

LIFESPAN OF AFRICANIZED HONEY BEES FED WITH VARIOUS PROTEIC SUPPLEMENTS

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S u m m a r y

This research was carried out to evaluate the nutritional quality of five supplements elaborated with linseed oil, palm oil, isolated soy protein and beer yeast. The study was performed about the life span of Africanized honey bees, confined into experimental cages maintained in incubator, at 32°C and relative humidity of 70% for 76 days. The supplements containing a mixture of polyunsaturated and saturated fatty acids with linseed oil, palm oil and/or isolated soy protein and beer yeast were frequently used, resulting in a larger life span and smaller mortality rate than supplements elaborated only with sources of polyunsaturated or saturated fatty acids. Variations of fatty acid sources are expected and desirable in supplements to Africanized honey bees.

Keywords: *Apis mellifera*, beer yeast, isolated soy protein, linseed oil, palm oil, supplementation.

INTRODUCTION

As soon as emerging, honeybees require different nutrients to complete the final development of their tissues, muscles and glands (Herbert Jr., 1997). Proteins, carbohydrates, minerals, lipids, vitamins and water are supplied primarily by nectar, pollen and water (Free, 1993). Nectar is their primary source of carbohydrates and pollen provides all the other essential nutritional elements (Winston, 1987). However, worker honeybees gradually use the resources stored in the combs in periods of low availability of pollen. When these are depleted, they use proteins and lipids of their own tissues to produce larval food and survive for a short period of time (Haydak, 1970; Winston et al., 1983).

For these occasions, several supplements made with soybean meal, fish flour, yeast and lactalbumin (Herbert and Shimanuki, 1980) have been evaluated. However, few studies have been conducted so far to estimate the nutritional quality of resources used and this has been a cause of concern for researchers and beekeepers. The supplement evaluation can be accomplished by a variety of measures and related observations: total honey production, daily production of larvae, individual productivity and longevity of worker honeybees (Winston et al., 1983).

A good supplement should be collected and after being ingested must provide the nutritional elements essential for growth, development of the colonies, longevity

and good productivity capacity (Haydak, 1945; Moeller, 1967; Standifer et al., 1973; Doull et al., 1980; Herbert and Shimanuki, 1980; Winston et al., 1983).

Depending on the chemical composition of the provided supplement, it is possible that the performance of the colonies may vary. Therefore, the objective of this study was to evaluate the nutritional quality of alternative supplements prepared with different sources of protein and oil through the assessment of intake, establishing preference, mortality rate and lifespan of Africanized honeybees confined in cages at 32°C and 70% relative humidity.

MATERIAL AND METHODS

The supplements were prepared: one mixture of them (Palm oil, Linseed oil, Isolated soy protein and Beer yeast), two sources of oil (linseed and palm), one non-supplemented (control I), two of protein (isolated soy protein and yeast), and one only polifloral pollen (control II). Three replications for each treatment were made with a total of 21 cages. Each experimental unit was represented by a cage with 125 newly-emerged Africanized workers. The distribution of treatments is presented in Table 1.

Supplement preparation

The supplements were produced using two protein sources (isolated soy protein and yeast), two energetic ones (sugar and

honey) and two lipids (linseed oil and palm oil). Energetic value, composition of essential fatty acids, crude protein, vitamins and minerals were considered for source selection (Tab. 2). Sufficient amounts of conservative ascorbic acid were added to all for adjusting pH to 5.1. Smell and flavoring substances were also added to make supplements more palatable and attractive, as well as pollen, lecithin and vitaminic nucleus.

The pollen used in the supplement composition was obtained by pollen traps placed in the hive entrance of Africanized honeybee colonies at the same place of this experiment. After harvesting, the pollen was dehydrated in the incubator with air circulation at 65°C for 24h, ground, sieved and packed into polyethylene bags and kept at -20°C.

The amount of ingredients selected for supplement preparation is presented in Table 3. Table 4 presents the calculated chemical composition of supplements.

Every three days, dead worker honeybees in each cage were removed and counted, and, also the water, syrup, pollen and supplement were replaced (Fig. 1).

