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Original article

Comparison of the nutrient composition of royal jelly and worker jelly of honey bees (*Apis mellifera*)

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Abstract – In this study, the chemical and mineral composition and trace elements in royal jelly (RJ) and worker jelly (WJ) and in royal jelly on particular days (only-2-day RJ [O2d], only-3-day RJ [O3d] and only-4-day RJ [O4d]) were determined. Significant differences in levels of moisture, protein, 10-hydroxy-2-decenoic acid (10-HDA), fructose (F) and glucose (G) were found between the RJ and WJ samples. The nutrient content was significantly higher in samples on O2d than the O3d and O4d samples. The results of this study add to the current knowledge of the nutritional value of RJ and WJ. These results also imply a strong relationship between nutritional effects and polyphenism in honey bees.

chemical / composition / royal jelly / worker jelly

1. INTRODUCTION

The characteristics of organisms are considered to be the comprehensive result of both genetics and the environment. The honey bee is a perfect model to explore the interaction of these two factors. Genetic factors are decisive in sex determination in honey bees. The females (workers or queens) are developed from fertilized eggs, and unfertilized eggs become drones (males). Although the queen and worker have the same genotype, they differ greatly in their behavior, physiology and development (Winston 1991), and this is most likely the result of environment factors. Both the queen and worker larvae are fed royal jelly for the first 3 days of that stage. The worker larvae are subsequently fed a mixture of royal jelly, pollen and honey, while the older queen larvae are provided with abundant royal jelly (Haydak 1943). The quality and quantity of larval food is believed to be the primary catalyst for the differentiation in development (Haydak 1943; Winston 1991). There are few reports on the differences between royal jelly (RJ) and worker jelly (WJ). Studies have shown that the moisture content of RJ is lower than that of WJ (Elser 1928; Smith 1959). Sugar is another important factor that may influence caste determination. Asencot and Lensky (1988) found that the food of 1- to 3-day-old queen larvae contained 12.4 % sugars, which was approximately four times that found in WJ. Studies have also found that adding sugars to WJ induced larvae to become queens (Asencot and Lensky 1985). Recent reports have noted that caste determination in honey bees is influenced by a 57-kDa protein in royal jelly through an epidermal growth factor receptor (EGFR)-mediated signaling pathway (Kamakura 2011). While these studies strongly suggest a close relationship between food nutrients and caste determination, we still have little knowledge regarding the differences in chemical composition between RJ and WJ, such as the content of proteins, lipids, 10-hydroxy-2-decenoic acid (HDA) and mineral elements.

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The composition of RJ has been the subject of investigation by numerous researchers for more than a century (Lercker et al. 1981; cf. reviews: Ramadan and Al-Ghamdi 2012). The content of moisture (Sabatini et al. 2009), carbohydrates (Asencot and Lensky 1988; Lercker et al. 1992), proteins (Simúth 2001; Boselli et al. 2003; Garcia-Amoedo and Almeida-Muradian 2007), lipids (Lercker et al. 1992; Noda et al. 2005), 10-HDA (Bloodworth et al. 1994; Koshio and de Almeida-Muradian 2003; Pamplona et al. 2004) and minerals (Benfenati et al. 1986; Stocker et al. 2005; Garcia-Amoedo and Almeida-Muradian 2007) has been thoroughly documented. However, it is important to note that almost all of these studies were focused on the quality of commercial RJ (i.e., the food of 4-day-old queen larvae). Furthermore, the food of queen larva in its cell is increasing daily, and the larva always eat the freshest food. It is doubtful that there is any change in the composition of the RJ that the queen larvae is fed daily by nurse bees. This study is based on the hypothesis that the composition of whole RJ obtained from a cell is different from that supplied to queen larva by nurse workers on a particular day.

If the nutrient composition of RJ and WJ are different, we will be forced to reexamine the relationship between nutrients and caste determination in honey bees. The aim of this study was the systematic investigation of the chemical composition (including moisture, total protein, 10-HDA, carbohydrates and minerals) of different-aged samples of RJ and WJ.

