Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1806-2636
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v35i2.16976

Alternative sources of supplements for Africanized honeybees submitted to royal jelly production

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ABSTRACT. This study was carried out to evaluate the effect of supplements with isolated soy protein, brewer's yeast, a mixture of isolated soy protein with brewer's yeast, linseed oil, palm oil and mixture of linseed oil with palm oil in the production of royal jelly by Africanized honeybee colonies. Total royal jelly production was higher ($p < 0.05$) in colonies fed with isolated soy protein and brewer's yeast (11.68 g colony^{-1}), followed by linseed oil and palm oil (11.30 g colony^{-1}) and palm oil (9.61 g colony^{-1}), being higher than control I (6.35 g colony^{-1}), and II (6.95 g colony^{-1}). The results demonstrated that the supplementation with a mixture of linseed oil plus palm oil, and isolated soy protein plus brewer's yeast is recommended to increase the royal jelly production on a commercial scale. Besides becoming an important tool for beekeepers by increasing the production, it also contributes to improve the acceptance rate of grafted larvae.

Keywords: *Apis mellifera*, honeybee supplementation, brewer's yeast, isolated soy protein, linseed oil, palm oil.

Fontes alternativas de suplementos para abelhas africanizadas submetidas à produção de geleia real

RESUMO. Este estudo foi realizado para avaliar o efeito de suplementos elaborados com proteína isolada de soja, levedo de cerveja, mistura de proteína isolada de soja com levedo de cerveja, óleo de linhaça, óleo de palma e mistura de óleo de linhaça com óleo de palma na produção de geleia real por colônias de abelhas africanizadas. O total de geleia real produzida foi superior ($p < 0,05$) nos tratamentos com proteína isolada de soja e levedo de cerveja (11,68 g colônia^{-1}), seguido do suplemento óleo de linhaça e palma (11,30 g colônia^{-1}) e suplemento palma (9,61 g colônia^{-1}), sendo estes superiores aos tratamentos-controle I (6,35 g colônia^{-1}) e II (6,95 g colônia^{-1}). Essas observações demonstraram que a suplementação feita com uma mistura de óleo de linhaça e de palma, e proteína isolada de soja e levedo de cerveja poderiam ser recomendadas para aumentar a produção de geleia real em escala comercial, além de se tornar uma importante ferramenta para os apicultores, por aumentar a produção, contribuir para aumentar a taxa de aceitação das larvas transferidas.

Palavras-chave: *Apis mellifera*, suplementação de abelhas, levedo de cerveja, proteína isolada de soja, óleo de linhaça, óleo de palma.

Introduction

Honeybees collect nectar and pollen from nature (WINSTON, 1987) and obtain nutritional requirements of protein (amino acids), carbohydrate (sugars), lipid (fatty acids, sterols), vitamin, mineral (salts) and water. Together, they play a key role on the proper development of pupae and adults, which includes the formation of the exoskeleton, glands, body fat and reproduction (MANNING; HARVEY, 2002). The quality and quantity of these nutrients in honeybee diet determine the optimal nutritional level of the colony and hence its productivity and longevity (SEREIA et al., 2010a).

Given the lack of pollen or other alternative resources, honeybees resort to their own spare source, metabolizing body tissue in order to prolong lives (HAYDAK, 1970). The artificial supplementation of the colonies during this period prevents a number of undesirable factors such as inadequate development of hypopharyngeal glands and fat, longevity reduction, imbalance between births and deaths, reduction in the flight distance and reduction in the disease resistance (KELLER et al., 2005).

Royal jelly is secreted in small quantities by hypopharyngeal and mandibular glands located at the head (HAYDAK, 1970) of young worker honeybees (MOURO; TOLEDO, 2004). For its synthesis,

honeybees require carbohydrates, vitamins, fatty acids, minerals and amino acids. Thus, the production has always been carried out in times of abundance of floral resources. To ensure the productivity and profit in shortage periods, supplementation is needed (PEREIRA et al., 2006), providing to the larvae both energy and protein supplements.

