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Royal jelly production in Africanized colonies with selected queens, use of Chinese model cups and supplementation

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ABSTRACT. This research was carried out to evaluate the royal jelly production in two trials. In Trial I, three genotypes of Africanized honeybees (*Apis mellifera* L.) were submitted to royal jelly production with two types of cups of different size and volume (conventional and Chinese model) and two types of supplements (commercial and formulated). A total of 24 colonies were used, two bars with different cups each, alternating between the upper or lower position of the frame. While in Trial II, 18 colonies, two genotypes, two types of cups and two types of supplement were tested. The evaluated parameters were: percentage of larvae accepted in upper and lower bars, royal jelly per cup (mg), and royal jelly per colony/collection (g). Chinese cups were not well accepted, fact that influenced in a negative way the production of general form, selected colonies were more sensible to the change of cups. Colonies of Africanized honeybees are not prepared to produce in cups of greater volume.

Keywords: genetic breeding; productivity; protein ration; performance test; larvae grafting.

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Introduction

Royal jelly is a substance composed of a mixture of secretions of hypopharyngeal and mandibular glands, located in the head of the honeybee workers. It is during the phase known as 'nurse', between the fourth and the twelfth day of life, that the workers reach the full development of these glands and secrete essential proteins that are part of the feeding of all the larvae in the initial phase and the queen throughout her life (Feng, Fang, & Li, 2009). In order to produce royal jelly, nurse workers need to consume pollen, depending also on internal factors such as brood area and population density, and external factors as foraging activity of the forager honeybees to stimulate production. This has a certain flexibility allowing honeybees a rapid adaptation to the needs and conditions of the colony, and a functional transition known as phenotypic plasticity (Ament, Wang, & Robinson, 2010).

Royal jelly production may be an alternative income for beekeepers in regions where honey production is low in periods of low flowering or sugarcane regions where honeydew does not reach the commercial value of floral honey, in addition to being less accepted by consumers. However, when analyzing production with Africanized honeybees, we observe values that are still far from those obtained in China, which currently accounts for 90% of world production, with colonies that produce up to 10 kg per colony per year (Blomstedt, 2013; Sereia et al., 2013).

The bases for improving production are genetic selection, nutrition and management, the technological level is a determining factor and innovations are essential for competitiveness gains (Khan, Matos, & Lima, 2009), supplementation for example increases the acceptance of grafted larvae and royal jelly production (Toledo, Nogueira-Couto, Malheiros, Faquinello, & Sereia, 2012).

As the world's largest producer of royal jelly, China uses in addition to a strain selected for decades, materials and techniques to maximize production such as larger cups that are filled with 650mg of royal jelly and can increase production by 20-30% (Chen, Su, & Lin, 2002). The Apiculture and Meliponiculture Sector of the State University of Maringa practices selection for royal jelly production since 2003,

accompanies the progress through the annual evaluation of the production and introduction of new techniques for adaptation in Africanized honeybees.

Thus, the objective of this research was to evaluate cup models used in China and the conventional ones used in colonies of Africanized honeybees with selected and unselected queens using protein and energetic supplementation.

Material and methods

The research was carried out at the Experimental Farm of Iguatemi of the State University of Maringá (FEI – UEM), in the Apiculture and Meliponiculture Sector, located at 554.9 m altitude, at the following geographical coordinates: 23° 25'South latitude and 52 20' of West longitude, the trial I in the period from November 2014 to May 2015 and trial II in the period from November 2014 to May 2016.

Trial I: A total of 24 mini-hives colonies with queens selected from colonies strains selected for honey production, royal jelly and without selection, two types of cups, conventional acrylic and Chinese model, two types of feed, one commercial (BeeFood®) and another formulated according to Sereia et al. (2010a) with adaptation, totaling 12 treatments (Table 1).

Trial II: It was used 18 mini-hive colonies with daughters of colonies selected to produce royal jelly with better results for acceptance of Chinese model cups obtained in the Trial I and daughters of colonies without selection from captured swarms, submitted to production with two types of artificial cups, conventional and Chinese model, as well as two types of commercial feed (BeeFood®) and another one formulated according to Sereia et al. (2010a) with modifications. Treatments were randomly assigned (Table 2).

For the production of queens, colonies were first standardized in both trials with five brood combs, two combs with storaged pollen, two combs with honey and a cup bar frame; queens were first produced from nine mother colonies selected by molecular marker MRJP3. Eight mini-hive colonies, initiator and terminators were used, each with two parts overlapping and separated by a queen excluder screen. The method used for the production of queens was that described by Doolittle et al. (1889), which consists of the grafting of larvae of workers from the brood comb to artificial acrylic cups containing royal jelly, diluted to 50%. Grafted larvae had less than 36 hours for better acceptance (Abd Al-Fattah, EI-Basiony & Mahfouz, 2003).

