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Development of colonies of uruçu stingless bees fed a vitamin-amino acid supplement

Desenvolvimento de colônias de abelhas sem ferrão uruçu alimentadas com o suplemento aminoácido vitamínico

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ABSTRACT

This study proposes to investigate the influence of a vitamin-amino acid supplement on the weight of colonies of uruçu stingless bees (*Melipona scutellaris*). The experiment was carried out with 24 colonies and three treatments, which consisted of a solution of different proportions of supplement (0, 3, and 5 mL) diluted in syrup (water and sugar). Although this supplement is effective and indicated for other species of domestic animals, analysis of variance with repeated measures over time did not reveal a significant effect (P > 0.05) of its dose on the weight of the hives, showing that the supply of the vitamin-amino acid supplement does not meet the nutritional requirements of the colony. The use of this product did not have a positive effect on the development of the uruçu bee colonies, so it should not be employed as a major source of amino acids and vitamins in the diet of bees. Beekeepers are suggested to provide uruçu bees with an abundant diversity of plants so that they have access to different types of pollen as a source of nutrients.

Keywords: Artificial feeding, *Melipona scutellaris*, Meliponini, Pollinators

RESUMO

O objetivo do presente estudo foi verificar a influência do suplemento aminoácido vitamínico no peso de colônias de abelhas sem ferrão Uruçu (*Melipona scutellaris*). O experimento foi realizado com 24 colônias e três tratamentos, consistindo na oferta de uma solução de xarope (água e açúcar) diluído com diferentes proporções de 0, 3 e 5 mL de suplemento. Embora este suplemento seja eficaz e indicado para outras espécies

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animais de domésticos, a análise de variância com medida repetida no tempo não apresentou efeito significativo (P> 0,05) do nível desse suplemento sobre o peso das colmeias, mostrando que a oferta do suplemento aminoácido vitamínico, não supre a necessidade nutricional para a colônia. Conclui-se que o uso desse produto não surtiu efeito positivo no desenvolvimento de colônias de abelhas Uruçu (*Melipona scutellaris*), indicando que não deveria ser utilizado como fonte majoritária de aminoácido e de vitaminas na alimentação das abelhas. Sugere-se que os meliponicultores proporcionem às abelhas Uruçu (*Melipona scutellaris*) uma abundante diversidade de plantas a fim de que tenham acesso a diferentes tipos de pólen como fonte de nutrientes.

Palavras-chave: Alimentação artificial, *Melipona scutellaris*, Meliponini, Polinizadores

INTRODUCTION

Melipona scutellaris bees (Apidae, Meliponini), popularly known as uruçu, are a species of stingless bees. Their rational exploitation constitutes an activity that generates products such as honey, propolis, geopropolis, and pollen, which can be used to promote health (Gabriele et al., 2015; Rao et al., 2016). However, the period of low flowering impairs bee survival due to nutritional deficiency, which compromises the development, maintenance, and lifespan of colonies (Arathi et al., 2018). These effects, in turn, can cause weakening or even loss of these colonies (Holanda-Neto et al., 2015; Leach & Drummond, 2018). Because pollen is the main source of proteins, amino acids, lipids, starch, sterols, vitamins, and minerals, its consumption is one of the factors that most influences the lifespan of bees (DI PASQUALE et al., 2013).

According to Tôrres et al. (2021), many beekeepers have been using artificial feeding to maintain the queen's egg-laying state and minimize colony losses during these low-flowering periods. Some of the most used alternative protein sources are soy extract, bee pollen, and food supplements containing amino acids, vitamins, and minerals (Contrera et al., 2015; Teixeira et al., 2020). Nevertheless, in *Apis mellifera* L. bees, some ingredients used have already been proven to be toxic. Among them are the sugars contained in the soy used as a pollen substitute (Barker, 1977) and lactose, which led to an increase in mortality of adult bees (Sylvester, 1979).

