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Stingless Bees Fed on Fermented Soybean-extract-based Diet Had Reduced Lifespan than Pollen-Fed Workers

ACM Queiroz[1], KL Leão[1,2], JCS Teixeira[1], FAL Contrera[2], C Menezes[1]

1 - Laboratório de Botânica, Embrapa Amazônia Oriental, Belém-PA, Brazil

2 - Laboratório de Biologia e Ecologia de Abelhas, Universidade Federal do Pará, Belém-PA, Brazil

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Corresponding author
Ana Carolina Martins de Queiroz
Laboratório de Botânica
Embrapa Amazônia Oriental
Travessa Enéas Pinheiro, s/nº
CP: 18 - CEP: 66095-100, Belém-PA, Brasil.
E-Mail: carolinamqueiroz@gmail.com

Abstract

Nectar and pollen are the basic food resources of stingless bees. The current advance of meliponiculture led to the search for supplementary feeding. Despite little is known about native bee supplementation, several alternative foods have been tested as protein substitutes, with soy being one of the most commonly used. In this study, we compared the effect of a semiartificial soy-based diet versus a natural diet on the longevity of adult worker of *Melipona flavolineata* Friese and *Scaptotrigona* aff. *postica* (Latreille). A total of 200 workers of each species (40 from each colony) were used, of which 100 comprised the control group (consumed honey and pollen) and 100 the experimental group (honey and a semiartificial food based on soybean extract). The workers were divided into groups of 20 individuals confined in MDF boxes not completely enclosed, without a queen, and kept in BOD incubators. Dead bees were counted and removed daily. Kaplan-Meier survival curves were plotted for each species. We found greater longevity in workers who consumed only natural pollen (71 days for *M. flavolineata*, 78 days for *S.* aff. *postica,* in average) compared to those consuming the soy-based diet (62 days for *M. flavolineata*, 61 days for *S.* aff. *postica,* in average). Workers of *M. flavolineata* that consumed pollen lived nine days more (21.8%) than those fed on the soy-based diet, while *S.* aff. *postica* workers lived seven days more (12.7%). As longevity was only slightly reduced, we can recommend a soy-based diet for stingless bees during dearth periods or for supplemental feeding of newly formed colonies.

Introduction

Stingless bees (Apidae, Meliponini) constitute a group of eusocial insects that form perennial colonies, and comprise over 500 species. These species occur in tropical and subtropical regions, where rational breeding for honey production and, more recently, for agricultural pollination is common (Cortopassi-Laurino et al., 2006; Slaa et al., 2006; Michener, 2013). The management of stingless bees for honey production is the most traditional, but the potential for agricultural pollination has greatly increased the demand for new colonies (Cortopassi-Laurino et al., 2006; Jaffé et al., 2015). However, several obstacles must be surpassed to enable the large-scale production of meliponine colonies, including the development of an efficient artificial diet (Venturieri et al., 2012; Menezes et al., 2013).

The bees obtain the nutrients necessary for their development and activities from nectar and pollen collected from the flowers (Roubik, 1989; Brodschneider & Crailsheim, 2010). The nectar is composed basically of sugars and, therefore, is the energy source of the adult workers. Pollen is much more nutritionally complex, being the main source of proteins, lipids, vitamins and minerals. Pollen is used to feed the larvae, as well as newly emerged workers, for the development of glands (mandibular and hypopharyngeal) needed to produce larval food (Cruz-Landim & Akahira, 1966; Nogueira-Neto, 1997).

The stingless bees store the pollen in cerumen ('wax') pots (Nogueira-Neto, 1997). Within the colonies, this pollen is subjected to the action of microorganisms that change considerably its flavor, odor, color and texture transforming it into what we know as "saburá" (Camargo et al., 1992; Souza et al., 2004; Menezes et al., 2018). The quality of pollen can affect bee longevity (Schmidt et al., 1987), ovarian activation (Hoover et al., 2006; Human et al., 2007; Pirk et al., 2010) and physiological mechanisms (Alaux et al., 2011; Di Pasquale et al., 2013). The lack of pollen, therefore, directly affects colony development by reducing larval food production, nutrient intake of egg-laying queens, and the number of offspring, consequently reducing colony health (Cruz-Landim, 2009).

