



Nutritional and mineral contents of honey extracted by centrifugation and pressed processes



Samir Moura Kadri, Rodrigo Zaluski, Ricardo de Oliveira Orsi *

Núcleo de Ensino, Ciência e Tecnologia em Apicultura Racional (NECTAR), Departamento de Produção Animal, Faculdade de Medicina Veterinária e Zootecnia, UNESP, Universidade Estadual Paulista, Distrito de Rubião Jr., s/n, Zip Code 18618-000 Botucatu, São Paulo, Brazil

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ABSTRACT

In this study, wild honey samples extracted by two different methods (centrifugation and pressed processing) were characterized and compared based on their physicochemical, and nutritional properties, macro- and micro-mineral contents, and pollen counts. Twelve colonies of Africanized *Apis mellifera* were used; six honey samples were obtained by centrifugation and six by honeycomb press. All physicochemical parameters of honey samples (moisture, pH, total acidity, ash, dry matter, and qualitative absence of hydroxymethylfurfural) were within the limits established by EU legislation, and all parameters in pressed honey were superior ($p < 0.05$). Nutritional contents (total carbohydrates, total lipids, total proteins, flavonoids, and ascorbic acid) and minerals (K, Ca, Mg, Na, Fe, Li, Zn) were also higher in pressed honey. The quantity of pollen in pressed honey samples was 5.6-fold higher than in centrifuged samples. Pressed honey, can be marked as a differentiated product with a higher mineral content and several nutritional properties.

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1. Introduction

Honey is the sweet natural substance produced by honey bees from the nectar of blossoms, the secretions of the living parts of plants, or the excretions of plant-sucking insects on the living parts of the plants. Honey bees collect, transform, and combine these materials with specific substances of their own and store them in honeycombs, where they ripen and mature (Codex Alimentarius Commission., 2001).

Honey is a nutritious food of worldwide economic importance. It is a complex mixture of carbohydrates, proteins, enzymes, amino acids, lipids, vitamins, volatile chemicals, phenolic acids, flavonoids and minerals (Ball, 2007). The composition, colour, aroma, and flavour of honey depend primarily on the flowers, climate, geographical regions, and honey bee species involved in its production. These properties are also affected by weather conditions, processing, manipulation, packaging and storage time (Escuredo, Dobre, Fernández-González, & Seijo, 2014; Tornuk et al., 2013).

Honey extraction is an important beekeeping practice and involves the removal of honey from combs and its isolation as a pure liquid (Maradun & Sanusi, 2013). Modern practices of honey

extraction involve harvesting and centrifuging the combs in stainless steel facilities designed for food processing. Honey can also be extracted using honey press machines or by draining honeycombs (Codex Alimentarius Commission, 2001).

The extraction process may influence the quality of honey, and we hypothesized that some physicochemical properties, nutritional and mineral contents may depend on the method used to extract honey from combs. Therefore, the objective of the present study was to characterize and compare honey extracted by standard centrifugation and pressing processes. Physicochemical parameters, nutritional properties, macro- and micro-mineral contents, and pollen quantity were used to evaluate honey extracted by these two procedures.

2. Material and methods

2.1. Sampling

The experiment was conducted at the apiary in the Beekeeping Production Area of the Edgárdia Experimental Farm, Faculty of Veterinary Medicine and Animal Science, UNESP, Botucatu, São Paulo, Brazil, 22°82'S and 48°39'W, with a humid subtropical climate and an average elevation of 488 m.

* Corresponding author.

E-mail addresses: samirkbr@yahoo.com.br (S.M. Kadri), rodrigozaluski@yahoo.com.br (R. Zaluski), orsi@fmvz.unesp.br (R.O. Orsi).

12 colonies of Africanized *Apis mellifera* housed in Langstroth beehives were used. In December 2014, the colonies received one super each above a queen excluder following standard apicultural methods. The honey supers of each colony were identified and harvested at the end of the wild blossom period (March). Honey was extracted from the supers only when 90% of the total area of frames was capped.

Honey from six colonies was extracted with a standard stainless steel centrifuge (centrifuged honey). Honeycombs from the other six colonies were cut with a clean knife, and the honey was extracted using a manual commercial stainless steel honey press (pressed honey). All procedures were performed using clean facilities and equipment, and handlers followed hygienic practices established by the [Codex Alimentarius Commission. \(2003\)](#). 500 g of honey from each colony was stored in sealed glass jars and frozen at -20°C until analysis.