Cage preparation

The worker honeybees were obtained from ten sealed brood combs taken from Africanized colonies, daughters of sisters-queens to reduce the genetic variation for lifespan test (Milne Jr., 1980). They were immediately placed into perforated paper

Table 1

Elaborated supplements with a mixture of palm oil, linseed oil, isolated soy protein and beer yeast (SLiPaPiLc), linseed oil (SLi), palm oil (SPa), isolated soy protein (SPi), beer yeast (SLc), non-supplemented (CI) and the polifloral pollen (CII) for evaluating the lifespan of Africanized honeybees

Treatments	Supplements	Number of cages
SLiPaPiLc	Palm oil, Linseed oil, Isolated soy protein and Beer yeast	3
SLi	Linseed oil	3
SPa	Palm oil	3
CI	Non-supplemented	3
SPi	Isolated soy protein	3
SLc	Beer yeast	3
CII	Polifloral pollen	3

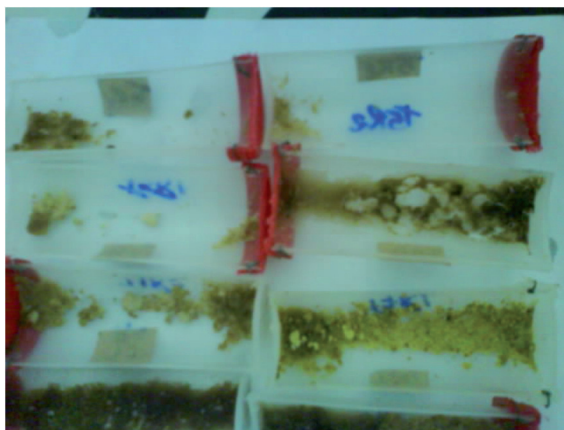


Fig. 1. Remnants of the supplements provided during the lifespan test

envelopes and left in the incubator at 32-35°C and at 70% relative humidity until to emerge.

The tests were conducted in triplicate, transferring 125 newly-emerged worker honeybees from the comb to each cage (9 x 6 x 15 cm). These cages were identified and in each one was supplied with water, a piece of beeswax foundation, sugar syrup and water (1:1) and the supplement (Fig. 2).

The supplements, approximately 3 g (Fig. 2), were supplied in polyethylene containers measuring 24 mm in diameter and 70 mm long, placed at the base of cages. The evaluated supplements were replaced every three days by fresh ones and the consumption was recorded.

Supplement intake

The average cumulative consumption of supplements was calculated as the sum of the differences in weight observed between the initial amounts provided (3 g on average) and the surplus recorded every three days during the experimental period. The consumption records were carried out until the 30th day of the trial period (which lasted 76 days). After the trial period, the amount of supplement intake by the bees in all treatments was negligible. Under normal conditions, at this age, the bees' nitrogen demand is reduced because their body structures (muscles, wings, glands) are fully developed and capable of carrying out work outside the hive (Haydak, 1970).

Test preference

This test was conducted to assess whether the worker honeybees would be able to select the supplements produced when offered simultaneously in the same experimental cage, depending on their preferences. Polyethylene containers divided into compartments were filled with approximately 1 g of each of the supplements studied. Every three days, the leftovers were recorded and the other supplements replaced by fresh ones, placed at different locations within the same cage.

Death Rate

The average mortality rates per treatment, expressed in percentages (%), were calculated approximately every six days by dividing the total number of dead worker honeybees by the initial number of treated worker bees (125). These observations were made until the 53th day of the trial period, when all the control worker bees were died (Tab. 5).

Longevity increase

The average increase in longevity was calculated by counting the additional days of life the worker honeybees of the different treatments after the worker death in the control (53th day).

Statistical analysis

The treatments were used for daughters of sisters-queens, similar nutritional and environmental conditions. Thus, excluding the influence of these variables, the best way of assessing the supplement quality was to compare the average amounts consumed

Table 2

Chemical composition of selected ingredients of supplements

Chemical Composition	Unit in 100 g	Selected Ingredients								
		Isolated soy protein	Linseed oil	Palm oil	Beer yeast	Sugar	Honey	Pollen	Soybean Lecithin	Vitaminic Nucleus
Water	g	4.20	0.00	0.00	8.90	0.03	17.10	16.80	0.00	0.00
Calories	kcal	350.00	900.00	900.00	269.00	387.00	304.00	405.00	850.00	0.00
Carbohydrates	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
Minerals	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 lipids	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 3

Quantity of ingredients in 100g of palm oil, linseed oil, isolated soy protein and beer yeast supplement (SLiPaPiLc), linseed oil supplement (SLi), palm oil supplement (Spa), control I (CI) - sugar syrup and water (1:1), isolated soy protein supplement (SPi), beer yeast supplement (SLc) and control II (CII) - polifloral pollen

