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1 **Different energetic diets affect the maintenance of *Apis mellifera* L. colonies during**
2 **off-season**

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13 Short title: Energetic diets for *Apis mellifera* L.

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21 **Abstract.** The aim of this study wasto evaluate the best energetic foodforuse in the
22 maintenance of honey bee colonies during the off-season. To do this, 20*Apis mellifera*
23 beehives were used(with five beehives per treatment): CTL,control (without feeding);
24 SJ,sugarcane juice; SS,sugar syrup; and IS,inverted sugar. We evaluated the food
25 consumption, population development, and physiological state (expression of
26 vitellogenin and hexamerin 70agenes)of eachcolony.The results showed that the
27 supplementation of colonieswith sugar syrup resulted in an intermediateconsumption
28 and thebetter development of the colony.In addition, this diet ensured that the colonies
29 were in a good physiological state,as beesfed this diet presentedthe highest relative
30 expression levels of vitellogenin and hexamerin 70ameasuredamong all thediets
31 tested.Therefore, sugar syrup was concluded to be the best artificial energetic food for
32 use in thesupplementation of honey bee colonies during the off-season.

33 **Key words: beekeeping / energetic foods / gene expression / stress**

34 **1. Introduction**

35 All of the nutritional needs of honey bees (*Apis mellifera*) for their complete
36 development, maintenance, reproduction, and longevity need to be supplied through
37 food sources available in the environment (Vaudo et al. 2015). In nature, *A.*
38 *mellifera*bees meet all their nutritional needs through the ingestion of nectar and pollen
39 (Degrandi-Hoffman and Chain 2015).Nectar has an undeniable importance to
40 colonydevelopment, since it is the main source of energetic food forbees and
41 permitstheir survivalthrough the off-season (Traynor et al. 2015). During the off-season,
42 however, there is a shortage of blossomsand, consequently, of food for the bees. Thus,
43 nutritional stress caused by food shortages or the availability of foods of only low

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44 nutritional value may lead to a reduction in the metabolic activity of the bees
45 (Crailsheim 1990; Wang et al. 2016). It has also been observed that whenthere is little
46 available natural food, there are reductions in the number of worker bees in the hive,
47 queen's layingand survival rates of individuals, and increases in escape or abandonment
48 rates (Paiva et al. 2016).

49 This nutritional stress can cause alterations in thebees' metabolic pathways that
50 influence several biological processes, including the expressionof such genes as
51 vitellogenin and hexamerin 70a. These genes are considered storage genes because they
52 give rise to proteins that are produced during the larval stage and remain stored in the
53 hemolymph and/or in the fat body (Martins et al. 2008).

54 Vitellogenin is important in the immune system and longevity of bees because
55 this protein is a zinc carrier, and thus protects many cell lines against oxidative stress
56 and apoptosis. Decreased expression of this gene product due to high expression levels
57 of juvenile hormone was also previously shown to be related to reductions in the
58 numbers of functional hemocytes in forager bees due to decreases in the quantity of zinc
59 carried by vitellogenin(Falchuk 1998; Pinto et al. 2000;Amdam et al. 2004; Harwood et
60 al. 2016).

61 Hexamerin 70a is a gene related to storage, which is expressed in the larval,
62 pupal, and adult stages of workers, queens, and drones, and is the only protein with a
63 hexamerin subunit present in significant quantities in the fat body and adult hemolymph
64 of*A. mellifera*. The synthesis of hexamerins, specifically the subunit 70a, like thatof
65 vitellogenin, is directly related to the quantity and quality of food intake. In older
66 worker bees, such as forager bees, the levels of this protein are reduced due to the fact

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67 thatsuch bees have a low-protein diet, since they do not collect and consume much
68 pollen and insteadpreferably consume nectar (Martins et al. 2008).