2.2. Physicochemical analysis

Moisture (%), pH, total acidity (meq kg^{-1}), ash (%), dry matter (%), and qualitative hydroxymethylfurfural (HMF) were measured for each honey sample as described by [Kadri, Zaluski, Lima, Mazzafera, and Orsi \(2016\)](#). All analyses were performed in triplicate.

2.3. Nutrient content

Total carbohydrate (g kg^{-1}), total lipid (%), total protein content (%), total flavonoids (mg of quercetin equivalents per kg; mg QE kg^{-1}), and ascorbic acid (mg kg^{-1}) were determined according to [Kadri et al. \(2016\)](#). All analyses were performed in triplicate.

2.4. Mineral analysis

A Varian model 12/1475 spectrometer was used to determine the concentrations of potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), zinc (Zn), copper (Cu), lithium (Li), aluminum (Al), and nickel (Ni). The limits of detection were 0.02, 0.10, 0.03, 0.05, 0.01, 0.04, 0.07, 0.03, 0.02, and 0.01 ppm, respectively. Sample preparation and spectrometric measurements were performed following the methodology of [Kadri et al. \(2016\)](#). A recovery experiment was performed by spiking two wild honey samples with known amounts of the analytical standards of the 10 minerals. The mean percentage recoveries of the analyzed minerals ranged from 96.38% to 99.81%. The results were expressed as mg kg^{-1} .

2.5. Absolute pollen count

The total number of pollen grains in the honey samples was determined by the method of [Song, Yao, and Yang \(2012\)](#). Briefly, 10 g of each honey was dissolved in 20 ml of warm water (40°C). The solution was centrifuged for 10 min at 3000g (Nova Técnica, Brazil), the supernatant was decanted, and the sediments were collected and treated with an acetolysis mixture (acetic anhydride:

concentrated sulphuric acid = 9:1 v/v) for approximately 30 min at $25 \pm 2^{\circ}\text{C}$. Pollen grains were counted under a microscope at $100\times$ magnification (Nikon, Eclipse E200) over a haemocytometer (counting chamber). The chamber was 0.1 mm high and had 25 medium-sized squares (0.04 mm^2 each), which were subdivided into 16 smaller squares of 0.0025 mm^2 each. These dimensions corresponded to a volume of $0.1\text{ }\mu\text{l}$ in the chamber, $0.004\text{ }\mu\text{l}$ in each medium-sized square, and $0.00025\text{ }\mu\text{l}$ in each small one. For each sample, the pollen grains were counted in five medium squares at the centre and at the left and right corners at the top and bottom of the chamber and were repeated until 100 individual observations were made. Based on the average of these 100 observations, the absolute pollen counts in the $100\text{-}\mu\text{l}$ suspensions of pollen sediment from 10 g of honey were calculated.

2.6. Statistical analysis

Data were analyzed using SPSS (version 9.0). Pearson's correlation coefficient test (r) was employed to determine the strength of linear relationships between the variables. Analysis of variance (ANOVA) followed by Tukey's test ($p < 0.05$) was used for comparison of means.

3. Results and discussion

3.1. Physicochemical parameters

The results of the physicochemical analyses (moisture, pH, total acidity, ash, dry matter, and qualitative presence of hydroxymethylfurfural) of centrifuged and pressed honey are summarized in [Table 1](#). A high correlation was found between total pollen content and moisture ($r = 0.985$), pH ($r = 0.995$), total acidity ($r = 0.986$), ash ($r = 0.986$), and dry matter ($r = 0.994$).

Moisture ranged from 17.67–17.98 and 18.98–19.05% in centrifuged and pressed honey, respectively. Moisture content in pressed honey was greater ($p < 0.05$). However, values in all samples were within the range (not more than 20%) required by the European Regulations of Quality ([European Union, 2002](#)). Water content depends on the botanical origin of the honey, the level of maturity achieved in the hive, processing techniques, and storage conditions ([Yücel & Sultanoglu, 2013](#)). Moisture content influences viscosity, colour, flavour, crystallization, taste, solubility, specific gravity, and conservation of honey ([Escuredo, Míguez, Fernández-González, & Seijo, 2013](#)). To avoid honey with high moisture, honey was extracted from supers only when 90% of the total area of frames was capped. Harvesting honey at the appropriate maturity is important, because this food is hygroscopic and can absorb moisture from the atmosphere during processing ([Karabagias, Badeka, Kontakos, Karabournioti, & Kontominas, 2014](#)).