Treatments	Components (g/100g)									
	Water	Isolated soy protein	Oils (g)		Beer yeast	Sugar	Honey	Pollen	Soybean Lecithin	Vitaminic nucleus
			Linseed	Palm						
SLiPaPiLc	-	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
Sugar syrup and water (CI)	50.0	-	-	-	-	50.0	-	-	-	-
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
Pollifloral pollen (CII)	-	-	-	-	-	-	-	100	-	-

Table 4

Chemical composition calculated of palm oil, linseed oil, isolated soy protein and beer yeast (SLiPaPiLc), linseed oil supplement (SLi), palm oil supplement (SPa), isolated soy protein supplement (SPi), beer yeast supplement (SLc), control (CI) - sugar syrup and water (1:1) and (CII) - polifloral pollen provided the Africanized honeybees to lifespan test

Chemical Composition	Unit in 100 g	Supplements					Syrup (CI)	Pollen (CII)
		SLiPa/PiLc	SLi	SPa	SPi	SLc		
Water	g	4.9	4.9	4.9	4.0	5.7	50.0	16.8
Calories	kcal	397.8	397.8	397.8	411.9	383.6	193.5	405.0
Carbohydrates	g	56.2	56.2	56.2	50.9	61.5	50.0	35.0
Total fiber	g	0.2	0.3	0.1	0.2	0.2	0.0	1.1
Minerals	g	2.5	2.5	2.5	2.2	2.8	0.0	2.6
Calcium	mg	89.7	89.7	89.7	84.1	95.3	0.0	260.0
Phosphorus	mg	419.7	419.7	419.7	258.2	581.2	0.5	430.0
Sodium	mg	291.3	291.3	291.3	360.4	222.2	0.0	200.0
Thiamine (B1)	mg	43.3	43.3	43.3	40.2	46.3	0.0	800.0
Riboflavin (B2)	mg	97.4	97.4	97.4	96.3	98.5	0.0	1920.0
Niacin (B3)	mg	8.4	8.4	8.4	2.4	14.4	0.0	20.0
Pantothenic Acid	mg	133.0	133.0	133.0	131.8	134.3	0.0	2600.0
Pyridoxine (B6)	mg	19.5	19.5	19.5	19.4	19.6	0.0	380.0
Cyanocobalamin (B12)	mg	25.0	25.0	25.0	25.0	25.0	0.0	500.0
Folic Acid	mg	92.5	92.5	92.5	92.6	92.5	0.0	1850.0
Biotin	mg	0.0	0.0	0.0	0.0	0.0	0.0	0.7
Vitamin A	mg	29.7	29.7	29.7	29.9	29.6	0.0	590.0
Vitamin E	mg	5.7	5.7	5.7	7.6	3.8	0.0	20.0
Total lipids	g	9.0	9.0	9.0	8.7	9.2	0.0	6.2
Palmitic Acid (C16:0)	g	11.1	9.4	12.9	3.3	19.0	0.0	28.7
Oleic Acid (C18:1)	g	8.8	8.4	9.2	2.9	14.7	0.0	2.9
Linoleic Acid (C18:2)	g	2.2	2.4	1.9	1.3	3.1	0.0	5.4
Linolenic Acid (18:3)	g	4.9	7.1	2.6	4.8	5.0	0.0	49.5
Crude Protein	g	25.7	25.7	25.7	32.8	18.5	0.0	26.2

Source: USDA (2006)



Fig. 2. Polyethylene containers approximately 3 g

from each one, verifying their preferences, determining the rate of mortality and further longevity increase through Tukey's test at 5% significance, using the Statistical Analysis System software (SAS, 2008).

RESULTS

Supplement intake - Table 6 presents the mean consumption of the supplements (n=3), over the 30th day of the experimental period and the average quantities consumed of each supplement.

Preference test - Table 7 presents the mean values of supplement consumption, with standard errors (n=3) in the preference test and the mean quantities consumed of each supplement.

Death rate - Table 5 presents the cumulative mortality rate (%; n=3) of confined, supplemented worker bees, from the emergence until 53th day of Africanized honeybees in lifespan test. Figure 3 presents the mortality curves in supplemented worker bees in cages, from emergence to the 53th day of observation.

Longevity increase - The values found were: three, eight, sixteen, sixteen and twenty-two days for linseed oil, palm oil, isolated soy protein, linseed oil with palm oil and beer yeast, respectively (Tab. 8). These values were converted into additional survival rate (%).