69 Beekeepersoften provide artificial food to their colonies in the off-season to
70 lessen the negative effects that occur to bee colonies during this period in which food
71 resources are drastically reduced, and thus ensure the survival and good performance of
72 the colony.Therefore, the objective of this study was to select the best energetic food to
73 be offered to bee colonies so that theirmaintenance is guaranteed during the off-season.
74 This was done whiletaking into account the food consumption, population development,
75 and physiological state of the colonyin terms of the expression of the genes for
76 vitellogenin and hexamerin 70a when bee colonies were provided different foods.

77 2. Materials and methods

78 2.1 Field experiment

79 For the field experiment, 20 *Apis mellifera* beehives were selected, and the
80 number of brooding and feeding frames in them were standardized. The food treatments
81 used were the following: control (CTL), in which no artificial food was provided;
82 feeding with sugarcane juice (SJ) produced by the research laboratory itself; feeding
83 with sugar syrup (SS) prepared using pre-boiled filtered water and commercial crystal
84 sugar; and feeding with inverted sugar (IS) purchased from Atrium Food Group,
85 Campinas, Sao Paulo, Brazil.

86 Food was supplied twice a week in the amount of 1 L per hive for a period of 60
87 days by means of a Boardman artificial feeder. The experiment was carried out in June
88 and July of 2015. During the experimental period, food consumption was measured
89 weekly.

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90 Population development, includingthe number and area of open and closed
91 brooding areas in the central nest structure, was measured monthly in the hives
92 throughout the experimental period usingthe methods used and described byLomele et
93 al. (2010).

94 The number of brooding and feedingframes was quantified weekly by visual
95 examination. Physicochemical analyses of the food provided were carried out at the
96 Laboratory of Bromatology of the Experimental Farm Lageado, College of Veterinary
97 Medicine and Animal Science, Universidade Estadual Paulista, UNESP, Botucatu, Sao
98 Paulo, Brazil. Total sugar reduction was performed according to Welke et al. (2008),
99 calorimetric and dry matter analysis according to Sodré et al. (2011), and ashing
100 according to Sodré et al. (2007).

101 **2.2 Genetic analysis**

102 **2.2.1 Honeybee collection**

103 The collection of bees for use in the analysis of the relative expression levels of
104 the selected genes was done on day 0, which was used as experimental control, as well
105 as on day 30, which was identified as "Moment 1" (M1) of the collection, and on day
106 60, which was designated as "Moment 2" (M2) of the collection. Five worker bees
107 developing nursing behavior were collected from the central group, which were
108 identified as nursing bees (N), and 5 worker bees were also collected that were carrying
109 pollen in their corbicle, which in turn were identified as forager bees (F). During the
110 experimental period, all ofthe colonies receiving the SJ treatment died, which made it
111 impossible to collect these bees for analysis. After collection, the bees were
112 immediately stored in a freezer at-80 °C for future RNA extraction.

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113 **2.2.2 Relative expression of storage genes**

114 To analyze the expression of vitellogenin and hexamerin 70a, 5 forager and 5
115 nursing bees were randomly collected and immediately frozen at -80 °C from each
116 experimental colony at days 0, 30, and 60. Total RNA was extracted from the heads of 5
117 bees of each type (Scharlaken et al., 2008) using 500 µL of TRIzol® Reagent (Life
118 Technologies) for each sample according to the manufacturer's instructions. The
119 extraction product was visualized on 1% agarose gel and quantified using aNanoDrop
120 Instrument (ND-1000 Spectrophotometer). The RNA was treated with DNase I,
121 incubated for 60 min at 37 °C and then for 10 min at 75 °C. A solution was then
122 prepared of 0.75 mM oligo dT,0.15 mM random oligos,0.75 mM deoxynucleotide
123 triphosphates, and 1 µLof RNA, and then it was incubated at 65 °C for 5 min, after
124 which it was placed on ice for 1 min. We added 1× buffer dithiothreitol
125 0.005,RNaseOUT (40 U/µL), and 100 U SuperScript® III Reverse Transcriptase
126 (Invitrogen) to this preparation. Complementary DNA synthesis was performed at 50 °C
127 for 60 min, followed by 15 min at 70 °C.