2. MATERIALS AND METHODS

2.1. Collection of RJ and WJ samples

All experiments and surveys were conducted in the experimental apiary of Shandong Agricultural University (Tai' an, Shandong Province, China). RJ and WJ sampling was conducted between September and October 2014. Five queen-right colonies (*Apis mellifera* L.) were used in our experiments. RJ and WJ production were conducted in the five colonies at the same time.

To obtain same-aged WJ and RJ samples, the queen of each colony was confined on an empty comb for 24 h using a queen spawning controller (Changge Jihong Beekeeping Equipment Co., He'nan, China). The laid-comb was then transferred to a super and the queen was kept in the hive body. A queen excluder was used to separate the queen from the super. After the eggs were deposited, the 1-day-old larvae were grafted into artificial cells using a standard royal jelly production technique (Chen et al. 2002). Every 24 h, 100 worker larvae of each colony were removed from the cells, and the WJ were obtained using a retractable Chinese-style grafting tool. The RJ samples of particular days were divided into two portions: the whole RJ samples and the RJ samples that the nurse bees fed the queen larvae on that particular day (see Figure 1). A mark was made on the surface of the artificial cells. To obtain each day's RJ samples, the RJ added on top of the mark from the previous day in each cell was collected (Figure 1). Each type of RJ sample was obtained from 33 queen cells in each colony. Sampling was performed on the following days: only-2-day RJ (O2d), only-3-day RJ (O3d) and only-4-day RJ (O4d). We collected five individual samples for

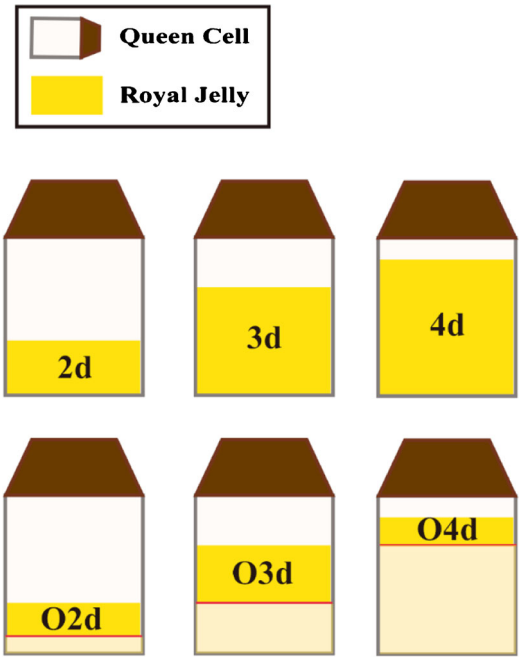

Figure 1. Schematic diagram of various RJ samples.

each sample type (2d-, 3d-, 4d-RJ and WJ, 5d-WJ and O2d-, O3d- and O4d-RJ) from each colony. We did not collect 1-day-old WJ or RJ samples, as there is very little 1-day WJ, and the 1-day-old larvae are too small to separate.

2.2. Analytical procedures

Moisture content was determined using a vacuum freeze-drying method (Messia et al. 2005). Total crude protein was estimated using the Kjeldahl method, as reported by Hoegger (1998). The 10-HDA content was analyzed using high-performance liquid chromatography (HPLC) as defined in the national industry standard for royal jelly (GB/T 9697–2008) of the General Administation of Quality Supervision, Inspection and Quarantine of the People's Republic of China (2008). The two main sugars of RJ and WJ (fructose and glucose) were quantified using HPLC, as reported by Sesta (2006).

To determine levels of trace elements, approximately 0.5 g (to the nearest 0.1 mg) of the RJ and WJ samples was first digested using a 20-mL mixture of 4:1, HNO_3: H_2O_2 for 24 h. The mixture was heated to 300 °C, and when the color of the mixture turned dark, several drops of H_2O_2 (30 %, v/v) were added until it became colorless. The solution was transferred to a 25-mL volumetric flask and filled to volume with 5 % HNO_3. Blank digestions of non-RJ or non-WJ samples were conducted in the same manner in order to eliminate reagent errors. The content of eight mineral elements was determined by atomic absorption spectroscopy (AA-6601 F; Shimadzu Corporation, Kyoto, Japan) using the method described by Chen et al. (2006).