At present, the market offers commercial products rich in amino acids, vitamins, and minerals for several species of domestic animals, which are indicated as a food supplement in abnormal situations in which supplementation may be required to meet their needs. In the case of beekeepers, many use these products empirically to feed their colonies by adding them to sugar syrup. However, no studies have been found that prove their effectiveness in stimulating the growth and development of stingless bee colonies.

The present study was thus undertaken to examine the influence of vitamin-amino acid supplementation on the weight and development of uruçu bee colonies.

MATERIAL AND METHODS

The experiment was conducted at the meliponary of the Federal University of Bahia (UFBA), in Salvador - BA, Brazil, from January 12 to March 8, 2020. This is a period of lower nectar and pollen availability in the region, which was chosen so that the nutrients derived

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mostly from the treatments offered to the colonies. At the site, a completely randomized design was set up with three treatments, each of which consisted of eight colonies, totaling 24 nuclei, installed in vertical wooden INPA-type hives. All colonies were formed by uruçu bees (*Melipona scutellaris*).

The "minimum disturbance" method was used to form the experimental nucleus colonies (Oliveira & Kerr, 2000). Twelve colonies with a strong population, with brood, honey, and pollen present in the nest and upper nest, were selected at random. By separating the lower nest from the upper nest, two nuclei were created per colony, with the queen being present in one of them, thus generating the 24 nucleus colonies that make up the experiment.

In dividing the colonies, all received two honey pots, two pollen pots, and three brood combs of approximate size. The lighter-weight nuclei were equalized with wax. To evaluate artificial diets in *Apis mellifera* L. bees, most studies adopt the parameter of the development of the ovaries or the hypopharyngeal glands of worker bees. However, the application of this methodology in meliponids is questionable, since there are biological-physiological differences between these two groups that are directly affected by protein diets (Fernandes-da-Silva et al, 1993; Gosto & Venturieri, 2009). Thus, we opted for the methodology of weighing the nuclei colonies, as we consider that the amount of brood between treatments would be more uniform. Moreover, the "minimum disturbance" method of forming new colonies causes less damage and represents less risk in the parent colonies, as it keeps the entire structure and internal constructions of the colony intact (Oliveira & Kerr, 2000). To prevent any treatment from having more nucleus colonies with queens and their performance being influenced, care was taken to ensure that both treatments had the same number of colonies with queens. Nucleus colonies without the queen remained in the place of origin, whereas nucleus colonies with queens were transferred to a distance greater than 10 m.

To determine the development of the experimental colonies, we chose not to adopt the method generally used to evaluate the development of A*pis mellifera* L. bee colonies, which consists of measuring the colony's brood area (Castagnino et al., 2006). In colonies of uruçu bees, this methodology would be unfeasible, since the broods of this species are fragile and vertically overlapped, and their removal would damage their internal structures (combs, honey pots, and pollen pots). For this reason, we decided to employ the methodology of daily weighing of colonies throughout the experiment, a method considered by Chiari et al. (2002) and Oliveira et al. (2015) as a viable criterion to assess the development of stingless bee colonies.

The treatments consisted of supplying 0, 3, and 5 mL of the amino acid-vitamin supplement Promotor ®L 47 added to a syrup solution that was prepared from a mixture of water and sugar (2:1 ratio) and agitated until no solid particles of sugar were observed. These supplement proportions were determined after previous trials discovered that supplement quantities greater than 5 mL in one liter of syrup resulted in leftovers in the feeders.

Every seven days, up to 56 days, the nuclei were fed 50 mL of their respective treatments in plastic pots inside the hive. Before each supply to the feeders, the

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nucleus colonies were weighed on a digital scale. There was no escape of swarms, dead bees inside the hives, or leftovers in the feeders in either treatment, as the solution was consumed in approximately 72 h.

For statistical analysis, the following model was used:

$$Y_{ijk} = \mu + X_i + T_j + \alpha_{ij}(XT) + \varepsilon_{ijk},$$

where Y_{ijk} is the colony weight record; X_i is the fixed effect of treatment i; T_j is the effect of time j; $\alpha_{ij}(XT)$ is the fixed effect of the interaction between treatment i and time j; and ε_{ijk} is the random residual effect.