Table 5

Cumulative mortality rate (%) of Africanized honeybees in lifespan test, supplied with mix supplement (SLiPa/PiLc), linseed oil (SLi), palm oil (SPa), isolated soy protein (SPi), beer yeast (SLc), pollen and control (sugar syrup and water 1:1), from emergency until honeybee death of control treatment

Cumulative mortality rate (%)							
Life day	SLiPa/PiLc	SLi	SPa	SPi	SLc	Pollen	Control
0	0.0	0.00	0.0	0.0	0.0	0.0	0.0
5	10.1	0.00	6.9	8.0	7.2	10.7	5.9
15	12.5	4.80	11.2	10.9	13.1	14.1	9.9
21	26.1	5.87	23.5	23.7	19.2	20.3	30.4
27	36.5	8.27	32.8	34.9	24.5	42.7	55.5
34	58.1	23.73	65.3	53.3	45.3	66.4	88.5
39	73.6	57.87	75.5	68.5	51.2	94.4	95.5
44	77.9	76.00	78.4	72.5	60.8	98.1	96.3
48	81.9	84.00	84.8	76.0	66.7	98.7	98.4
53	88.3c	92.00b	92.8b	88.5c	73.9d	100.0a	100.0a

Different small letters indicate significant differences at $p \leq 0.05$

Table 6

Quantity consumed (mean $\pm$ standard errors) of mix supplement (SLiPa/PiLc), oil linseed (SLi), oil palm (SPa), Isolated soy protein (SPi), beer yeast (SLc) and pollen, by Africanized honeybees in lifespan test, from emergence until the 30th day of age

Accumulated time	SLiPa/PiLc	SLi	SPa	SPi	SLc	Pollen
0 - 3 rd day	2.42 $\pm$ 0.96	0.40 $\pm$ 0.18	0.54 $\pm$ 0.30	1.83 $\pm$ 0.46	2.62 $\pm$ 0.22	1.55 $\pm$ 0.01
4 - 8 th day	2.12 $\pm$ 0.69	1.42 $\pm$ 1.30	1.49 $\pm$ 1.30	2.21 $\pm$ 0.55	1.85 $\pm$ 0.14	0.83 $\pm$ 0.71
9 - 15 th day	1.40 $\pm$ 0.22	0.56 $\pm$ 0.33	1.61 $\pm$ 0.57	1.72 $\pm$ 0.20	1.70 $\pm$ 0.43	1.22 $\pm$ 0.05
16 - 21 th day	2.78 $\pm$ 0.40	0.71 $\pm$ 0.24	1.16 $\pm$ 0.71	1.73 $\pm$ 0.27	2.38 $\pm$ 0.48	1.55 $\pm$ 0.01
22 - 26 th day	1.93 $\pm$ 0.34	0.86 $\pm$ 0.19	0.45 $\pm$ 0.12	1.50 $\pm$ 0.45	0.11 $\pm$ 0.02	0.53 $\pm$ 0.46
27 - 30 th day	0.48 $\pm$ 0.47	0.23 $\pm$ 0.02	0.27 $\pm$ 0.16	0.12 $\pm$ 0.10	0.15 $\pm$ 0.06	0.11 $\pm$ 0.01
Accumulated mean	11.12a	4.18d	5.53c	9.11b	8.80b	5.79c

Different small letters indicate significant differences at $p \leq 0.05$

Table 7

Intake (mean $\pm$ standard errors) of mix supplement (SLiPa/PiLc), linseed oil (SLi), palm oil (SPa), isolated soy protein (SPi), beer yeast (SLc), and pollen intake by Africanized honeybees in lifespan test for preference, from emergence until the 30th day of adult life

Accumulated time	SLiPa/PiLc	SLi	SPa	SPi	SLc	Pollen
0 - 3 th day	0.81 $\pm$ 0.26	0.47 $\pm$ 0.18	0.18 $\pm$ 0.15	0.61 $\pm$ 0.46	0.87 $\pm$ 0.12	0.52 $\pm$ 0.1
4 th - 8 th day	0.71 $\pm$ 0.19	0.47 $\pm$ 0.50	0.50 $\pm$ 0.30	0.74 $\pm$ 0.35	0.62 $\pm$ 0.24	0.28 $\pm$ 0.21
9 th - 15 th day	0.47 $\pm$ 0.12	0.19 $\pm$ 0.13	0.54 $\pm$ 0.27	0.57 $\pm$ 0.10	0.57 $\pm$ 0.23	0.41 $\pm$ 0.10
16 th - 21 th day	0.93 $\pm$ 0.20	0.24 $\pm$ 0.14	0.39 $\pm$ 0.11	0.58 $\pm$ 0.17	0.79 $\pm$ 0.28	0.52 $\pm$ 0.21
22 th - 26 th day	0.64 $\pm$ 0.14	0.29 $\pm$ 0.12	0.15 $\pm$ 0.12	0.50 $\pm$ 0.15	0.70 $\pm$ 0.01	0.18 $\pm$ 0.16
27 th - 30 th day	0.16 $\pm$ 0.17	0.09 $\pm$ 0.02	0.09 $\pm$ 0.06	0.04 $\pm$ 0.00	0.50 $\pm$ 0.06	0.04 $\pm$ 0.01
Accumulated mean	3.71b	1.74d	1.84d	3.04c	4.05a	1.93d