Nectar has a simpler composition, and therefore has been easily replaced by beekeepers by sugar-rich artificial feed made from sugar cane (Nogueira-Neto, 1997). However, pollen is not so easily replaced because of its nutritional complexity, and fully replacing it is still a challenge (Venturieri et al., 2012). The pollen collected and processed by stingless bees is indeed very complex but very little is known about it yet. The knowledge is still limited to their general composition such as protein, carbohydrates and lipids content; nothing is known about micronutrients. This is an important gap to find better ingredients to complement their nutritional needs (Silva et al., 2006; Rebelo et al., 2016; Hartfelder & Engels, 1989; de Oliveira Alves & Carvalho, 2018; de Oliveira Alves et al., 2018). Several alternative foods have been tested as protein substitutes. Penedo et al. (1976) observed a regular hypopharyngeal glands development of *Scaptotrigona postica* (Latreille) workers fed with a mixture of yeast (25%) and pollen (75%). Zucoloto (1976), also studying *S. postica* workers, chose a mixture of yeast (18%) and sucrose (82%) as a good alternative for pollen among eight substitute compositions tested. Still for this species, a mixture of 75% pollen from *S. postica* and 25% pollen from *A. mellifera* was a satisfactory pollen substitute (Testa et al., 1980). A fermented mixture of yeast, pollen and sucrose solution (50%) showed equivalent results in hypopharyngeal glands development to *Scaptotrigona depilis* (Moure) workers, compared to the natural diet (Fernandes-da-Silva & Zucoloto, 1990).

Soybean is one of the most commonly ingredient used, because of the low cost and wide availability in the market. In the genus *Melipona,* studies showed an efficient semi-artificial composition based on soybean extract, sucrose solution and pollen. Costa and Venturieri (2009) pointed out no differences in the acini and oocytes sizes of *Melipona flavolineata* Friese workers, compared to natural diet (pollen). Pires et al. (2009) recommended the use of a similar composition (soybean extract, 30% sucrose solution and pollen) to the *Melipona fasciculata* Smith workers, in scarcity periods.

The main parameters evaluated so far were the development of the ovaries and of the hypopharyngeal glands of the workers. However, other parameters should also be used to efficiently evaluate the nutritional value of protein diets for stingless bees, especially at the colony level (e.g., the effect on worker longevity, colony brood cell production rate and larval development), despite the importance of the aforementioned factors in evaluating an artificial protein diet (Menezes et al., 2012).

Therefore, the objective of this study was to compare the effects of a semi-artificial soybean extract diet on the longevity of workers of two stingless bee species, *M. flavolineata* and *S.* aff. *postica*. These species are widely kept by local beekeepers in the Brazilian state of Pará. *Melipona flavolineata* is a species of restricted distribution in Brazil, occurring in the states of Pará, Maranhão and Tocantins, while *Scaptotrigona* aff. *postica* has a much broader distribution (Bahia, Ceará, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Pará, Pernambuco, Piauí, São Paulo, Tocantins), being commonly called "canudo" (Camargo & Pedro, 2013).

Material and Methods

Experimental design

The experiments were conducted at the Meliponiculture Laboratory, Eastern Amazon Embrapa, Belém, Pará, Brazil (1°26'11.52''S, 48°26'35.50''W), from June to October 2013. Newly emerged workers obtained from 10 nests of the scientific bee nursery (herein 'meliponary') of the Eastern Amazon Embrapa were used to evaluated the effect of the consumption of a semiartificial soy-based diet on the survival of workers of the species *M. flavolineata* and *S.* aff. *postica* (five nests per species). A total of 200 bees were used for each species (40 per colony), of which 100 were used in the control group (natural pollen) and 100 for each experimental group (soy).