Given the correlation between the weights obtained in the same colony at different times, analysis of variance with repeated measures over time was used. The choice of the covariance matrix for this analysis was based on the analysis of different matrices, and the matrix that resulted in the lowest BIC (Bayesian Information Criterion) was chosen. The significance level was set at 5%. When the treatment or time effect was significant, first- and second-degree polynomials were analyzed.

RESULTS AND DISCUSSION

Analysis of variance with repeated measures over time did not identify a significant effect ($P > 0.05$) of vitamin-amino acid supplementation level on hive weight. On the other hand, the time and the treatment × time interaction had a significant effect ($P < 0.05$) on this variable (Table 1). Regression analysis indicated that the time had a linear effect in the 0-mL treatment, but a quadratic effect in the 3- and 5-mL treatments (Figure 1).

Table 1. Colony weight means (standard error) and p-values of the F test for the fixed effects of treatment (T), time (Ti), and treatment × time interaction (T × Ti)

Time (days)	Treatment			P-value		
	0 mL	3 mL	5 mL	T	Ti	T × Ti
7	3781.25 (114.3)	3778.25 (114.3)	3765.86 (122.2)	0.1851	<0.0001	0.0004
14	3587.37 (114.3)	3565.12 (114.3)	3533.43 (122.2)			
21	3534.50 (114.3)	3494.88 (114.3)	3485.00 (122.2)			
28	3613.50 (114.3)	3474.25 (114.3)	3472.57 (122.2)			
35	3780.63 (114.3)	3441.38 (114.3)	3416.86 (122.2)			
42	3835.00 (114.3)	3455.63 (114.3)	3423.43 (122.2)			
49	3940.88 (114.3)	3468.63 (114.3)	3439.43 (122.2)			
56	4108.00 (114.3)	3581.38 (114.3)	3490.29 (122.2)			

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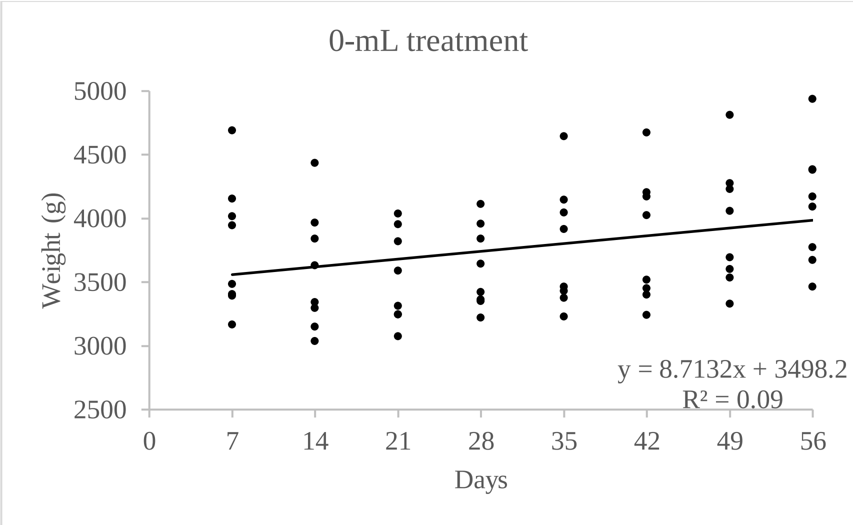

Figure 1. Regression analysis showing colonies in the treatment that did not receive vitamin-amino acid supplementation in artificial feeding

As illustrated in Figure 1, the colonies that received syrup without supplement showed a linear increase in colony weight throughout the experiment. Because these colonies did not receive supplement, the only food resource for their development were sucrose syrup in the feeders, nectar, and pollen from the plants. These results suggest that these colonies searched for pollen in the flowers around the meliponary—the only source of protein for the development of their offspring. This is because, as highlighted by Morgado et al. (2011), pollen is a vital food for the development of larvae, and when stored in the colony, it stimulates the queen's egg-laying behavior and population growth. Protein-deficient diets during the larval stage can lead to malformation of the hypopharyngeal glands of worker bees; therefore, the consumption of pollen or diets with equivalent composition is essential (Pereira et al., 2015).