Food supplementation reduces the mortality rate, increases lifespan (SEREIA et al., 2010a), profits (SEREIA et al., 2010b) and ensures a continuous development of colonies in places and times of nectar and pollen shortage. Various supplements have been developed to provide nutritional requirements for the royal jelly producers (MANNING; HARVEY, 2002; PEREIRA et al., 2006). Perlin (1999) recommended the utilization of one part of milky meal and three parts of sugar to increase the royal jelly production from December to January, in southern Brazil. Toledo and Mouro (2005) and Faquinello et al. (2011) using colonies and supplements made with other sources of oils and proteins, observed values from 1.68 to 4.70 g for the royal jelly production. Toledo et al. (2003) reported more royal jelly produced per colony (42.04%) receiving a supplement with sunflower oil than others did not receive.

Considering that research on bee nutrition is recent in Brazil (TOLEDO et al., 2010) and that honeybees of this country are result of the cross between European and African honeybees, called Africanized, it is understandable that these differences justify the fact that some studies performed in other parts of the world, may not adequately reflect the needs of these honeybees. Thus, only with the development of production techniques appropriate to the Africanized honeybees, the beekeeping in Brazil may grow and expand, becoming thus competitive.

This research was undertaken to evaluate supplements to Africanized honeybee colonies for royal jelly production and effects on the acceptance of grafted larvae, amount of royal jelly deposited per cell and overall production per colony, investigating a possible association of these variables with the supplements provided, contributing to the knowledge of nutritional requirements of Africanized honeybees in the performance of this activity.

Material and methods

Two experimental assays were developed to test the supplements: the first, from December 2007 to February 2008 and the second, from March to May 2008. The experiment was conducted in three phases:

Preparation of the colonies

Twenty colonies from the beekeeping sector of State University of Maringá, Paraná State, Brazil was used. Another 15 colonies were used to support the experimental units, providing pupae to keep the population of nurse bees and larvae for the grafting. Queen-daughters were randomly produced from several matrix colonies before starting the experiment. Virgin queens, at the same age, were introduced in the experimental colonies and were mated naturally in the air.

The rearing systems were composed of two overlapping nests, separated by a queen excluder. The lower nest had ten combs and the upper nest had eight combs and a cup-holder frame with three bars. The cups were attached to the bar with beeswax: 33 on top bar, 33 on the middle bar and 34 on the lower bar, totaling 100 cups in each experimental unit.

Every ten days the rearing systems were managed: four open brood combs were transferred from the lower nest to the upper nest to keep enough larvae and nursing bees - which care for and feed the grafted larvae, besides making room in the lower nest so that the queen could continue laying eggs.

In both assays, twenty rearing hive (each with 100 cups) were randomly divided into four treatments, with five repetitions per treatment. Hive systems were submitted to royal jelly production for 15 consecutive times (Table 1), totaling 60,000 larvae grafting and 75 observations per treatment during the experimental period.

Table 1. Experimental groups and number of observations for the evaluation of supplements with a mixture of palm oil + linseed oil (SLiPa), linseed oil (SLi), palm oil (SPa), isolated soy protein + brewer's yeast (SPiLc), isolated soy protein (SPi), brewer's yeast (SLc) and controls (C I and C II) from December 2007 to May 2008, in Maringá, Paraná State, Brazil.

Treatment			
Experimental group		Supplement	Number of observations / colonies
Assay I oil group	SLiPa	Palm oil + linseed oil	15
	SLi	Linseed oil	15
	SPa	Palm oil	15
	C I	Non-supplemented	15
Assay II protein group	SPiLc	Isolated soy protein + brewer's yeast	15
	SPi	Isolated soy protein	15
	SLc	Brewer's yeast	15
	C II	Non-supplemented	15

Seventy-five grams of fresh supplement were provided inside the larval rearing system, except

for the control. In each grafting, regardless the treatment, all larval rearing systems were supplemented with 900 mL sugar syrup (1:1).

Preparing the supplements

Supplements were produced using two protein sources (isolated soy protein and yeast), two energy sources (sugar and honey) and two lipid sources (linseed and palm oil).

All supplements had equal proportions of honey, sugar, pollen, lecithin, and vitamin nucleus in their composition. The amounts of ingredients selected to prepare the supplements are listed in Table 2.