Table 1. Distribution of the 12 treatments among the 24 colonies of Trial I.

Genetic	Position of the Chinese cups	Feed
Selected for royal jelly production (n = 8)	Upper bar (4)	Formulated (2)
		Commercial (2)
	Lower bar (4)	Formulated (2)
		Commercial (2)
Selected for honey production (n= 8)	Upper bar (4)	Formulated (2)
		Commercial (2)
	Lower bar (4)	Formulated (2)
		Commercial (2)
Unselected (n = 8)	Upper bar (4)	Formulated (2)
		Commercial (2)
	Lower bar (4)	Formulated (2)
		Commercial (2)

Table 2. Distribution of the eight treatments among the colonies of Trial II.

Genetic	Position of the Chinese cups	Feed
Selected for royal jelly production (n = 8)	Upper bar (4)	Formulated (2)
		Commercial (2)
	Lower bar (4)	Formulated (2)
		Commercial (2)
Unselected (n = 8)	Upper bar (4)	Formulated (2)
		Commercial (2)
	Lower bar (4)	Formulated (2)
		Commercial (2)

In the upper part of each mini-hive used to produce queens, a cup bar frame was placed, containing 45 cups with larvae of different genealogies properly identified and randomly distributed. Ten days after the

larvae grafting, queen cells were removed from the mini-hives and placed vertically in 20 mL glass bottles with paper and candy-type food, identifying the genealogy and the mini-hive number. Then, they were placed in an incubator to raise queens with temperature between 34 and 35ºC (Medrzycki et al., 2010) and humidity of 60%.

The newly emerged queens were anesthetized with CO_2 and recorded weight at emergence (mg) on a 0.001 g precision scale, selecting queens without defects and weighing more than 200 mg, marking them on the dorsal torax with chosen colors according to their genealogy. They were then housed in JZsBZs™ cages and randomly distributed in queenless hive for fecundation 24 to 72 hours before the introduction of the new queen. At the time of introduction, the combs were inspected for the presence of queen cells and these were destroyed. The queens were accepted and confirmed egg laying after ten to 15 days.

For royal jelly production, the system evaluated in this research was that of mini-hive, composed of two overlapped parts, were formed from the reproduction hive that developed in the period of 50 days in which the population of workers was replaced by daughters of new queen, so that the population evaluated was from the genetics of the introduced queen, the production of royal jelly per colony was evaluated after this period.

The process was similar to that of queen production, in which case the process is interrupted 68-72h after grafting and the royal jelly is collected. To obtain appropriate larvae for the grafting, an empty comb was introduced four days before grafting in the center of different colonies. Thus, at the time of grafting, the comb contained newly hatched larvae with one day of age and/or eggs. After larvae grafting, the grafted cup bars were carefully returned each to their respective mini-hive. Trial I carried out 18,144 larvae grafting and 288 collected samples, and in Tiral II 10,080 larvae grafting and 160 collected samples.

Each mini-hive was composed of nine combs, five combs in the lower body, separated by the queen excluder screen of the second with four combs plus a cup bar frame with two bars. The bars consisted of conventional acrylic cups (10/6 mm diameter x 11 mm height, volume about 600 µL) with 30 cups each or Chinese model (10/10 mm x 13.5 mm, with volume of about 1000 µL) of 33 cups, in alternate positions (upper or lower bar) according to Figure 1, and cover feeder.

Royal jelly collection was performed 68 to 72 hours after grafting with a vacuum pump suction system, without manual contact with the product. The percentage of acceptance of larvae grafted in the upper and lower bars (% of accepted larvae on each bar), weight of the discarded larvae (mg), royal jelly production per colony/collection (g) and royal jelly per cup (mg). The royal jelly collected was weighed in a 0.0001g precision scale, packed in 100g pots and frozen. Then, a new larvae grafting was made.

When the grafted bars were returned to their respective colonies, energy supply was provided with a 1:1 solution of water and sugar (sucrose), plus 38 g of a supplement based on linseed and palm oils, concentrated soybean protein and beer yeast adapted from Sereia et al. (2013), with 16.2% of crude protein (CP) or another commercial (BeeFood®) with 23.7% of CP (Table 3). The feed intake was calculated based on the dry matter, initial dry weight difference and dry weight of leftovers.

The data were analyzed using the statistical software R Development Core Team (R, 2018) with beta regression for cup acceptance variables and multiple regression to explain the other variables. Means were compared by Tukey's test with significance level of 5%.