128 Amplification was performed with real-time quantitative polymerase chain
129 reaction (RT-qPCR) in a 25µL reaction mixture using the SYBR® Green PCR Master
130 Mix (Applied Biosystems) and 0.2 µM of each primer. The sequences and details of the
131 primers used are provided in Table I. The RT-qPCR reactions were performed using
132 ABI 7300 (Applied Biosystems) equipment under the following conditions: 1 cycle at
133 50 °C for 2 min; 1 cycle at 94 °C for 10 min; and 40 cycles of 94 °C for 15 s and 60 °C
134 for 1 min. The dissociation curve was obtained under the following conditions: at 95 °C
135 for 15 s, 60 °C for 30 s, and 95 °C for 15 s. The determination of the expression levels of
136 vitellogenin and hexamerin 70a was performed in triplicate, andthe expression of the

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137 actin gene was used as the control (Scharlaken et al. 2008). For each reaction, a negative
138 control consisting of a mixture of reagents and water was also used.

139 The efficiency of the oligonucleotides (E) was calculated from 4 dilutions of
140 complementary DNA samples (1:5, 1:25, 1:125, and 1:625) using the formula E = 10 (–
141 1/inclination). The quantification of a gene's relative expression (R) was determined
142 according to Pfaffl (2001), defining the crossing point as the point at which the detected
143 fluorescence was appreciably above the background fluorescence and using the formula:

144
$$R = \frac{E_{target} \times \Delta CP_{target}(control - sample)}{\Delta CP_{endogenous}(control - sample) \times E_{endogenous}}$$

145 **2.3 Statistical analyses**

146 The data obtained for food consumption, population development, and gene
147 expression were first tested for normality (Anderson-Darling test) and homogeneous
148 variances (Levene'stest).When significant deviations (P<0.05) from these assumptions
149 were detected, the data were compared using the non-parametric Mann-Whitney test,
150 and the median and interquartile intervals (Q1_Q3) were presented. When no significant
151 deviations from normality and homoscedasticity were detected, the data were analyzed
152 with one-way ANOVA, and the mean ± standard deviation values were presented. P-
153 values below 0.05 were considered significant. All statistical analyses were performed
154 using Minitab statistical software.

155 **3. Results**

156 On mean ± standard deviation, 0.994.8 ± 310, 894.6 ± 291, and 433.9 ± 227.6
157 mL of SJ, SS, and IS, respectively, were consumed weekly. The CTL, SJ, SS, and IS
158 treatments underwent 100, 75, 0, and 0% colony losses, respectively.

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159 The results of the physicochemical analyses of the different foods are presented
160 in Table II. Significant differences were observed in the analysis ofSJ ash (0.27 ±
161 0.02%),in that this value differed significantly from that for SS (0.01 ± 0.001%), but not
162 from that for IS (0.11 ± 0.04%).

163 The calorimetric analysis of the foods showed that SS presented the highest
164 energetic value (4155.0 kcal kg^{-1}) of all the foods tested (3903.0 kcal kg^{-1}forSS and
165 3895.0 kcal kg^{-1}forIS).The dry matter values ofSJ and SS were lower than that of IS,
166 indicating they had higher moisture content. The analysis of the total reducing sugars in
167 each food type showed a higher value for SS (41.52 ± 2.8%), which differed
168 significantly from those found for SJ (21.15 ± 1.6%) and IS (0.82 ± 0.01%).

169 The data contained in Table III represent the number of brooding and feeding
170 frames in colonies under different treatments. The number of brooding frames was
171 higher for colonies in theSS and IS treatments compared to that in the CTL and SJ
172 treatments. However, the number of feedingframes showed no differences among
173 treatments.The data shown in Table IV represent the areas of open and closed brooding
174 areas (cm^2) observed in colonies subjected to different treatments. The treatments SS
175 and IS presented larger closed brooding areas than the other treatments did. For the
176 open brooding areas, the largest observed area was recorded in theIS treatment.

177 The mean and standard deviation of the relative expression levels of the
178 vitellogenin and hexamerin 70a genes in each treatment were calculated, and these are
179 presented in TablesI and II and Figures 1 and 2.