The following criteria were used to select the sources: energy, composition of essential fatty acids (DeGROOT, 1953), crude protein, vitamins and minerals (Table 3).

The amount of each ingredient was defined as a function of the chemical composition of pollen collected by honeybees (KELLER et al., 2005) and the chemical composition of ingredients selected taking into consideration that the supplements would be metabolized together with the nectar by nursing bees and secreted in the form of royal jelly. Table 4 shows the calculated chemical composition of supplements.

All supplements received sufficient amount of ascorbic acid to set the pH at 5.1 (MARCHINI et al., 2006) as well as flavours and flavouring to make supplements more palatable and attractive.

Table 2. Quantity of ingredients in 100 g of linseed oil + palm oil supplement (SLiPa), linseed oil supplement (SLi), palm oil supplement (SPa), isolated soy protein + brewer's yeast supplement (SPiLc), isolated soy protein supplement (SPi), brewer's yeast supplement (SLc) and controls I and II, supplied to Africanized honeybees colonies under royal jelly production from December 2007 to May 2008, in Maringá, Paraná State, Brazil.

Treatments	ingredients (g 100 g^{-1})								
	Isolated soy protein	Oils (g)		Brewer's yeast	Sugar	Honey	Pollen	Soybean Lecithin	Vitamin mix
		Linseed	Palm						
				Assay I					
SLiPa	17.5	4.0	4.0	17.5	40.9	10.0	5.0	1.0	0.1
SLi	17.5	8.0	-	17.5	40.9	10.0	5.0	1.0	0.1
Spa	17.5	-	8.0	17.5	40.9	10.0	5.0	1.0	0.1
Control I	-	-	-	-	50.0	-	-	-	-
				Assay II					
SPiLc	17.5	4.0	4.0	17.5	40.9	10.0	5.0	1.0	0.1
SPi	35.0	4.0	4.0	0.0	40.9	10.0	5.0	1.0	0.1
SLc	-	4.0	4.0	35.0	40.9	10.0	5.0	1.0	0.1
Control II	-	-	-	-	50.0	-	-	-	-

Table 3. Chemical composition of ingredients selected to compound the supplements.

Chemical Composition	Unit in 100 g	Selected Ingredients								
		Isolated soy protein	Linseed oil	Palm oil	Brewer's yeast	Sugar	Honey	Pollen	Soybean Lecithin	Vitamin mix
Water	g	4.20	0.00	0.00	8.90	0.03	17.10	16.80	0.00	0.00
Calorie	kcal	350.00	900.00	900.00	269.00	387.00	304.00	405.00	850.00	0.00
Carbohydrate	g	0.00	0.00	0.00	30.40	99.90	82.40	35.00	0.20	0.00
Total Fiber	g	0.00	2.40	0.00	0.00	0.00	0.20	1.10	0.00	0.00
Mineral	g	5.70	0.00	0.00	7.40	0.00	0.60	2.60	0.00	0.00
Calcium	mg	200.00	0.00	0.00	232.00	1.00	6.00	260.00	10.00	0.00
Phosphorus	mg	674.00	0.00	0.00	1597.00	0.00	4.00	430.00	36.00	0.00
Sodium	mg	1000.0	0.00	0.00	605.00	0.00	4.00	200.00	0.00	0.00
Thiamine (B1)	mg	0.30	0.00	0.00	17.60	0.00	0.01	800.00	12.00	22.30
Riboflavin (B2)	mg	0.30	0.00	0.00	6.60	0.02	0.04	1920.00	4.00	160.00
Niacin (B3)	mg	0.40	0.00	0.00	34.60	0.00	0.12	20.00	25.00	980.00
Pantothenic Acid	mg	4.20	0.00	0.00	11.30	0.00	0.07	2600.00	0.00	323.40
Pyridoxine (B6)	mg	0.80	0.00	0.00	1.60	0.00	0.02	380.00	0.00	81.70
Cyanocobalamin (B12)	mg	0.00	0.00	0.00	0.00	0.00	0.00	500.00	0.00	0.80
Folic Acid	mg	0.10	0.00	0.00	0.00	0.00	0.00	1850.00	0.00	8.00
Biotin	mg	0.00	0.00	0.00	0.00	0.00	0.00	0.70	0.00	1.60
Vitamin A	mg	1.00	0.00	0.00	0.00	0.00	0.00	590.00	0.40	70.00
Vitamin E	mg	10.80	0.00	0.00	0.00	0.00	0.00	20.00	240.00	400.00
Total Lipid	g	0.00	100.00	100.00	1.40	0.00	0.00	6.20	40.00	0.00
Palmitic Acid (C16:0)	g	0.00	0.00	43.50	44.90	0.00	0.00	28.70	11.70	0.00
Oleic Acid (C18:1)	g	0.00	27.00	36.60	33.90	0.00	0.00	2.90	18.00	0.00
Linoleic Acid (C18:2)	g	0.00	16.00	9.10	5.10	0.00	0.00	5.40	0.00	0.00
Linolenic Acid (C18:3)	g	0.00	57.00	0.20	0.60	0.00	0.00	49.50	0.00	0.00
Crude Protein	g	90.00	0.00	0.00	49.00	0.00	0.30	26.20	0.00	0.00