Different small letters indicate significant differences at $p \leq 0.05$

Table 8

Increase in longevity (n=3) of Africanized honeybees confined in cages and fed a supplement mixture (SLiPa/PiLc), linseed oil (SLi), palm oil (SPa), isolated soy protein (SPi) and beer yeast (SLc), from the 54th day until the 76th day

Lifetime	SLiPa/PiLc	SLi	SPa	SPi	SLc
54 th day	15	10	9	14	33
57 th day	10	0	5	10	23
62 nd day	5	0	0	5	15
66 th day	3	0	0	2	9
70 th day	0	0	0	0	6
76 th day	0	0	0	0	0
Total (days)	16b	3d	8c	16b	22a

Different small letters indicate significant differences at $p \leq 0.05$

Table 8 presents the average increase in worker bees longevity (in days) confined in cages.

The total gain in the lifetime of worker honeybees supplemented with different diets ranged from 3 to 22 days, with an average of 13 days. The lifespan of worker bees in the control was 26 days, with a high mortality rate after the third day of life. In this group, all worker bees died before the 53th day of life (Figs. 3-A). In pollen treatment, there was no additional time in longevity, with the same mortality rate of the control (Figs. 3-B). In palm oil and linseed oil treatments, worker bees presented a light increase in lifespan, three and eight days respectively, with an average of 5.5 days. The treatments with linseed oil, palm oil, isolated soy protein and beer yeast presented an increase of

16, 16 and 22 days, respectively, with an average of 18 days.

An additional longevity of 5.7, 15.1, 30.2, 30.2 and 41.5% was observed in linseed oil, palm oil, isolated soy protein, mixed supplement and beer yeast, respectively. The isolated soy protein, beer yeast and mixed supplements were the most consumed (Tab. 7), favored (Tab. 6), had the lowest mortality rates (Tab. 5 and Figs. 3-C, 3-D and 3-G) and the highest increase in longevity (Tab. 8). The beer yeast promoted the longest additional lifespan (41.5%) differing from the other supplements ($P < 0.05$).