180 In terms of the relative expression of the vitellogenin gene, from the comparison
181 between nursing and foragerbees at M1 and M2 (Table V and Figure 1) it can be

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182 observed that the nursing bees in the CTL, SJ, and IS treatments at M1 and in the CTL
183 and IStreatments at M2 presented significantly lower relative expression levels of this
184 gene (P<0.05). However, the inverse was observed in the bees analyzed from the SS
185 treatment at both M1 and M2 (P <0.05), with the nursing bees expressingthis gene at
186 levels 2862 times greater than those in the foragerbeesat M2.

187 At M1, when analyzing only the nursing bees in relation to the diets provided,
188 the treatments CTL, SJ, and IS resulted in the downregulation of this gene (i.e. a
189 decrease in the relative expression level of the gene in comparison to that in the control
190 group), whereas in the SStreatment the upregulation of this gene (i.e. increased relative
191 expression) was noted. For the foragerbees sampled at the same time, only those treated
192 with IS presented downregulation of this gene, which showedthat there were differences
193 in the patterns of gene expression between the nursing and forager bees.

194 When analyzing nursing bees at M2, the expression of this gene was upregulated
195 in bees in all treatments, although the CTL and SS treatments presented relatively
196 higher levels of expression compared to those in the IStreatment (67,92 and 205,63
197 times more than in the IS treatment, respectively). It was observed that bees fed with SS
198 showed greaterexpression of this gene in comparison to those in the other treatments,
199 similarly to what was noted above for the nursing bees collected at M1. However, for
200 the foragerbees collected at the same time, only those treated with SS presented
201 downregulation of this gene in relation to the control.

202 When changing the focus of the data analysis and comparing the results obtained
203 for nursing andforager bees between M1 and M2 in the different treatmentsto check for
204 changes in the expression pattern of this gene after 60 days of feeding, it was observed

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205 that all the analyzed treatments showed differences in their relative expression levels
206 (P<0.05). In the CTL and IS treatments, both nursing andforager bees showed
207 increasedexpression of the vitellogenin gene during the experiment (P<0.05). However,
208 with the SS treatment, the inverse occurred and there was greater relative expression at
209 M1 than at M2 in both nursing and forager bees.

210 For the comparison between nursing and forager bees at M1 and M2 in relation
211 to their relative expression levels of the hexamerin 70a gene (Table VI and Figure 2), in
212 all treatments except SS there were lower relative expression levels of this gene in the
213 nursing bees than the in the foragerbees at M1 thismeantthat in the nursing beesthis
214 gene was downregulated, whereas in the forager bees it was upregulated. However, the
215 inverse patternoccurred in the SStreatment, in which the nursing bees presented greater
216 relative expression levels of hexamerin 70a than the forager bees did, and in the nursing
217 bees a very extensive upregulation was observed; specifically, the expression of this
218 gene reached a value 33483 times higher than that in nursing bees at M1, whereas in the
219 forager bees this gene's expression was downregulated.

220 At M2, this gene was upregulated in both the foragerand nursing bees in all
221 treatments. At this time, it was also possible to observe thatthe nursing bees had
222 relatively higher levelsof expression of the hexamerin 70a gene than the foragerbees
223 didin all treatments.

224 4. Discussion

225 Feeding honey bee colonies with artificial energetic foods during the off-season
226 ensures the correct annual operation of the colony. For this to be effective, it is
227 necessary to choose the best energetic food to offerthe bees to guaranteetheproper

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228 development of a colony for the producer.The lower consumption of inverted sugar in
229 the present study may suggest that this energy supply was less attractive to the bees
230 when compared to that offered in the other treatments. This fact can be attributed to the
231 higher viscosity of the inverted sugar, which was supplied to the hives at its full
232 concentration (Gratão et al. 2004).