Source: USDA (2006).

Table 4. Chemical composition calculated of linseed oil + palm oil supplement (SLiPa), linseed oil supplement (SLi), palm oil supplement (SPa), isolated soy protein + brewer's yeast supplement (SPiLc), isolated soy protein supplement (SPi), brewer's yeast supplement (SLc) and controls (C I e C II), supplied to Africanized honeybee colonies under royal jelly production from December 2007 to May 2008, in Maringá, Paraná State, Brazil.

Chemical Composition	Unit in 100 g	Supplement					
		SLiPa	SLi	SPa	SPiLc	SPi	SLc
Water	g	4.9	4.9	4.9	4.9	4.0	5.7
Calorie	kcal	397.8	397.8	397.8	397.8	411.9	383.6
Carbohydrate	g	56.2	56.2	56.2	56.2	50.9	61.5
Total Fiber	g	0.2	0.3	0.1	0.2	0.2	0.2
Mineral	g	2.5	2.5	2.5	2.5	2.2	2.8
Calcium	mg	89.7	89.7	89.7	89.7	84.1	95.3
Phosphorus	mg	419.7	419.7	419.7	419.7	258.2	581.2
Sodium	mg	291.3	291.3	291.3	291.3	360.4	222.2
Thiamine (B1)	mg	43.3	43.3	43.3	43.3	40.2	46.3
Riboflavin (B2)	mg	97.4	97.4	97.4	97.4	96.3	98.5
Niacin (B3)	mg	8.4	8.4	8.4	8.4	2.4	14.4
Pantothenic Acid	mg	133.0	133.0	133.0	133.0	131.8	134.3
Pyridoxine (B6)	mg	19.5	19.5	19.5	19.5	19.4	19.6
Cyanocobalamin (B12)	mg	25.0	25.0	25.0	25.0	25.0	25.0
Folic Acid	mg	92.5	92.5	92.5	92.5	92.6	92.5
Biotin	mg	0.0	0.0	0.0	0.0	0.0	0.0
Vitamin A	mg	29.7	29.7	29.7	29.7	29.9	29.6
Vitamin E	mg	5.7	5.7	5.7	5.7	7.6	3.8
Total Lipid	g	9.0	9.0	9.0	9.0	8.7	9.2
Palmitic Acid (C16:0)	g	11.1	9.4	12.9	11.1	3.3	19.0
Oleic Acid (C18:1)	g	8.8	8.4	9.2	8.8	2.9	14.7
Linoleic Acid (C18:2)	g	2.2	2.4	1.9	2.2	1.3	3.1
Linolenic Acid (18:3)	g	4.9	7.1	2.6	4.9	4.8	5.0
Crude Protein	g	25.7	25.7	25.7	25.7	32.8	18.5

Source: USDA (2006).

Royal jelly production

The modified Doolittle's method (DOOLITTLE, 1899) was used for royal jelly production, which consists of the grafting of larvae from their original cells to the acrylic cells. Twenty-four hours before grafting, the frames with cup bars were introduced into the colonies so that the material would have the smell of the colony and waxing of cups.