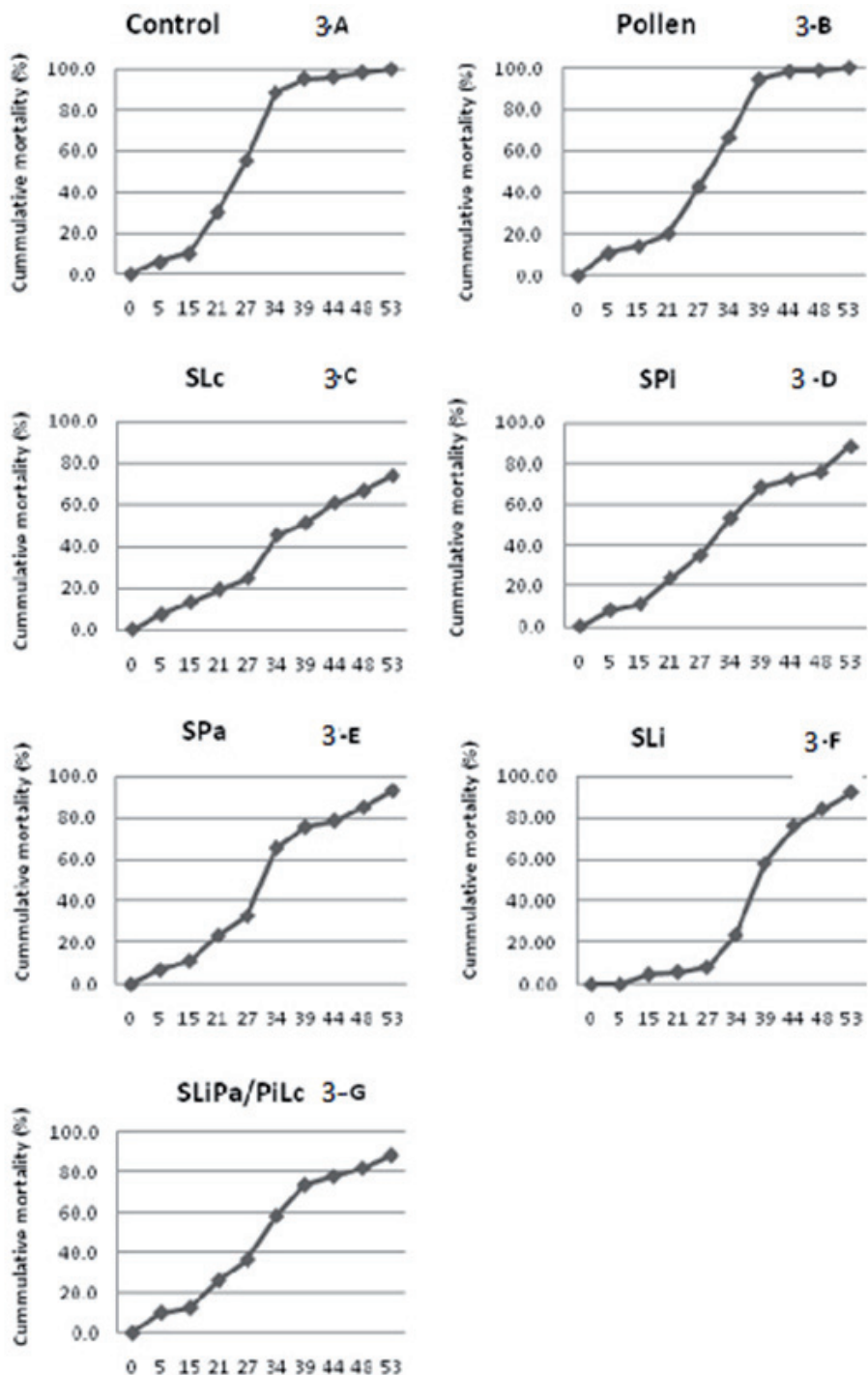


Fig. 3. Mortality curves (n=3) of Africanized honeybees confined in cages from emergence until 53th day of life fed with mix supplement (SLiPa/PiLc), linseed oil (SLi), palm oil (SPa), isolated soy protein (SPi) and yeast (SLc), pollen and control (sugar syrup and water 1:1)

DISCUSSION

Supplement intake

When comparing the intake data, the results ranged from ($P < 0.05$) 4.18 g (linseed oil supplement) to 11.12 g (mixed supplement) as presented in Table 6.

The linseed oil supplement consumption fell sharply around the 15th day, showing essentially a supplement that was less accepted by worker bees, while the palm oil supplement consumption decreased gradually until the 30th day of observation (Tab. 7), both supplements being prepared with only one source of oil. The low consumption of the linseed oil supplement may be attributed to the strong fishy smell of the supplement. According to Manning (2001) and Manning and Harvey (2002), worker bees had tolerance to the linoleic acid present in linseed oil, while excessive consumption of oleic acid present in this same source of oil may be associated with reduced lifespan by causing changes in the supplement palatability.

In this study, supplements linseed oil and palm oil were the least intake while the supplements prepared from the two sources of oil (linseed oil supplementation with palm oil, isolated soy protein and beer yeast) were the most consumed. This observation shows that mixtures of polyunsaturated and saturated fatty acids became balanced supplements with pleasant sensory characteristics, stimulating the intake in Africanized honeybees (Tab. 7).

The beer yeast supplement was prepared with a mixture of linseed oil, palm oil and beer yeast and resulted in a rich B-vitamin supplement, polyunsaturated and saturated fatty acids (Tab. 4). These ingredients became nutritional resources that ensured optimal consumption and acceptance, low mortality and greater longevity rates. When prepared, it was a pleasant aroma, fine-grained and high hygroscopicity, forming a paste within the cage that probably made its collection and consumption easier. This supplement offers no risk and can be offered to the worker bees as a supplement especially in periods of shortage or poor quality of nectar and/or pollen.

Worker bees supplemented with pollen had relatively low consumption and low longevity than other supplements (Tab. 7 and Fig. 3-B). The cause of early death was not determined, however, this suggests that although ingested, some components present in the supplied pollen possibly did not provide real nourishment benefit, causing high mortality (Tab. 5 and Fig. 3-B).