233 The dry matter data followed opposing trends among treatments to thedifferent
234 food types' viscosities, since the moisture content of the food is inversely proportional
235 to its viscosity (Yanniotis et al. 2006; Cui et al. 2008). Therefore, sugar syrup and
236 sugarcane juice had lower viscosities, which may have favored their consumption since
237 nectar, a natural energetic food of bees, has a low viscosity and high humidity (Garcia et
238 al. 2005; Hazlehurst and Karubian 2016). Furthermore, the higher reducing sugarand
239 caloriccontent and the lower ash content of sugar syrup detected in the analyses carried
240 out in this study, along with this food having an adequate dry matter content, indicated
241 that this was the food that we supplied to the bees that most closely resembled honey,
242 which is the main natural source of energy reserves for bee colonies. Thus, because it
243 has a composition closer to that of the bees' natural food, it was, at least in
244 bromatological terms, the best source of artificial food for bees that was tested in this
245 study.

246 Castagnino et al. (2006) showed that energetic supplementation during the off-
247 season increases the queen's posture. In addition to supplying an energetic diet, a
248 protein diet is also essential for colonymaintenance and improving the queen's posture
249 (Frias et al. 2016).However, under the conditions of this experiment, the colonies had
250 bee bread reserves,and thus no protein supplementation was required. In this case, the
251 energetic supplementation provided assisted in the maintenance of the colonies, and was

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252 able to explainthe greater number of brooding frames observed in the SS and IS
253 treatments, suggesting that these energetic foods provided the necessary energetic
254 support for the queen's posture during this period. Castagnino et al. (2006) obtained a
255 large brooding area incolonies fed with sugar syrup, which was similar to the results of
256 the present study. The energy provided by the consumption of the sugar syrup and
257 inverted sugar probably stimulated the queen's laying.

258 The loss of all of the colonies subjected to the SJ treatment over the
259 experimental period probably occurred because the sugarcane juice (the diet offered to
260 bees in the SJ treatment) may have undergone fermentation at ambient temperature
261 (Pedroso et al. 2005).Given this, it was not possible to obtain data on the relative
262 expression of the tested genes at Moment 2 in the nursing and forager bees in this
263 treatment.

264 The analysis of nursing bees at M1 demonstrated an upregulation in vitellogenin
265 expression in the SS treatment only. This shows that sugar syrup had a more direct
266 influence on nursing bees than the other foods provided throughout the experimental
267 period. This result may be related to several factors, such as food viscosity, energetic
268 value, and the maintenance of foodintegrity and quality at room temperature.

269 The vitellogenin expression levels observed after 60 days of feedingsuggested
270 that the SS treatment had a greater influence on the expression of this gene than the
271 other diets, and it also facilitated better population development of the colony since the
272 values of almost all of the performance parameters observed were higher in this
273 treatment compared to those in the other treatments. The forager bees, which live
274 forapproximately 21 days, presented less relative expression of this gene than the

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275 nursing bees, which were less than 15 days old, at both M1 and M2. This possibly
276 occurred due to the fact that there was a higher concentration of juvenile hormone in the
277 hemolymph of the older bees, which may have influenced their biosynthesis of
278 vitellogenin. As noted earlier, vitellogenin is a protein that is related to the prevention of
279 oxidative stress since it is a zinc carrier, andthe occurrence of low levels of this protein
280 can compromise the immune system (Dallacqua et al. 2007).

281 Therefore, the results of this study demonstrated that the supplementation of
282 honey bee colonies in the field during the off-season with sugar syrup resultsin an
283 intermediate level of consumption of this food by them and greater colony development,
284 and also ensured that the bees were in a better physiological state. This showed that this
285 wasthe most beneficial artificial energetic food tested in this study.

286 **5. Acknowledgements**

287 Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the scientific
288 initiation scholarship provided (FAPESP process: 2017/12001-2).

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383 **7. Tables**

384 **Table I:** Oligonucleotides used in gene expression study of *Apis mellifera* that were fed
385 with different energetic foods during the off-season.