The larvae for grafting were selected from brood combs of rearing colonies and were younger than 36 hours old.

When removed from the colonies they were immediately covered with clean, damp cloth and carefully carried to the lab, thereby maintaining the quality of the larvae until the end of grafting. Meanwhile, the lab temperature was kept at 34 ± 2ºC and humidity 50 to 60%. At grafting each cup received a drop of royal jelly diluted in distilled water (1:1).

The cup bar frame was removed from the colonies 62-68h after grafting. The number of accepted larvae in the upper, middle, and lower bars as well as the total number were counted and recorded for assessing the acceptance and the amount of royal jelly produced. The wax was removed from the cups, and the larvae were removed with the aid of tweezers; later on, the bars were weighed on an analytical scale. The royal jelly was removed with a vacuum suction system, and then the empty bars were weighed again.

The royal jelly yield was calculated after each harvest, obtaining in this way the ratio between the amount of supplement consumed (g) per the amount of royal jelly produced (g). The royal jelly was stored in labeled vials, protected from light at -20°C.

The data for the supplemented larvae were calculated based on the adding up of the provided amounts with a total of 5,625.00 g of supplement per treatment, except the control.

Statistical analysis was run using the Statistical Analysis System (SAS, 2008). The data regarding the treatment effects on the number of cups accepted on the upper, middle, and lower bars as well as the total number (%), total acceptance (%), total weight of royal jelly produced by treatment (g) and the amount of royal jelly deposited per cup (mg) were subjected to an analysis of variance (ANOVA). The mean values were compared by the Tukey's test at 5% probability.

Results and discussion

The Table 5 shows the means and standard deviation of the number of grafted larvae in cup bar frame in the upper, middle, and lower (%), total weight of royal jelly produced by treatment (g), amount of royal jelly per cup (mg), F values with respective probabilities (P), coefficients of variation (CV%) of 20 Africanized honeybee colonies supplemented and not supplemented (controls).

Table 5. F values with respective probabilities (P), coefficients of variation (CV%) and means of the number of accepted larvae in cup holder on the upper, middle, and lower bars (%), acceptance (%), royal jelly produced per treatment (g), royal jelly per colony (g) and royal jelly per cup (mg) in Africanized honeybees colonies in two experimental assays supplemented with a mixture of palm oil + linseed oil (SLiPa), linseed oil (SLi), palm oil (SPa), isolated soy protein + brewer's yeast (SPiLc), isolated soy protein (SPi), brewer's yeast (SLc) and controls (C I and C II) from December 2007 to May 2008, in Maringá, Paraná State, Brazil.

Treatments	Number of accepted larvae / bar			Acceptance (%)	Royal jelly / Treatment (g)	Royal jelly / Colony (g)	Royal jelly / cup (mg)
	Upper (%)	Middle (%)	Lower (%)				
				Assay I (December 2007 to February 2008)			
F values	3.59 p = 0.0001	4.92 p = 0.0001	8.50 p = 0.0001	7.71 p = 0.0001	9.83 p = 0.0001	7.46 p = 0.0001	3.52 p = 0.0001
CV (%)	36.86	34.62	34.94	28.83	4.98	34.97	42.91
SLiPa	20.11a[1] (± 5.95)[2]	21.12a (± 6.37)	22.23a (±6.32)	63.45a (±15.86)	847.21a (± 6.87)	11.30a (±3.68)	182.93a (± 51.03)
SLi	16.56b (± 5.79)	17.53b (± 6.09)	15.61b (± 7.48)	49.71b (± 15.69)	698.17b (± 6.87)	9.31b (±2.85)	198.67a (± 64.66)
SPa	14.91b (± 6.76)	15.88b (± 6.80)	15.01b (± 6.83)	45.80 b (± 16.65)	630.01b (± 6.87)	8.40b (±3.39)	199.33a (± 110.55
C I	15.67b (± 6.87)	16.73b (± 6.93)	17.20b (± 6.36)	49.60b (± 17.43)	476.60 c (± 6.87)	6.35c (±2.61)	136.53b (± 75.27)
Average I	16.81	17.82	17.51	52.14	663.00	8.84	179.37
				Assay II (March to May 2008)			
F values	2.57 p = 0.0008	2.04 p = 0.0097	4.88 p = 0.0001	3.89 p = 0.0001	7.56 p = 0.0001	5.83 p = 0.0001	2.74 p = 0.0003
CV (%)	34.86	32.94	36.67	28.64	4.87	33.53	42.77
SPiLc	20.76a (± 5.98)	21.32a (± 6.21)	21.67a (± 6.85)	63.75a (± 16.32)	876.06a (± 3.72)	11.68a (±3.72)	190.65a (± 60.00)
SPi	16.81b (± 5.55)	18.03b (± 5.50)	15.48b (± 7.48)	50.32 b (± 14.97)	720.71b (± 2.75)	9.61b (±2.75)	201.07a (± 60.00)
SLc	15. 96b (± 6.85)	18.07b (± 6.38)	16.92b (± 6.50)	50.95b (± 15.52)	708.73b (± 3.29)	9.45b (±3.29)	200.05a (±110.00)
C II	17.03b (± 6.21)	17.81b (± 6.74)	17.33b (± 6.62)	52.17 b (± 16.73)	521.20c (± 2.76)	6.95c (± 2.61)	141.77b(± 70.00)
Average II	17.64	18.81	17.85	54.30	706.68	9.42	183.05