These findings are in line with results described by Pinto et al. (2018), who tested different protein sources in swarms of Africanized *Apis mellifera* bees in formation. The authors showed that those supplemented with pollen had a significantly higher mean laying area value than those fed invert sugar associated with a vitamin-amino acid supplement.

In terms of the average weight reached by the colonies at the end of the experiment (56 days), the control group reached 4.108 kg with a growing population that allowed the use of an upper nest or a new colony division. This suggests that uruçu colonies fed a syrup of sucrose, nectar, and plant pollen can be divided during this period.

The colonies that received 3 mL of supplement showed a drop in weight soon after hive division, which lasted up to 35 days, followed by an ascending curve (Figure 2). At the end of the experiment, this group reached an average weight of 3.581 kg and had not yet fully occupied the nest, meaning that a longer period of development was necessary to insert an upper nest or divide the swarm.

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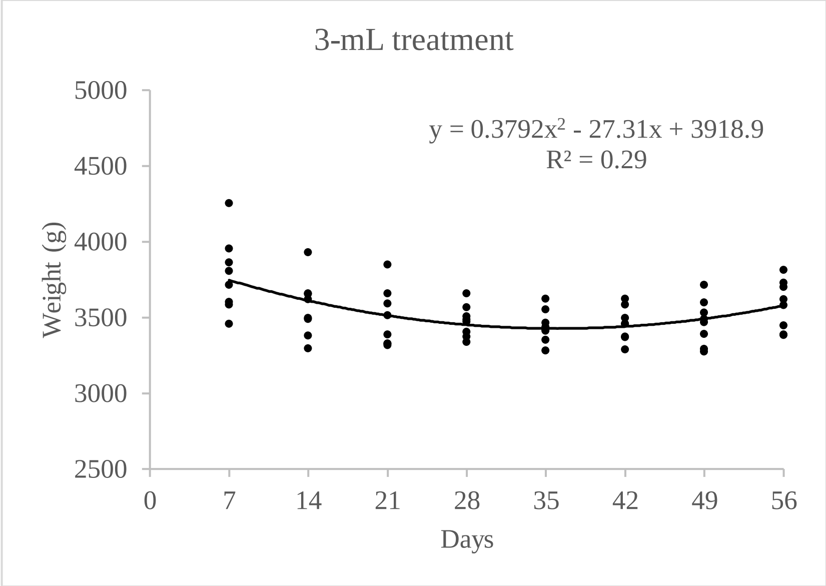

Figure 2. Analysis of colonies that received the vitamin-amino acid supplement

After division, the colonies that received 5 mL of supplement exhibited a decline in weight for 35 days. Thereafter, their weight behaved in a slow upward curve, reaching an average value of 3.490 kg at the end of the experiment, which was numerically smaller than the average weight of the treatment with 3 mL of supplement. This population had also not yet fully occupied the nest (Figure 3).