2.3. Statistical analysis

Before analyses were performed, Bartlett's and Levene's tests were used to ensure that the data conformed to the assumptions of ANOVA. Data were statistically analyzed using the one-way ANOVA procedure in SAS (version 9.1, SAS Institute Inc. 2003; Cary, NC, USA). Duncan's multiple-range test was used to compare differences between means, and differences were deemed significant at the $P<0.05$ level. All values are reported as mean±SD.

3. RESULTS

The proximate composition and mineral element content of the RJ and WJ samples are shown in Tables I and II, respectively.

The moisture content of WJ was significantly higher than that of RJ for all 3 days (2d: F=321.5, $P<0.01$; 3d: F=66.0, $P<0.01$; d4: F=300.6, $P<0.01$) (Table I). For WJ, the moisture was higher on 2d, 3d and 4d, but decreased rapidly on d5 (F=110.1, $P<0.01$). However, unlike that of the WJ, the moisture content of the RJ increased with time and was significantly lower on d2 (F=31.1, $P<0.01$).

The proportion of total protein in the RJ was significantly higher than that in the WJ on d2 and d3 (2d: F=987.9, $P<0.01$; 3d: F=9.1, $P<0.05$); however, similar protein content was found in the d4 RJ and WJ samples (F=0.7, $P>0.05$). The WJ maintained a relatively stable protein content at d2, d3 and d4, but it decreased significantly on d5 (F=27.4, $P<0.01$). The protein content in the RJ significantly decreased from d2 to d4 (F=73.1, $P<0.01$).

WJ and RJ had similar trends in decreasing proportion of 10-HDA over time; however, significant differences were found between WJ and RJ on d2, d3 and d4 (2d: F=63.2, $P<0.01$; 3d: F=12.0, $P<0.05$; d4: F=33.8, $P<0.01$). The 10-HDA content of WJ was maintained at a relatively stable level of approximately 1.50 to 1.80 % during the first 3 days, and was then reduced significantly at d4 and d5 (F=48.6, $P<0.01$). Although RJ had the lowest 10-HDA content on d4, it was still at a higher level than that of WJ.

Both the fructose and glucose levels in the RJ were significantly higher than those for the WJ at d2, d3 and d4 (2d: F=266.7, $P<0.01$; 3d: F=59.7, $P<0.05$; d4: F=48.8, $P<0.01$ and 2d: F=43.5, $P<0.01$; 3d: F=1080.5, $P<0.05$; d4: F=85.8, $P<0.01$, respectively). In the first 3 days, the content of fructose and glucose in the RJ was three times that of the WJ. The food of the worker larvae had the highest levels of fructose and glucose at d5 (F=39.3, $P<0.01$ and F=21.6, $P<0.01$, respectively). The fructose and glucose content of RJ were maintained at a relatively stable level during these 3 days, despite the significantly increased fructose level on 4d (F=8.9,

Table I. Analyzed chemical composition of the RJ and WJ samples (g/100 g, fresh weight) from different days. Data are reported as mean±SD. An asterisk (*) next to the higher value of RJ or WJ indicates a significant difference between the RJ and WJ samples on the corresponding day at $P < 0.05$. Different lowercase letters (a, b, c) in a column indicate significant differences among RJ or WJ samples at $P < 0.05$. Different uppercase letters (A, B) in a column indicate significant differences among the O2d, O3d and O4d RJ samples at $P < 0.05$.