[1]Means followed by the same letter in the column are not statistically different ($p > 0.05$) by Tukey's test. [2]Numbers in parenthesis show the standard deviation of the mean.

The total amount of supplements provided and consumed during the six-month experimental period was 5,625.00 g treatment^{-1}, which corresponds to an average consumption of 25 g of supplement colony^{-1} day^{-1}. This result is much higher than reported by Faquinello et al. (2011) with 2.21 g day^{-1}. In this study, honeybees have accepted the six supplements indiscriminately, without presenting any evidence of rejection.

Various supplements have been developed to provide the nutritional requirements for royal jelly producers, (MANNING; HARVEY, 2002; PEREIRA et al., 2006). Avilez and Araneda (2007), working with five types of supplements, reported that honeybees adequately consume syrup, pollen and milk substitute, but not soy and quinoa. These authors attributed these variations to digestibility and particle size between studied supplements.

Observations made in this research allow inferring that harvesting, storage and consumption of supplements by honeybees were similar in all treatments, possibly because: a) they had equal levels of lipids and energy in the composition (Table 4); b) provided feeding stimulation due to the addition of pollen and honey; c) were mixtures with sensory characteristics suitable for taste, consistent granulometry; d) presented in the composition all essential amino acids required by honeybees (DeGROOT, 1953) and, finally, protein levels close to those of pollen, according to Costa et al. (2007) in the same region of this study.

There were differences ($p < 0.05$) for the percentage of total acceptance of grafted larvae with a mixture of linseed oil + palm oil and isolated soy protein + brewer's yeast that had respectively 63.45 and 63.75% of accepted cups when compared with palm, linseed, isolated soy protein, yeast and control I and II (45.80, 49.71, 50.32, 50.95, 49.60 and 52.17% respectively).

The percentage of accepted cups in the upper, middle and lower bars differed ($p < 0.05$) between studied treatments. Supplementation for colonies subjected to royal jelly production helps to increase the acceptance of grafted larvae (GARCIA; NOGUEIRA-COUTO, 2005). Toledo et al. (2010) reported the larvae acceptance and royal jelly production per cup had a positive correlation with the maximum relative humidity and negative correlation with the minimum relative humidity.

The average amount of royal jelly/colony and total amount of royal jelly/treatment had differences ($p < 0.05$) between treatments. The values observed in assays I and II, respectively, for mixed isolated soy protein + brewer's yeast (11.68 g colony^{-1}; 876.06 g treatment^{-1}) and linseed oil + palm oil (11.30 g colony^{-1}, 847.21 g treatment^{-1}) were the highest ones. With the exception of the palm oil supplement, all the others had a higher average production per colony and total than the control I and II (6.35 g colony^{-1}; 476.60 g treatment^{-1} and 6.95 g colony^{-1}, 521.20 g treatment^{-1}, respectively). These results represent a production increase of 40.50 and 43.74% for the mixed treatment when compared with control I and II, respectively.