Gene	Accession number in Gene Bank	Sequence of primers 5'-3'	Amplified (pb)	Temperature[a] (°C)	Efficiency of the oligonucleotides[b] (%)
Actin	AB023025	TGCCAACACT GTCCTTTCTG AGAATTGACCCA CC AATCCA	156	61	91,17
vitellogenin	AJ517411	GCAGAATACA TGGACGGTGT GAACAGTCTTCG GA AGCTTG	146	61	110,17
hexamerin 70a	Martins et al, (2008)	AAAGCCAATCAC GCTCTGAT AATCGTGATTCA GATACCAGC	119	61	116

386 [a]Specific optimal annealing temperature for each primer.
387 [b]Measurement of the efficiency or real-time quantitative polymerase chain reaction (RT-
388 PCR: calculated using the standard curve)
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393 **Table II.** Physicochemical analyses of different energetic foods (sugarcane juice, sugar
394 syrup and inverted sugar).

	Ashing (%)	Calorimetric (Kcal Kg^{-1})	Drymatter (%)	Reduced sugars (%)
SugarcaneJuice	0.27±0.02a	3903.0	15.94±0,00a	21.15±1,6a
Sugar syrup	0.01±0.00b	4155.0	53.84±0.41b	41.52±2.8b
Inverted sugar	0.11±0.04ab	3895.0	75.66±0.75c	0.82±0.0c

395 Different lowercase letters on the same line indicate statistical differences between
396 means ($p < 0.05$).
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409 **Table III.** Mean and standard deviation of the number of tables occupied in the nest
410 containing brooding and feeding frames for control, sugarcane juice, sugar syrup and
411 inverted sugar treatments during the experimental period.

Occupied frames in the nest with brooding and feeding area				
Frame	**Control**	**Sugar cane Juice**	**Sugar syrup**	**Inverted sugar**
Brooding	2,00±0,90a	1,90±0,31a	3,60±0,67b	3,00±1,10c
Feeding	4,52±2,60a	3,84±1,40a	4,00±1,40a	4,32±2,10a

412 Different lowercase letters on the same line indicate statistical differences between
413 means (p <0.05).
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425 **Table IV.** Mean and standard deviation of the open and closed (cm^2) brooding area
426 referring to the treatments control, sugarcane juice, sugar syrup and inverted sugar,
427 during the experimental period.

Population development (cm^2)				
Brooding	**Control**	**SugarcaneJuice**	**Sugar syrup**	**Inverted sugar**
Closed	188,4±132,2a	216±167,5a	384,9±237,3b	401,7±194b
Open	82,9±100,5a	100,9±102,1ab	158,3±171,6ab	174,2±126,3b

428 Different lowercase letters on the same line indicate statistical differences between
429 means ($p < 0.05$).
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441 **Table V.** Relative expression of the vitellogenin gene in nursing bees (N) and forager
442 bees (F) of the different treatments used after 30 days (Moment 1 - M1) and 60 days
443 (Moment 2 - M2).

	CTL	SJ	SS	IS
M1 N	0,0967±0,10 Aa α	0,26±0,21 Ab	3653,57±461,62 Ac α	0,071±0,0078 Ad α
M1 F	121,88±30,20 Ba α	10,67±1,9 Bb	127,25±16,16 Bc α	0,52±0,134 Bd α
M2 N	378,32±50,29 Aa β	---	1145,38±376,25 Ab β	5,57±3,23 Ac β
M2 F	619,9±25,95 Ba β	---	0,40±0,13 Bb β	6,15±0,219 Ac β

444 CTL: control; SJ: sugarcane juice; SS: sugar syrup; IS: Invert sugar.
445 Different capital letters in the same column indicate statistically significant difference
446 between averages compared to the comparison between nursing and forager bees within
447 the same collection day ($p < 0.05$).
448 Different lowercase letters on the same line indicate statistical difference between
449 averages in relation to the comparison of nursing and forager bees between treatments
450 on the same collection day ($p < 0.05$).
451 Different Greek letters (α and β) in the same column indicate statistically significant
452 difference between means compared to M1 and M2 nursing bees, and M1 and M2
453 forager bees ($p < 0.05$).
454 --- Data not obtained due to the death of the colonies during the experimental period.
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459 **Table VI.** Relative expression of the hexamerin 70a gene in nursingbees (N) and
460 forager bees (F) of the different treatments used after 30 days (Moment 1 - M1) and 60
461 days (Moment 2 - M2).