Type and main wt. (g/100 g, fresh weight)	Moisture		Total protein		10-HDA		Fructose	
	WJ	RJ	WJ	RJ	WJ	RJ	WJ	RJ
2d	73.22±0.10*a	51.03±2.77b	13.63±0.14a	17.93±0.27*a	1.79±0.04a	3.72±0.30*a	1.52±0.18c	5.35±0.37*b
3d	72.85±0.62*a	61.55±2.40a	13.91±0.45a	14.33±1.05*b	1.78±0.14a	2.58±0.15*b	1.93±0.26bc	5.93±1.13*b
4d	71.73±1.11*a	61.28±2.95a	14.05±0.35a	13.73±0.44c	1.50±0.067b	2.19±0.07*c	3.70±0.70b	6.95±1.15*a
5d	57.43±3.00b	–	11.21±0.98b	–	0.76±0.065c		9.50±2.06a	–
O2d		49.01±6.59B		17.78±1.74A		2.46±0.15A		8.37±1.23A
O3d		61.75±2.27A		13.39±0.84B		2.29±0.54A		6.16±1.40B
O4d		61.09±3.19A		13.83±0.49B		2.10±0.10A		5.11±1.42B

Type and main wt. (g/100 g, fresh weight)	Glucose		F/G		G+F	
	WJ	RJ	WJ	RJ	WJ	RJ
2d	1.06±0.050b	5.12±1.03*	1.43±0.22b	1.08±0.24	2.58±0.14c	10.47±1.30*b
3d	1.60±0.14b	5.48±0.80*	1.21±0.20b	1.08±0.11	3.54±0.30bc	11.41±1.87*b
4d	2.33±0.59b	6.06±0.97*	1.60±0.11*a	1.15±0.053	6.03±1.29b	13.01±2.09*a
5d	5.80±1.60a	–	1.66±0.11a	–	15.29±3.65a	–
O2d		7.37±0.64A		1.13±0.072A		15.74±1.85A
O3d		5.62±0.88B		1.09±0.10AB		11.77±2.25B
O4d		4.97±1.22B		1.02±0.034B		10.08±2.64B

Table II. Mineral and trace elements in RJ and WJ samples (mg/kg, fresh weight) from different days. Data are reported as mean±SD. An asterisk (*) next to the higher value of RJ or WJ indicates a significant difference between the RJ and WJ samples on the corresponding day at $P < 0.05$. Different lowercase letters (a, b, c) in a column indicate significant differences among RJ or WJ samples at $P < 0.05$. Different uppercase letters (A, B) in a column indicate significant differences among the O2d, O3d and O4d RJ samples at $P < 0.05$.

Type and main wt.(mg/kg, fresh weight)	Fe		Zn		Cu		Mn	
	WJ	RJ	WJ	RJ	WJ	RJ	WJ	RJ
2d	13.22±1.68b	25.43±8.55	17.33±0.51a	24.80±1.32*a	3.88±0.30a	4.74±0.28*a	1.87±1.51	0.52±0.16b
3d	16.52±3.64b	23.66±4.44	17.47±0.78a	24.91±3.25*a	3.88±0.14a	4.66±0.38a	1.49±0.62	1.43±0.64a
4d	21.30±4.68b	38.04±0.90*	16.72±1.85a	18.23±2.59b	3.29±0.65a	4.24±0.53*b	2.37±0.18*	1.59±0.26a
5d	30.73±6.66a	–	9.75±1.63b	–	1.43±0.35b	–	2.93±1.47	–
O2d		38.99±4.24A		22.48±3.24A		5.57±1.05A		2.48±0.96A
O3d		35.63±1.37A		17.56±1.61B		3.67±0.25B		0.95±0.54B
O4d		35.94±3.78A		19.28±3.45AB		4.37±0.54B		1.46±0.089AB