These observations corroborate Faquinello et al. (2011), who reported that given the unavailability of nectar, providing supplement benefits the production of royal jelly.

Szczêsna (2006) reported that fatty acids stored in tissues of insects in the form of oils or fats, with few exceptions, are usually long-chain, and can be saturated such as the palmitic and stearic, or unsaturated as oleic, linoleic and α-linolenic acid, and also observed high concentrations of fatty palmitic, oleic and α-linolenic acid in the pollen collected by honeybees. These data suggest that the supplementation with palm + linseed oils could provide to Africanized honeybees a normal metabolism with several positive consequences, such as, improved royal jelly production. Experiments of Manning and Harvey (2002) evidenced that the addition of polyunsaturated fatty acids in diets prepared with pollen and flours significantly promoted the acceptance and royal jelly production. The polyunsaturated fatty acids are essential components of honeybee diet (SOMERVILLE, 2005).

Therefore, Sereia et al. (2010b) concluded that is economically feasible to supplement Africanized honeybees in royal jelly production.

The mean results (Table 5) were higher than observed by Mouro and Toledo (2004), Garcia and Nogueira-Couto (2005). Toledo and Mouro (2005) and Faquinello et al. (2011) using colonies and supplements made with other sources of oils and proteins, observed values of 33.15 to 43.40% of acceptance; 1.68 to 4.70 g for the royal jelly production per experimental unit and 119.90 to 234.00 mg of royal jelly per cup. Nevertheless, Toledo et al. (2010) reported that by adding protein supplement (35%) has not increased the royal jelly production by Africanized honeybee colonies, and its use for this purpose was unprofitable.

The average amount of royal jelly deposited every 68h per cup was higher with isolated soy protein, brewer's yeast, palm oil, and linseed oil, and lower with mixed sources of oils and proteins. With the exception of the colonies supplemented with a mixture of linseed oil + palm oil, all other treatments differed ($p < 0.05$) from controls I and II (Table 5). Thus, the percentage of acceptance had more influence on the total amount of royal jelly produced than the amount of royal jelly per cup (Table 5).

Faquinello et al. (2011) used honeybees in overlapped nucs and a supplement with 24% of crude protein, to estimate genetic, phenotypic and (co)variance components for royal jelly production. They obtained means of 1.60 and 62.31% lower than obtained in this study for the percentage of total larvae acceptance and the royal jelly production per colony, respectively, and 20.77% higher for the royal jelly production per cup.

Ballesteros and Vásquez (2007), comparing the royal jelly production in colonies of different sizes without supplementation, found a production of 6.36 g per grafting, an acceptance of 51% and an average production of 208.00 mg in rearing systems with ten combs. These values were lower probably owing the difference in the type of larval rearing system and the variations of oil sources and protein used in the supplementation.

Throughout the assay period, in the absence of queen or a virgin queen, caused by accidental death or deliberate change before starting of each experimental phase, the supplement intake was virtually insignificant when compared with larval rearing system under normal conditions.

From the production data reported in Table 5, the treatment yield was calculated (ratio of supplement consumption/royal jelly production) which was 6.48, 6.70, 7.87, 8.01, 8.13 and 9.01 g g^{-1} for the isolated soy protein + brewer's yeast, linseed oil + palm oil, isolated soy protein, brewer's yeast, linseed oil and palm oil, respectively.

Conclusion

Although the six supplements had been similarly consumed, they provided different benefits in terms of royal jelly production. Our observations demonstrated that the supplementation with a mixture of linseed oil + palm oil, and isolated soy protein + brewer's yeast is recommended to increase the royal jelly production on a commercial scale. Besides becoming an important tool for beekeepers by increasing the production, it also contributes to improve the acceptance rate of grafted larvae.

Acknowledgements

The authors thank to the Brazilian National Research Council (CNPq, process no. 303345/2008-0 and 479329/2009-5) and Araucaria Foundation (Fa, protoc. #15095 conv. 422) for financial support.

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Received on April 28, 2012.
Accepted on June 21, 2012.