According to DeGroot (1953), for some pollen types, the lower consumption may be associated with the presence of a large amount of oil in the outer layer or bitter chemical taste. According to the same author, pollen usually contains all the essential amino acids in satisfactory amounts, but these quantities may vary in some species of plants. Longevity studies performed by Schmidt and Johnson (1984) showed that low consumption of pollen is due to the lack of attractive substances, presence of natural toxic elements or poor nutrient balance. Several factors may be combined, including the inadequate amount of essential amino acids and proteins. Schmidt et al. (1987) fed *Apis mellifera* bees with 25 varieties of unifloral pollen and various blends as the sole source of protein and assessed the longevity of these worker bees compared to controls that received only water and sugar. A wide variation in the amount of consumed pollen and the worker bees longevity was observed by these authors. They attributed these differences to the protein concentration in the consumed pollen.

The mixed supplement (Tab. 3, 6 and 8) was acceptable not only to bring the composition of the supplement close to the composition of a natural balanced diet, but also for maximizing the consumption providing a greater lifespan for the treated worker bees (Fig. 3). The consumption seen was a partial measure of how attractive or how phage-stimulating to the worker bees these supplements were. Supplements readily consumed, such as the beer yeast, probably contained more such factors.

Preference test

The preference test presented different values, ranging from ($P < 0.05$) 1.74 g (linseed oil) to 4.05 g (beer yeast) (Tab. 7). These results indicate that worker bees, under natural nutritional conditions, are able to distinguish between sources of pollen and non-pollen (Levin and Bohart, 1955; Doull, 1966; Schmidt, 1982; Schmidt and Johnson, 1984). Likewise, when confined, they can actively distinguish between different sources of supplements, preferring those with a better balance of nutrients, in particular, those mixtures containing mixed sources of fatty acids, amino acids and B vitamins (Tabs. 4, 6 and 7).

According to Doull (1966), Robinson and Nation (1968) and Schmidt and Buchmann (1985), the basis on which the worker bees discriminate against non-floral sources and some pollen types, or as the worker bees almost always recognize the majority of pollen as food, is not yet known, although there is evidence that attractiveness and feeding stimulation factors are involved.

Death rate

Table 5 and Figures 3-C, 3-D and 3-G present that the beer yeast, isolated soy protein and linseed oil with palm oil supplements, prepared with two sources of oil (Tab. 3) stand out, respectively. These supplements were the most consumed (Tab. 7), resulted in the lowest cumulative mortality rates (Tab. 5) and provided the greatest increase in longevity different from the other treatments ($P < 0.05$).

Figure 3-G shows that worker bees treated with the mixed supplement, died more evenly until the 53th day of adulthood, while other, fed with linseed supplementation (Fig. 3-F) died slowly at a 50% lower rate than the other treatments in the first 30 days, then, dying quickly displayed a shorter lifespan ($P < 0.05$) than the other treatments (Tab. 8).

Figure 3-E-F presents the mortality curves of worker bees treated with the palm and linseed supplements, respectively. Both supplements were prepared with only

one type of oil, the mortality curves were very similar ($P > 0.05$) and they presented a high mortality rate, close to that observed in the control (Fig. 3-A).

Comparing the cumulative death rate (Tab. 5) of worker bees treated with supplements, prepared with only one type of oil or with the mixture, it is possible to suggest some favorable effects of linseed oil when mixed with palm oil and an increase in the mortality rate when both were used pure in supplements (Figs. 3-E-F).

As for the sources of protein used, the lifespan was longer for worker bees receiving beer yeast as the sole source of protein (yeast supplement), than isolated soy protein. According to Haydak (1935), the supply of dried yeast ensures the development of hypopharyngeal glands in newly emerged worker bees. The supply of soybean meal caused an abrupt reduction in pupae emergence, attributing this to the lack of niacin amino acid in soybean meal (Haydak, 1949).

Increasing longevity

A mixture of equal parts of linseed oil and palm oil in supplements provided the best results for increasing the longevity of confined worker bees, possibly by the nutritional requirements of worker bees during the confinement period. According to Robinson and Nation (1968, 1970) and Manning and Harvey (2002), the predominant lipid concentration in worker bees is oleic fatty acid followed by linoleic, palmitic and stearic. The linseed supplement gave the Africanized honeybees the shortest time of increased longevity, differing from the other supplements ($P < 0.05$). Manning et al. (2007) reported that diets containing an excess of linoleic fatty acid cause high mortality of worker bees and attributed this to the collateral effect this fatty acid has on the diet palatability.

It could be concluded that the mixed supplement, isolated soy protein and beer yeast were attractive sources of food, probably because of presenting a chemical composition rich in saturated, unsaturated fatty acids, essential amino acids and B vitamins that possibly induced all stages

of feeding: intake, digestion and nutrients use, seen in significant worker bees consumption and preference, reduction in the mortality rate and increase in lifespan.

