, but they are usually based on energy and protein sources. Oliveira *et al*. (2020) observed that sugar syrup in concentrations of 50% had better results when compared to sugar cane and inverted sucrose syrup in the maintenance of colonies. Sugar syrup is also recommended for colonies where management aimed at wax production is applied, although it is not the least expensive when compared with sugar cane syrup supplementation (Carrillo *et al*., 2015).

One of the precautions when providing sugar syrup is to avoid prolonged periods of heating in its preparation and not to use lemon, as it acidifies the solution and enhances the production of HMF, reducing the lifespan of honeybees (Frizzera *et al*., 2020). A rather common practice among beekeepers is the provision of dry sugar as an energy supplement. However, the use of dry sugar instead of sugar syrup reduces the intake rate of individuals by 50% because the tongues of honeybees are adapted for the consumption of a liquid diet (Liao *et al*., 2020).

Supplementation is also used when products are desired that require protein sources for their production, such as royal jelly (Toledo *et al*., 2012) and queens (Mahbobi *et al*., 2012; Njeru *et al*., 2017; Souza, 2019). Tawfik *et al*. (2020) concluded that colonies supplemented with artificial diet cake + sucrose syrup supplemented with vitamin C for at least 4 months of the year had

Table I. **Type of supplement, composition and effect on an *Apis mellifera* colony** (Tipo de suplemento, composición y efecto sobre una colonia de *Apis mellifera*).

Type of supplementation	Feedstuff	Effects	Reference
Energetic	Milk flour (25%) and refined sugar (75%)	Royal jelly production	Perlin (1997)
Energetic	Sugarcane juice	Production of beeswax	Carrilo et al. (2015)
Energetic	Sugar syrup (50% sugar and 50% water, produced freshly on the same day it was offered to the colony	Colony development in the off-season	Oliveira et al. (2020)
Protein	Sugar powder (40%), milk meal powder (40%) and honey (20%)	Queen honeybee production	Mahbobi et al. (2012)
Protein	Wheatmeal (30%), soymeal (27.20%), cornmeal (33.44%), soy oil (5.00%), limestone (1.32%), salt (0.50%), dicalcium phosphate (1.54%), vitamins (0.50%) and BHT (0.01%)	Increased acceptance of larvae used for the production of royal jelly	Toledo et al. (2012)
Protein	Isolated soy protein (17.50%), linseed oil (4.00%), palm oil (4.00%), brewer's yeast (17.50%), sugar (41.90%), honey (10.00%), pollen (5.00%), lecithin (1.00%) and vitamin nucleus (0.01%)	Royal jelly production	Sereia et al. (2013)
Protein	Soy milk powder (50%), albumin (33.33%), sucrose (16.67%) and sufficient 50% sucrose syrup to make a paste	Colony development in the off-season	Morais et al. (2013)
Protein	brewer's yeast (28.57%), soy milk powder (28.57%), rice bran (14.28%), sucrose (28.57%), and sufficient 50% sucrose syrup to make a paste.	Colony development in the off-season	Morais et al. (2013)
Protein	Corn (19.29%), brewer's yeast (4.60%), soybean meal (38.58%), sucrose (31.99%), alpha-linolenic acid (4%), antioxidant (0.09%), fungicide (0.02%), premix (0.29%), sodium citrate (0.46%), choline chloride (0.46%) and calcium carbonate (0.22%), enough water was added to make a moist patty.	Colony development of colony and prolonged the life of the worker bee	Ma et al. (2015)
Protein	Isolated soy protein (17.50%), linseed oil (4.00%), palm oil (4.00%), brewer's yeast (17.50%), sugar (41.90%), honey (10.00%), pollen (5.00%), lecithin (1.00%) and vitamin nucleus (0.01%) with fermented by microorganisms	Queen honeybee production	Souza, 2019. adapted de Lima et al. (2017)
Protein	Corn (19.45%), corn gluten (12.00%), soybean meal (61.72%), sugar (0.83%), soybean oil (6.00%)	Colony development in the off-season and the mandibular gland	Camilli et al. (2020)

* Laboratory evaluated. ** Not indicated by authors (Some supplements in paste form may be provided on the combs).

better productive parameters, such as brood-rearing and adult honeybee populations.

Among the alternatives used for supplementation to produce royal jelly is milk flour mixed with refined sugar (Perlin, 1999); however, this has been contested due to the presence of milk sugars. Focusing on royal jelly, the mixture of isolated protein (isoflavone, 90% PB), brewer's yeast, honey, sugar, pollen, flaxseed oil and palm oil, among other ingredients, resulted in exceptional colony development (Sereia *et al*., 2013). The use of protein sources in honeybee feeding directly benefits the quality of royal jelly and improves its antioxidant response (Escamilla, 2019).

In lines selected to produce royal jelly, protein supplementation is necessary to optimise the productive potential of the colonies (Santos *et al*., 2019). Toledo *et al.* (2010) did not find satisfactory results from supplementation of the colonies to produce royal jelly using a commercial supplement with 35% crude protein. This negative effect may have occurred due to environmental conditions not being favourable to produce royal jelly and consumption of the supplement or because in this formulation the supplement contained milk sugar. Li *et al*. (2012) stated that the ideal supplement for the maintenance of colonies should have a protein value of around 30%–35%, with a supplement formulated with pollen, sugar and honey. In addition, microalgae are presented as alternatives in the substitution of pollen; these are mixtures with 50% syrup and supplied in the form of paste to bees and have shown results in improving the nutritional status of nursing honeybees (Ricigliano, 2020; Ricigliano & Simone-Finstrom, 2020).

Rousseau & Giovenazzo (2016) supplemented colonies in the spring with a mixture of pollen and soy flour with syrup and obtained heavier drones with a greater abdomen volume and a higher reproductive capacity. Mahbobi *et al*. (2012) found that supplementation with honey, sugar and powdered milk significantly increased most of the queen's morphometric characteristics, such as weight, chest width and length, wing length and spermatheca volumes. Currently, supplements have been sought with a composition as close as possible to that of bee bread. Araneda *et al*. (2014) obtained a supplement like bee bread in 20 days of fermentation. However, Lima *et al*. (2020) obtained fermented protein supplements with characteristics like those of bee bread in 5 days of fermentation, which was an advance in obtaining these supplements. This fermented food and the presence of probiotics promoted an increase in longevity and a lower mortality rate in honeybees kept in the laboratory (Lima, 2017).

Szymaś *et al*. (2012) concluded that probiotic supplements can contribute to better use of nutrients by honeybees. Ricigliano & Simone-Finstrom (2020) reported that the addition of microalgae such as *Arthrospira platensis* as a prebiotic additive promotes health benefits to honeybees. However, further research with honeybees should be carried out to identify the impact of these fermented supplements on the productivity of colonies, both for maintenance in periods of scarcity and for the production of royal jelly and queens.

CONCLUSION

The use of supplementation provides healthier honeybees and a larger population and enables greater gains in the productive period while reducing the cost of replacing colonies.

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