The workers were collected as soon as they emerged (first day of age) to form groups of 20 individuals from different colonies, because few workers emerge from a single comb in a single day, especially in *M. flavolineata* combs. They were placed in MDF (medium density fiberboard) boxes (8.2 x 8.2x 3.5 cm), without a queen. Boxes were kept in BOD incubators (model DL-SEDT 02) at 28 ± 1 °C and Relative Humidity between 70% and 80%, from emergence to death of all the bees. The number of live bees was monitored and dead bees were removed daily. In addition, the trash was removed and replaced, and water added to maintain moisture (adapted from Costa & Venturieri, 2009).

The workers were submitted to two diets, soy-based and pollen-based, according to the treatment used. Bees on the soy-based diet consumed its own honey and semi-artificial food based on soybean extract (recipe below) stored in a refrigerator at 4 °C for the experimental period, while those on the pollen-based diet consumed honey and pollen from the colonies of the species itself, stored in a freezer at – 6 °C throughout the experimental period. Bees received approximately 0.1 mL of honey and 0.15 g of soy-based food or pollen per bee per day, depending on the treatment. Food stocks were renewed daily.

Semi-artificial food used in experiments

The production of the semi-artificial food based on soybean extract followed Costa and Venturieri (2009), and Pires et al. (2009), with some modifications. The food consists of 500 g soybean extract, 500 mL sucrose syrup diluted to 50% and 50 g fermented pollen. Pollen was collected from the colony pots of the study species and used immediately. The 500 g of soybean extract was homogenized in 500 mL of diluted syrup at about 60 °C. 50 g fermented pollen was added when the temperature of the mixture was reduced to 28 °C. The food was kept in an oven at 28 °C for 15 days and homogenized daily to enable oxygen penetration and facilitate the fermentation process. Once ready, the food was refrigerated at 4 °C during all the experiment period.

Data analysis

Kaplan-Meier survival curves were plotted for each treatment, per species. The log-rank test was applied using the software Statistica® 8.0, to compare the survival curves of the treatments, for each species. A 5% significance level was considered for all analyses.

Results

The bees used the protein food (pollen or soy) for consumption throughout the experimental period. Despite consumption was not measured, we could observe a higher consumption in the first days of bee life. In some boxes food pots were built by bees, in these cases, they were filled of honey.

The effect of the semi-artificial diet on the longevity of the bee species *M. flavolineata* and *S.* aff. *postica* was observed, and a higher mean longevity was recorded for the control groups (pollen treatment) than for the experimental groups (soy treatment) (Table 1).

The *S.* aff. *postica* individuals that consumed pollen exhibited a greater longevity, surviving on average 17 days more than individuals on the soy treatments. Similarly, individuals of the species *M. flavolineata* survived on average 9 days more than individuals on the soy treatment (Table 1).

Table 1. Longevity in days (mean ± standard error) for bees of the species *Melipona flavolineata* and *Scaptotrigona* aff. *postica* confined in group of 20 individuals and submitted to dietary treatments. Pollen (control): bees fed honey and its own pollen, Soybean: bees fed honey and a semi-artificial food based on soybean extract. It was applied a Log rank test using 5% significance level.

Species/Treatment	n	Pollen (days)	Soybean (days)	p
Scaptotrigona aff. *postica*	200	78 ± 22.50	61 ± 11.90	<0.001
Melipona flavolineata	200	71 ± 43.56	62 ± 30.07	<0.001

The maximum longevity of *S.* aff. *postica* was recorded in an individual submitted to pollen treatment (control) (121 days), while the maximum in the soy treatment was 83 days. Bee mortality occurred from the 9th day for the soy treatment, and from the 19th day for the control. Comparison of survival curves showed that a highly significant difference in results (log-rank test, $p < 0.01$; Fig 1).