	CTL	SJ	SS	IS
M1 N	0,3818±0,02 Aa α	1,557±0,34 Ab	2554,82±243,59 Ac α	0,2689±0,02 Ad α
M1 F	125,46±2,64 Ba α	1217,16±383,91 Bb	0,0763±0,003 Bc α	20,818±1,43 Bd α
M2 N	911,30±49,52 Aa β	---	233,83±30,94 Ab β	19,256±1,78 Ac β
M2 F	2,276±0,21 Ba β	---	8,437±1,04 Bb β	9,531±0,60 Bb β

462 CTL: control; SJ: sugarcane juice; SS: sugar syrup; IS: Invert sugar.
463 Different capital letters in the same column indicate statistically significant difference
464 between averages compared to the comparison between nursing and forager bees within
465 the same collection day ($p < 0.05$).
466 Different lowercase letters on the same line indicate statistical difference between
467 averages in relation to the comparison of nursing and forager bees between treatments
468 on the same collection day ($p < 0.05$).
469 Different Greek letters (α and β) in the same column indicate statistically significant
470 difference between means compared to M1 and M2 nursing bees, and M1 and M2
471 forager bees ($p < 0.05$).
472 --- Data not obtained due to the death of the colonies during the experimental period.
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476 **8. Figures**

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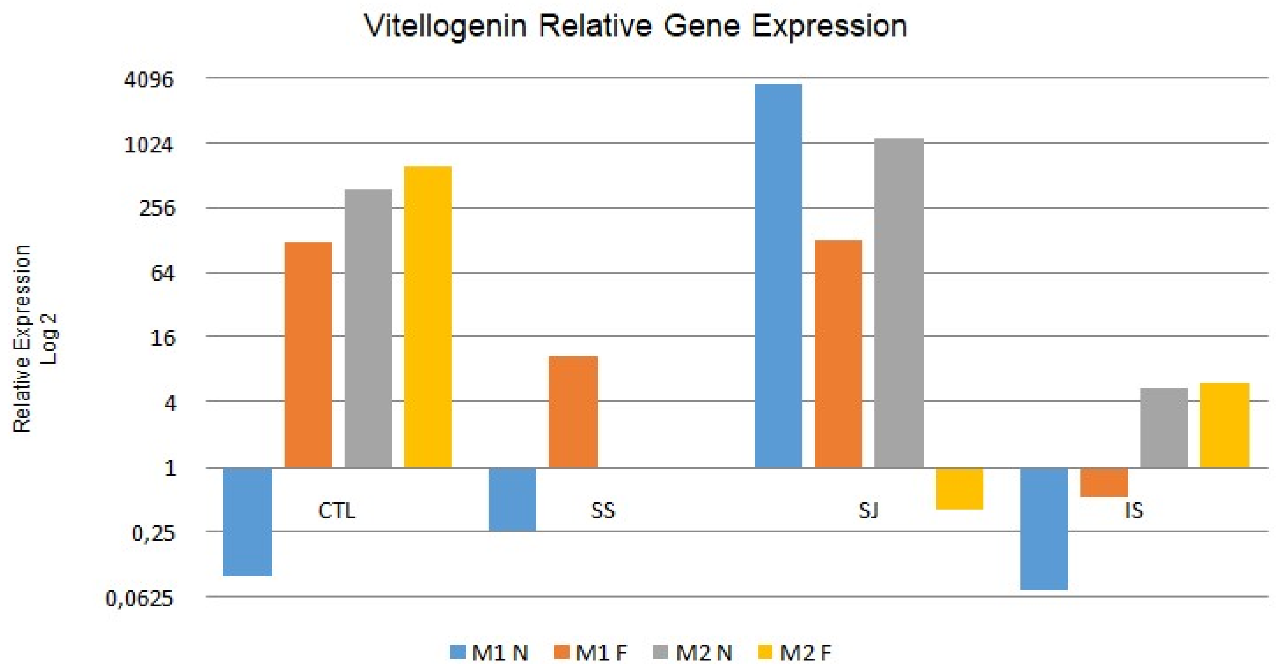