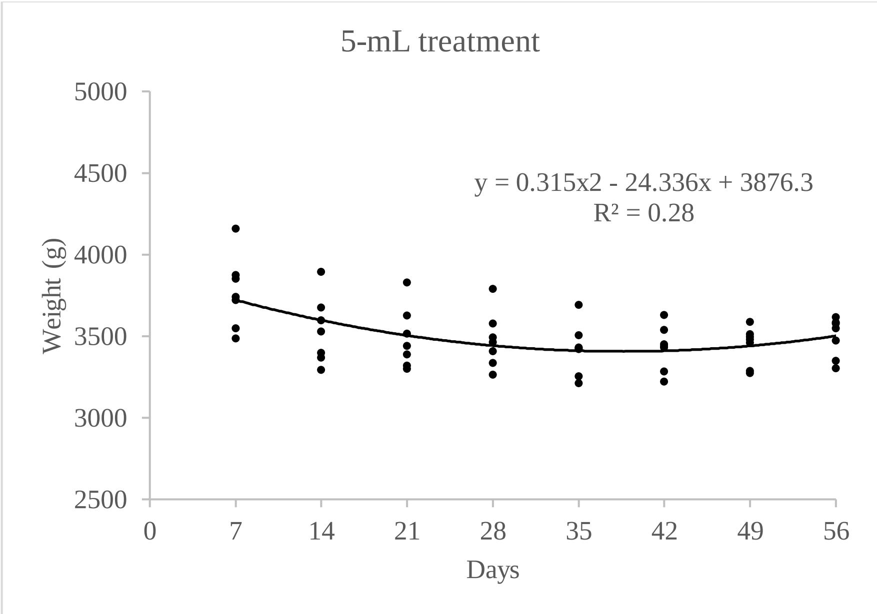

Figure 3. Analysis of colonies that received vitamin-amino acid supplementation after colony division

The data from this study support the theory that at the evaluated doses, the tested **supplement** did not have a positive effect on the weight or

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development of colonies of uruçu stingless bees. Other authors that used the same vitamin-amino acid supplement associated with invert sugar in Africanized *Apis mellifera* swarms found similar results. Castagnino et al. (2006) concluded that the supposedly beneficial effects of its use are actually due to the supply of invert sugar that is offered along with the amino acid supplement. Although the bees are of different sexes, these results corroborate those found in the present study, in which the average weight of developing colonies artificially fed the supplement was significantly lower than that of the colonies fed only sucrose syrup. This result shows that pollen is a vital food for the development of bees and that the supply of the vitamin-amino acid supplement is not capable of replacing it as a source of nutrients necessary for the perfect development of the uruçu colony. These findings are in line with those described by Morais et al. (2013), who stated that complementary feed must contain all the essential nutritional elements to enable the development of the colony, the longevity of the adult bee, and good reproductive capacity. They are also supported by Silveira Neto (2017), who tested the use of syrup with vitamin-mineral supplement in developing colonies. According to these authors, the practice of providing syrup with a vitamin-mineral supplement to bees remains inconclusive, and this product should not replace pollen as the major source of protein and amino acids for colony development.

According to De Groot (1953), bees need ten essential amino acids when their food comes from pollen of varied origins. In *A. mellifera*, a deficiency of any of the essential amino acids interferes with the protein biosynthesis necessary for its development and production (Haydak, 1970). On the other hand, Paoli et al. (2014) stated that high concentrations of amino acids in the diet can be toxic, causing damage to the development of both *A. mellifera* bees and meliponids.

Based on the results of the present study, further research is warranted to investigate vitamin-amino acid supplements that meet the nutritional requirements of stingless bees, in the formulation of specific artificial diets. Our results are in agreement with Pinheiro et al. (2009), who concluded that information on the nutritional needs of stingless bees is scarce and there are no consistent studies to determine nutritional requirements, much less of amino acids, which denotes a window for further research. Another aspect to be considered is that the present results may be an indication that the supplement was not physiologically assimilated by the organism of the stingless bees in a positive manner, as might occur in other species of domestic animals for which the product is indicated.

It is also worth mentioning that although artificial feeding is used in stingless bees as a viable management strategy during natural food shortages, artificial diets never fully replace natural food sources, as demonstrated in the studies by Teixeira et al. (2020) and Goulson et al. (2015).

CONCLUSION

In the dose range tested here, the supply of a vitamin-amino acid supplement to developing colonies of uruçu (*Melipona scutellaris*) stingless bees does not positively influence their development or weight.

In this respect, for a balanced and sustainable nutrition, beekeepers are

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suggested to provide uruçu bees with access to a diversity of plants that offer different and abundant types of pollen as a source of nutrients. The use of supplementation—even if of inferior quality to natural pollen—would only be recommended in case this is not possible.

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