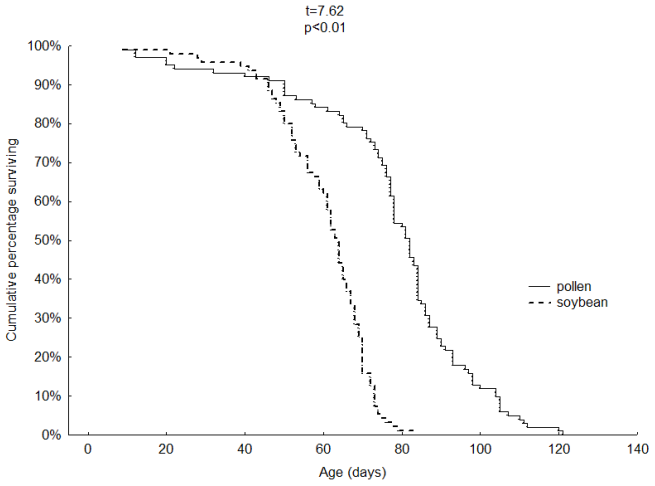

Fig 1. Survival curves for *Scaptotrigona* aff. *postica* confined in group of 20 individuals and submitted to dietary treatments. Pollen (control): bees fed honey and its own pollen, Soybean: bees fed honey and a semi-artificial food based on soybean extract. The value t refers to log rank test using 5% significance level (p).

The longest that *M. flavolineata* lived in the control treatment was 141 days compared to 138 days in the soy treatment, Bee mortality began on the 5th day in the pollen (control) treatment, and on the 6th day in the soy treatment. The difference among survival curves of each treatment was highly significant (log-rank test, $p < 0.01$; Fig 2).

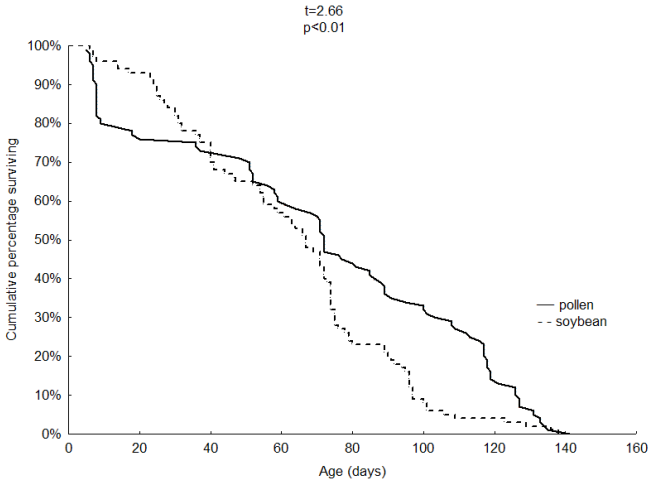

Fig 2. Survival curves for *Melipona flavolineata* confined in group of 20 individuals and submitted to dietary treatments. Pollen (control): bees fed honey and its own pollen, Soybean: bees fed honey and a semi-artificial food based on soybean extract. The value t refers to log rank test using 5% significance level

Discussion

In this study, the longevity of workers that consumed exclusively natural pollen was greater than of those that consumed the soy-based diet. The *M. flavolineata* workers fed on pollen lived 22% more than those that consumed soy-based diet, while the *S.* aff. *postica* workers lived 13% more. On the one hand, it is a positive result as it shows that the soy-based diet did not drastically affect the longevity of adult bees (Camargo, 1982; Gomes et al., 2015), and therefore it is a good basis for the elaboration of an artificial diet. However, it is also evidence that the diet still requires enhancements to fully meet the nutritional needs of stingless bees.

Improvement of artificial diets is essential in the breeding activity of these bees, to assist the growth of newly formed colonies and to strengthen them during periods of food shortage. Pollen availability is essential for the development of the colony and to increase population size. Pollen availability also plays a key role in bee's morphology and physiology and may affect longevity if it is not suitable (Ramalho et al., 1998). As in other social insects, an altered longevity of the workers will affect the growth rate and final size of the colony, which can be increased both by a higher birth rate and/or by a decreased mortality rate of workers (Carey, 2001).