The introduction or removal of isolated soy protein and beer yeast did not have much influence on the parameters evaluated for the introduction or withdrawal of linseed oil and palm oil, and the presence of both presented the best results, especially if combined with beer yeast.

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DŁUGOŚĆ ŻYCIA PSZCZÓŁ AFRYKAŃSKICH KARMIONYCH RÓŻNYMI SUPLEMENTAMI BIAŁKOWYMI

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Celem badania była ocena wartości odżywczej suplementów białkowych i ich wpływu na długość życia pszczoł afrykańskich, przetrzymywanych w klatkach eksperymentalnych. Przygotowano następujące suplementy: jedną mieszaną (olej palmowy, olej lniany, izolowane białko sojowe i drożdże piwne), dwa surowce wykorzystywane do produkcji oleju (len i palma), jeden pokarm bez dodatku suplementu (kontrola I), dwa z dodatkiem białka (izolowane białko sojowe i drożdże), jeden z dodatkiem tylko pyłku wielokwiatowego (kontrola II). Pszczoły robotnice pozyskano z dziesięciu zasklepionych plastrów z czerwem, pobranych z rodzin pszczoł afrykańskich, pochodzących od matek sióstr. Plastry, bezpośrednio po zabranii z uli zostały umieszczone w perforowanych kopertach papierowych i pozostawione w inkubatorze w temperaturze 32-35°C i wilgotności 70% do wygryzienia. Nowo wygryzione robotnice (n=125) umieszczono w 21 klatkach (9 x 6 x 15 cm). Do każdej z opisanych klatek dostarczono wodę, kawałek wosku pszczelego, syrop cukrowy i wodę (1:1) oraz suplement. Suplementy w ilości ok. 3 g zostały dostarczone w pojemnikach z polietylenu. Pojemniki były wymieniane co trzy dni, a spożycie suplementów było rejestrowane. Analizowane cechy obejmowały: przeciętne skumulowane spożycie suplementów, test preferencji, wskaźnik śmiertelności i wzrost długowieczności pszczoł. Spożycie suplementu wahało się od 4,18 g (suplement oleju lnianego) do 11,12 g (suplement składający się z oleju lnianego i palmowego), a mieszanina wielonienasyconych i nasyconych kwasów tłuszczowych stanowiła zbilansowany suplement o przyjemnych właściwościach sensorycznych, pobudzających konsumpcję przez pszczoły afrykańskie. Wyniki testu preferencji wahały się od 1,74 g (olej lniany) do 4,05 g (drożdże piwne). Wyniki te wskazują, że w warunkach naturalnych robotnice są w stanie odróżnić źródła pyłku kwiatowego od niepyłkowych źródeł pokarmu. Również w niewoli potrafią dokonać aktywnego rozróżnienia między źródłami suplementów, wybierając te o lepszym bilansie składników odżywczych. Porównując skumulowany wskaźnik śmiertelności wśród pszczoł, którym podawano suplementy, przygotowane z jednego rodzaju oleju lub z ich mieszaniny, można wskazać korzystne efekty stosowania oleju lnianego w mieszaninie z olejem palmowym oraz wzrost wskaźnika śmiertelności, gdy oba były stosowane w suplementach w stanie czystym. U pszczoł afrykańskich, którym podawano olej lniany, zanotowano najmniejszy wzrost długości życia, przy istotnej różnicy między tym olejem a innymi suplementami ($P < 0,05$). Podsumowując, można stwierdzić, że mieszany

suplement, izolowane białko sojowe i drożdże piwne były atrakcyjnymi źródłami, prawdopodobnie dzięki składowi chemicznemu bogatemu w nasycone i nienasycone kwasy tłuszczowe, niezbędne aminokwasy i witaminy z grupy B, które prawdopodobnie stymulowały wszystkie etapy żywienia: pobór pokarmu, trawienie i wykorzystanie składników pokarmowych. Wprowadzenie lub usunięcie izolowanego białka sojowego lub drożdży piwnych nie wpłynęło istotnie na wartość parametrów ocenianych dla wprowadzenia lub usunięcia oleju palmowego i oleju lnianego, a najlepsze wyniki osiągnięto, gdy obecne były oba, szczególnie w połączeniu z drożdżami piwnymi.

Słowa kluczowe: pszczoła miodna, drożdże piwne, izolowane białko sojowe, olej lniany, olej palmowy, suplementacja.