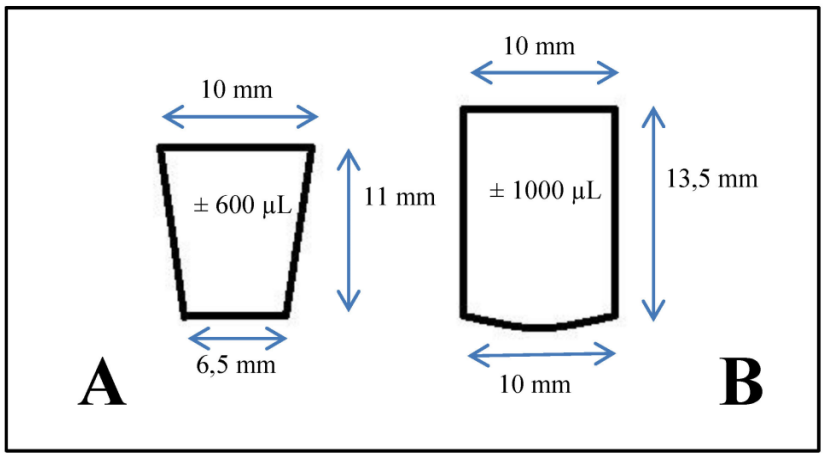

Figure 1. Measures of conventional "A" cups and Chinese model "B".

Table 3. Analyzed chemical composition of the feed provided, dry matter (DM), ash (AS), crude protein (CP), crude fiber (CF), ethereal extract (EE) and gross energy (GE).

Feed	DM (%)	AS (%)	CP (%)	CF (%)	EE (%)	GE (cal g^{-1})
Formulated	87.77	1.86	16.24	0.85	4.22	4133.70
Commercial	74.86	2.90	23.75	1.48	6.89	4080.21

Results and discussion

The inclusion of Chinese cups influenced the data collected in relation to the productive parameters. For either upper or lower bar, with commercial or formulated feed, with selected or unselected honeybees, whenever we had the Chinese cups, larvae acceptance, the amount of royal jelly per cup and the total amount of royal jelly produced per colony in each were low, when they were not zero.

The mean value for weight per cup of royal jelly in Trial I when used the traditional cups was 210.5 mg, slightly lower than 213.5 mg found by Toledo et al. (2010), and the percentage of accepted larvae was 42.4%, almost 45.0% higher than that found by the same authors using unselected Africanized honeybees. While the Chinese cups were not well accepted and had the mean value of 126.42 mg of royal jelly per cup and 7.66% of accepted larvae. Jianke and Aiping (2005) reported that royal jelly production per colony has a positive correlation with the number of larvae accepted ($r = 0.95$), but the amount of royal jelly per cup has a negative correlation with the number of larvae accepted, a fact not observed with this type of cup ($r = 0.14$).

These strains used in this experiment were the same as those tested by Toledo and Mouro (2005) in comparison with Carniolan honeybees, which were superior to accepted larvae, but these did not differ in royal jelly production, which can be attributed to a genetic correlation for these characteristics, which confirms the results of Faquinello et al. (2011), who found a genetic correlation of 0.42 for the selection of better queens for these two characteristics.

In the Trial I the type of feed had no effect on royal jelly production per colony (Table 4). Şahinler, Gül, and Şahin (2005) working with colonies of *Apis mellifera caucasica* obtained for the treatment with supplementation an increase of 19.0% and 28.4% for royal jelly weight per cup and colony, respectively. In this study the commercial and formulated feed influenced larvae acceptance, amount of jelly deposited per cup and feed consumption.

The feed used by Sereia et al. (2010a) was tested for cost benefit and showed improvements in royal jelly production. In another study, Sereia et al. (2010b) found that supplements such as this one, containing a mixture of polyunsaturated fatty acids and saturated fatty acids with linseed oil, palm oil, isolated soy protein and brewer's yeast, resulted in longer longevity and lower mortality rate than supplements made only with sources of polyunsaturated or saturated fatty acids. This justifies the data found by Toledo, Alves, Oliveira, Ruvolo-Takasusuki, and Faquinello (2010) in which the addition of protein supplement (35%) did not increase royal jelly production in colonies of Africanized honeybees. Studying the development of colonies in the region of Maringa, Costa et al. (2007) found crude protein content between 20 and 25% for the period from April to May, justifying the low consumption of protein supplementation at that time.

Garcia and Nogueira-Couto (2005) reported that there are differences in the acceptance of larvae and royal jelly production at different periods throughout the year and this measure is correlated with the production per colony, this increase may be more related to the growth of the colonies that at the end of Trial I had large number of nursing honeybees.

In Trial II, we verified that the genetic group did not influence larvae acceptance and the royal jelly production per colony by collection, only royal jelly per cup ($p < 0.001$), 197 mg/cup with honeybees selected for royal jelly production and 155 mg/cup with colonies unselected (Table 5).