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479 **Fig. 1** Relative expression of the vitellogenin gene in nursing bees (N) and forager bees
480 (F) of the different treatments used after 30 days (Moment 1 - M1) and 60 days
481 (Moment 2 - M2). CTL: control; SJ: sugarcane juice; SS: sugar syrup; IS: Inverted
482 sugar. M2 N and M2 F: Data not obtained due to death of the colonies during the
483 experimental period

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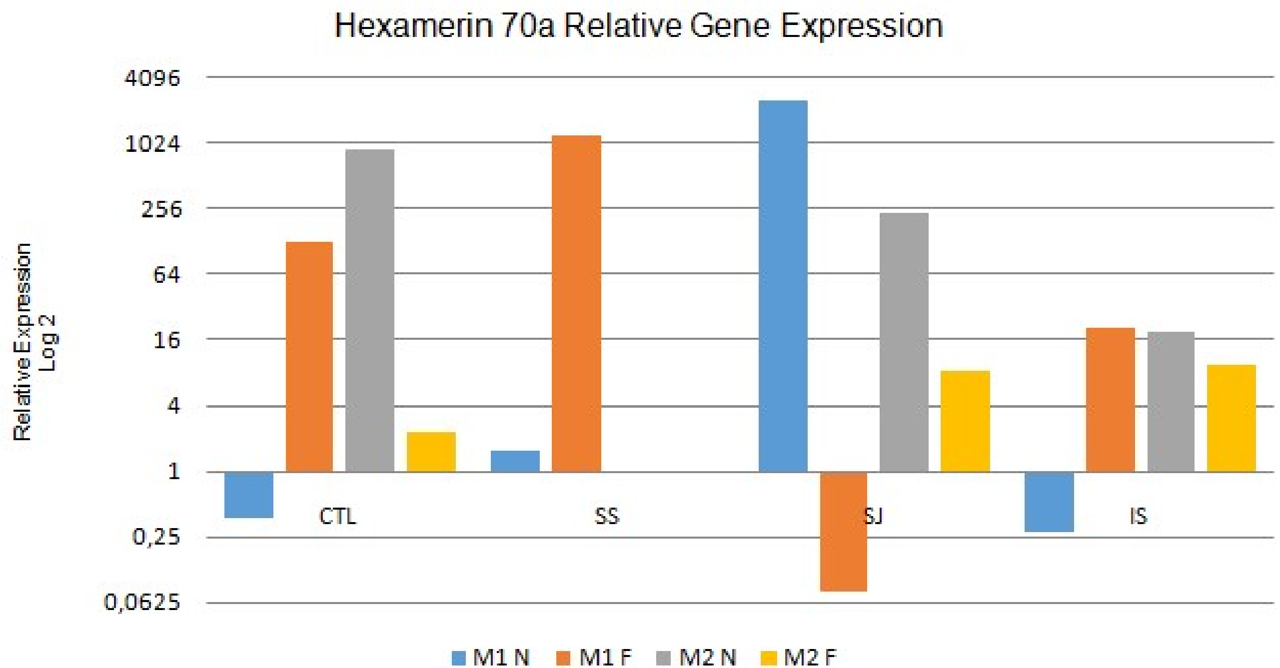

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491 **Fig. 2** Relative expression of the hexamerin 70a gene in nursing bees (N) and forager

492 bees (F) of the different treatments used after 30 days (Moment 1 - M1) and 60 days

493 (Moment 2 - M2). CTL: control; SJ: sugarcane juice; SS: sugar syrup; IS: Inverted

494 sugar. M2 N and M2 F: Data not obtained due to death of the colonies during the

495 experimental period