Type and main wt.(mg/kg, fresh weight)	Na		K		Ca		Mg	
	WJ	RJ	WJ	RJ	WJ	RJ	WJ	RJ
2d	563.38±47.38a	689.79± 31.37*a	1968.7± 147.42a	2852.13± 70.27a*	216.94± 14.62ab	257.91± 23.68a	316.22± 36.57a	424.55± 20.28*a
3d	689.29± 174.75*a	184.10± 19.63b	2126.54± 87.56a	2562.61± 48.83b*	247.29±60.75a	276.29± 12.90a	343.07± 28.66ab	337.71± 64.62a
4d	673.38± 63.78*a	191.62± 57.53b	1744.76± 228.26a	1937.87±20.32c	216.94± 43.34*ab	108.71± 16.31b	264.46± 56.52b	309.92± 32.34b
5d	133.85±46.07b	–	1049.82± 76.05b	–	147.76±38.26b	–	147.44±8.98c	–
O2d		170.02± 35.08A		2457.16± 246.23A		175.97± 46.42A		436.33± 21.94A
O3d		169.69±9.72A		1961.87± 121.81B		217.49± 70.79A		334.99± 30.95B
O4d		173.39± 33.14A		1977.95± 79.37B		166.92± 39.04A		305.11± 15.48B

$P<0.01$). The content of F+G in RJ was significantly higher than in WJ during the first three stages (2d: F=103.4, $P<0.01$; 3d: F=182.5, $P<0.01$; d4: F=66.4, $P<0.01$). The F/G ratios of WJ and RJ were similar at 2d and 3d (2d: F=4.37, $P=0.082$; 3d: F=1.55, $P=0.26$). The F/G ratio of 3d-WJ was significantly higher than that of 3d-RJ (F=52.86, $P<0.01$). The F/G ratios for 4d- and 5d-WJ were significantly higher than those for 2d- and 3d-WJ (F=6.04, $P<0.05$). No significant difference was found among 2d-, 3d- and 4d-RJ samples (F=0.43, $P=0.66$), but O2d and O3d RJ samples had significantly higher F/G ratios than O4d RJ (F=7.03, $P<0.05$).

Mineral and trace element content in the WJ and RJ samples is shown in Table II. For the 2d jellies, relatively high levels of zinc, copper, sodium, potassium and magnesium were found in RJ samples compared to WJ samples. On the third day, the RJ contained higher levels of zinc and potassium and lower levels of sodium than the WJ. On day 4, the content of iron and copper was higher in RJ than in WJ, but the WJ contained significantly higher levels of manganese, sodium and calcium than the RJ. Most notably, zinc, copper, potassium and magnesium were found in the lowest concentrations in 5d-WJ samples, whereas the content of these minerals on 2d, 3d and 4d-WJ or 2d and 3d-RJ samples was similar. The iron content of WJ was similar during the first 3 days and reached the highest levels at the 5d, but no significant difference was found for the RJ samples. With time, the manganese levels in RJ were lower on 2d and increased significantly from the 3d, but no differences were found for WJ samples.

The O2d RJ contained significantly lower levels of moisture (F=13.14, $P<0.01$) and higher levels of protein, glucose, fructose, Cu, K and Mg than the O3d and O4d RJ samples (total protein: F=13.14, $P<0.01$; glucose: F=8.44, $P<0.01$; fructose: F=7.57, $P<0.01$; Cu: F=7.73, $P<0.01$; K: F=11.95, $P<0.01$; and Mg: F=39.32, $P<0.01$). F+G of O2d RJ was significantly higher than that of O3d RJ and O4d RJ samples (F=8.11, $P<0.01$). Significant differences were found in the content of 10-HDA, Fe, Na and Ca among these three RJ samples (10-HDA: F=1.9, $P>0.05$; Fe: F=1.21, $P>0.05$; Na: F=0.02, $P>0.05$; and Ca: F=1.05, $P>0.05$). The lowest levels of Zn and Mn were found in the O3d RJ samples (Zn: F=3.32, $P>0.05$; and Ca: F=6.05, $P<0.05$).