It is clear that one generation was not enough to generate positive effects on all parameters, the great difference being that Africanized honeybees are a poly-hybrid with little selection but adapted to the environment, different from the European ones that are subspecies selected by many years, like the Italian honeybees selected in China for royal jelly production for over 40 years (Ament et al., 2010). Faquinello et al. (2011) observed that royal jelly production suffers great influence of the environment and that the selection to increase the production per colony will increase larvae acceptance and the amount of royal jelly per cup. The type of feed had a significant effect on the amount of royal jelly deposited per cup ($p = 0.0197$) and royal jelly per colony/collection ($p = 0.0353$), with better results on the colonies that consumed the formulated feed.

Table 4. Trial I: Regression analysis for variables of royal jelly production with Africanized honeybees of different genetic groups, with cups of the Chinese model in different positions in the cup bar frame and two types of feed

	Regression models	Significant variables at 5%	R^2 Ajusted
	Beta regression		
TLA	1.36638+0.0392(PC)+0.5158(GEN)+0.2711(SUP)+0.5756(CUP) - 0.0591(Max.T)+0.2538(Min.T)-0.0436(RH)+0.0326(RF)	GEN, SUP, CUP, Min.T, RH, RF	24.07
	Multiple regression		
RJCup	0.95048-0.0243(PC)-0.001(GEN)-0.025(SUP)+0.034(CUP) -0.021(Max. T)-0.06(Min. T)+0.001(RH)+0.001(RF)	PC, SUP, CUP, Max. T	27.24
RJC	14.9619-0.0365(PC)+1.2091(GEN)+0.2513(SUP)+1.1534(CUP) -0.4243(Max. T)+0.6663(Min. T)-0.01682(RH)+0.0933(RF)	GEN, CUP, Max. T, Min. T, RH, RF	26.63
AFI	29.2888-2.1884(PC)-1.4671(GEN)-4.2250(SUP)+1.1838(CUP)-0.8489(Max. T)-1.2345(Min. T)+0.5270(RH)+0.1063(RF)	PC, SUP, Min.T, RH	44.72

TLA- total larvae acceptance (upper and lower bars); RJCup- royal jelly per cup; RJC- royal jelly per colony; AFI- actual feed intake; GEN- genetic group; PC- production cycle; CUP- position of Chinese model cups (upper and lower bar); SUP- type of supplement (formulated or commercial); Max.T- maximum temperature; Min.T- minimum temperature; RH- relative humidity; RF-rainfall (mm).

Table 5. Trial II: Regression analysis for variables of royal jelly production with Africanized honeybees of different genetic groups, with cups of the Chinese model in different positions in the cup bar frame and two types of feed.

	Regression models	Significant variables at 5%	R^2 Ajusted
	Beta regression		
TLA	-2.6712 - 0.0320(PC) - 0.1774(GEN) - 0.2018(SUP) - 0.0546(CUP) + 0.2814(Max.T) - 0.3046(Min.T) + 0.0019(RH) + 0.0203(RF)	Max.T, Min.T	28.92
	Multiple regression		
RJCup	-0.0530 + 0.0011(PC) + 0.0383(GEN) - 0.0219(SUP) - 0.0019(CUP) + 0.0187(Max.T) – 0.0180(Min.T) + 0.001(RH) + 0.0005(RF)	GEN, SUP, Max.T, Min.T	22.63
RJC	-1.3216 – 0.1453(PC) + 0.0498(GEN) – 0.6844(SUP) – 0.4398(CUP) + 0.9144(Max.T) – 0.9566(Min.T) – 0.0093(RH) + 0.0714(RF)	SUP, Max.T, Min.T, RF	36.33
AFI	-1.7886 – 2.2004(PC) + 1.6979(GEN) – 2.2383(SUP) + 1.6850(CUP) - 0.0333(Max.T) + 0.3800(Min.T) + 0.3606(RH) – 0.20005(RF)	PC	47.97

TLA- total larvae acceptance (upper and lower bars); RJCup-royal jelly per cup; RJC-royal jelly per colony; AFI- actual feed intake; GEN- genetic group; PC- production cycle; CUP- position of Chinese model cups (upper and lower bar); SUP- type of supplement (formulated or commercial); Max.T- maximum temperature; Min.T- minimum temperature; RH- relative humidity; RF-rainfall (mm).

Conclusion

One generation was not sufficient to improve the acceptance of larvae in cups of the Chinese model. However, the selected honeybees deposit more jelly per cup than unselected colonies. The bees did not present royal jelly production that would justify the use of higher volume cup and the selection should identify the colonies most suitable for this type of cup. The formulated feed generated greater stimulation to royal jelly production.

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