Bees require basic nutrients such as carbohydrates and lipids (non-essential nutrients), amino acids, vitamins and minerals throughout their life stages. Pollen is the main protein source (Souza et al., 2004; Menezes et al., 2018), and acts directly on offspring production, larval development and longevity of adult individuals (Sagili & Pankiw, 2007; Brodschneider & Crailsheim, 2010; Alaux, 2011; Frias et al., 2016). In this study, differences in the nutrient concentration in the food offered to the bees could justify the increased longevity found for bees fed on natural food (pollen). The higher protein concentration in natural food may be one of the causes of the differences in longevity found between treatments.

Little is known about chemical composition of pollen collected by stingless bees (Rebelo et al., 2016), included these species studied. Bee pollen usually has a high protein content. Three Amazonian species of *Melipona* showed protein content between 15.7 and 23.8 (mean 19.5%) (Souza et al., 2004). Rebelo et al. (2016) obtained the protein percentage mean values of 24 (for *M. interrupta*) and 37.63 (*M. seminigra*).

Costa and Venturieri (2009) verified different results due to protein content in diets. Using a semi-artificial food soy-based (25 g of soybean extract, 25 g of sucrose, 50 mL of water, 2.4 g of processed pollen from *M. flavolineata*) similar to the one used in this study, they reported a protein content of 12%, lower than protein bees requirement of 20% (Souza et al., 2004; Somerville, 2005). *M. flavolineata* workers fed with this diet had smaller acini than workers fed with natural pollen, however no differences were reported in oocytes development (Costa & Venturieri, 2009). On the other hand, *M. flavolineata* workers fed with a different composition soy-based diet (43 g soybean extract, 14 g of sucrose, 43 mL of water, 2.4 g of pollen) that reach a protein content of 18%, presented greater development of the hypopharyngeal glands and oocytes, being even superior to the control treatment (pollen). Probably it is due to the higher energetic value of soybean compared to pollen.

The higher protein concentration in natural food may be one of the causes of the differences in longevity found between treatments. Somerville (2000) observed that higher protein levels in the body of *A. mellifera* workers resulted in greater longevity. In another important species for beekeeping in the states of Pará and Maranhão, *M. fasciculata*, a higher longevity has been reported in workers submitted to a semi-artificial diet (a mixture of soybean extract, sucrose syrup diluted to 30% and pollen) compared to bees that consumed natural pollen (Pires et al., 2009). These authors present a formula that can be offered during periods of flower shortage with no apparent damage to colonies.

Semi-artificial food based on soybean extract has proven to be a viable option for critical periods of scarcity of natural resources, or even to be used in large-scale colony production despite the reduction in longevity. Teixeira (personal communication, April 02, 2018) compared the size of individuals submitted to the aforementioned treatments and reached the conclusion that individuals fed on soy were larger than those that consumed natural food (pollen) and reported no differences in the survival or size of immature *M. flavolineata* individuals when compared to those that consumed natural food (pollen).

Similar longevity-reduction results were obtained for *S.* aff. *postica* individuals that consumed soy-based food compared to natural food consumption (pollen), although precise data for the species are lacking. The physical and chemical compositions of the food tested possibly resemble those found for *M. flavolineata* (Costa & Venturieri, 2009).

Besides the concern of ensuring a minimum percentage of components (e.g., proteins), an excess of elements must also be considered. The minerals sodium, sodium chloride and calcium, for example, may be toxic to honey bees (*A. mellifera*), affecting the production of offspring (Somerville, 2005). Therefore, the addition of new ingredients to the artificial diet needs to be done with caution. Future studies are important in trying to match the components to the nutritional needs of bees.

Until this optimal composition is reached, the results obtained in this study contribute by reinforcing the recommendation to use semi-artificial food based on soybean extract for stingless bees in periods of scarcity of natural resources or for newly formed colonies.

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Author's Contribution

ACMQ, KLL, JCST and CM conceived the experimental design; ACMQ, KLL and JCST collected and analyzed data; FALC assisted with data analyses; ACMQ and KLL wrote initial draft of the manuscript and all authors contributed to subsequent revisions and gave final approval for publication.

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