4. DISCUSSION

It has long been believed that caste determination in honey bees is driven by the quantity and quality of brood food (Shuel and Dixon 1960). Although previous research has shown that RJ and WJ differ in some nutrients, this study showed a more significant difference in chemical composition between RJ and WJ. We found that RJ had lower moisture content than WJ. The WJ had high moisture content during the first 4 days, which decreased sharply on day 5, while an increasing trend was found in the RJ. This is in agreement with previous studies (Elser 1929; Smith 1959; Dietz 1966). Elser (1929) found a decrease in the percentage of protein in queen larvae food, and a decreasing total protein content in RJ was also found in our study. In a study by von Planta (1888), higher protein and lower glucose content was found in the food of worker larvae less than 4 days old compared with older larvae. We also found significantly lower protein content in d5-WJ and an increasing trend of glucose content in WJ. As Kamakura (2011) found that a single protein in royal jelly could induce queen differentiation, the royalactin content in RJ and WJ may also differ. Although numerous studies have confirmed the 10-HDA content of RJ (Takenaka and Echigo 1980; Lercker et al. 1992; Bloodworth et al. 1994; Koshio and de Almeida-Muradian 2003; Zheng et al. 2011), we have found no reports analyzing the 10-HDA content of WJ. This study provides compelling evidence that queen larvae are fed significantly higher amounts of 10-HDA than are worker larvae. The 10-HDA content in WJ was no more than 2 % during the first 4 days, and this declined to the lowest level at 5d. Although the proportion of 10-HDA in RJ also showed a downward trend, the 10-HDA content remained at high levels. Previous studies found that the proportion of sugars in RJ for female larvae under 4 days old was approximately 34 % (dry mass), whereas only 12 % sugars were found in WJ fed to worker larvae 0–1.5 days old (Shuel and Dixon 1959). Similarly, Asencot and Lensky

found that sugars in RJ for 1–3-day-old queen larvae were at levels approximately 4 times higher than those found in WJ (12.4 vs 3.1 %, dry mass) (Asencot and Lensky 1988). In our study, we found that the G+F levels in RJ were approximately 4, 3 and 2 times higher than those in WJ at d2, d3 and d4, respectively. These results are consistent with previous reports. Interestingly, the glucose and fructose content in the WJ maintained low levels at d2, d3 and d4, and increased sharply at d5. This suggests that a higher proportion of honey is supplied to worker larvae at d5. Overall, RJ and WJ had similar Fe, Mn, Ca and Mg content in each age sample. The RJ contained higher levels of Zn, Cu and K and a lower level of Na than that of WJ. However, the Na levels in the 2d-RJ were higher, although they were significantly lower in the O2d-RJ. Because the 2d-RJ consists of 1d-RJ and O2d-RJ, the additional Na content worker bees feed female larvae the same food during their first 3 days of larval development (Haydak 1943, 1970; Nijhout and Wheeler 1982; Wilde and Beetsma 1982). Zheng et al. (2011) found that the composition of RJ harvested at different times varied significantly. Our results are in agreement, as RJ samples collected at 2d, 3d and 4d varied significantly. Interestingly, when the mixture of RJ samples was separated into each corresponding day, we found that the composition of RJ samples fed to 3-day-old (O3d) and 4-day-old (O4d) queen larvae was nearly identical. This may provide insight into the basic biology of honey bees. The chemical composition of food fed to 2-, 3- and 4-day-old worker larvae was similar.

In mammals, gene expression and development can be influenced by nutrition (Dolinoy et al. 2007). Previous studies have shown that the rate of food intake is influenced by the level of sugars (Asencot and Lensky 1975, 1976; Lensky 1985), most likely associated with hormonally regulated mechanisms (Lensky et al. 1978; Asencot and Lensky 1984). Although Kamakura's work (2011) is based on the effects of a single protein on queen differentiation, it is unquestionably a good start in understanding the relationship between nutrition and caste determination. In this study, tremendous variations in chemical composition were observed between RJ and WJ samples. We still know very little about the role of these nutrients in honey bee caste determination. Therefore, much still remains to be explored of the relationship between nutritional effects and polyphenism in honey bees.

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Comparaison de la composition en nutriments de la gelée royale et de la gelée pour ouvrières chez les abeilles (*Apis mellifera*)

substance chimique / valeur nutritionnelle / protéines / fructose / glucose

Vergleich der Nährstoffzusammensetzung von Königinnen- und Arbeiterinnenfuttersaft der Honigbiene (*Apis mellifera*)

chemische Zusammensetzung / Gelée royale / Arbeiterinnenfuttersaft / Nahrungseffekt

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