The pH of centrifuged and pressed honey samples ranged from 4.06–4.15 and 4.19–4.33, respectively. These results are consistent with values obtained for honey from different origins ([Gomes, Dias, Moreira, Rodrigues, & Estevinho, 2010](#); [Saxena, Gautam, & Sharma, 2010](#)). This parameter is important during the extraction and storage of honey, because it influences stability, texture, and shelf life

Table 1
Physicochemical parameter analyses of wild honey samples extracted by centrifugation and pressed processes.

Process	Moisture%	pH	Total acidity (meq kg^{-1})	Ash%	Dry matter (%)	Qualitative HMF
Centrifuged	$17.76 \pm 0.11\text{b}$	$4.11 \pm 0.03\text{b}$	$16.13 \pm 0.05\text{b}$	$0.16 \pm 0.01\text{b}$	$80.56 \pm 0.41\text{b}$	Negative
Range	17.67–17.98	4.06–4.15	16.10–16.23	0.15–0.17	79.98–81.12	–
Pressed	$19.01 \pm 0.03\text{a}$	$4.26 \pm 0.06\text{a}$	$17.22 \pm 0.06\text{a}$	$0.23 \pm 0.01\text{a}$	$87.51 \pm 0.54\text{a}$	Negative
Range	18.98–19.05	4.19–4.33	17.12–17.24	0.22–0.23	86.90–88.14	–

Data were expressed as average $\pm$ standard deviation based on three measurements ($n = 6$ honey samples/process). Different letters in the same column indicate a significant difference between values according to Tukey's test ($p < 0.05$).

(Terrab, Diez, & Heredia, 2002). The pH of pressed honey was significantly higher ($p < 0.05$) than that of honey harvested using centrifugation. However, the values found were consistent with the inhibition of micro-organism growth, because the optimum pH for most organisms is between 7.2 and 7.4 (da Silva, Gauche, Gonzaga, Costa, & Fett, 2016; Karabagias et al., 2014). According to Kamal et al. (2002), a variation in acid or mineral content can influence the pH of honey. A high correlation was found between total acidity and pH ($r = 0.828$) and between total mineral content and pH ($r = 0.846$). Thus, the higher pH of pressed honey may be due to the greater quantities of minerals and total acidity (Table 3).

The total acidity of honey ranged from 16.10–16.23 and 17.12–17.24 meq kg⁻¹ in centrifuged and pressed honey, respectively; the values in pressed honey were higher ($p < 0.05$). These values are lower than those reported by Azeredo, Azeredo, Souza, and Dutra (2003) and by Almeida-Muradian et al. (2013). All samples showed acidity levels within the limit of 50 meq kg⁻¹ established by the European Union (2002). Free acidity is an important parameter related to the deterioration of honey and is associated with many factors, including floral sources, mineral content, time of harvesting, and amount of gluconic acid resulting from the enzymolysis of glucose (Karabagias et al., 2014).

The ash content of samples ranged from 0.15–0.17 and 0.22–0.23% in centrifuged and pressed honey, respectively; values were higher ($p < 0.05$) in pressed honey. These results are within the ranges reported by Almeida-Muradian et al. (2013) and by Yücel and Sultanoglu (2013). Ash content correlates with the mineral content of honey (da Silva et al., 2016), and the ash content reported in this study is consistent with the mineral content (Table 3). A high correlation was found between ash and mineral content ($r = 0.987$). Studies have shown that the average ash content in honey is 0.17% with a range of 0.02–1.03% (Chakir, Romane, Barbagianni, Bartoli, & Ferrazzi, 2011).

The dry matter of honey samples ranged from 79.98–81.12 and 86.90–88.14% in centrifuged and pressed honey, respectively; values were higher ($p < 0.05$) in pressed honey. The majority of dry matter in honey consists of sugars (Wang & Li, 2011), which are the predominant compounds in honey (Table 2). A linear correlation was found between the dry matter and total carbohydrates ($r = 0.969$).

All centrifuged and pressed honey samples had negative levels of hydroxymethylfurfural (HMF). HMF is an indicator of the

freshness; it is absent in fresh honey and tends to increase during processing and/or ageing. HMF is formed by the decomposition of monosaccharides (Maillard reaction), when honey is heated or stored for a long time (da Silva et al., 2016). HMF is also indicative of adulteration with inverted sugar syrup (Wang & Li, 2011).

3.2. Nutrient analysis

The results of nutrient analyses of centrifuged and pressed honey (total carbohydrates, total lipids, total proteins, total flavonoids, and ascorbic acid) are summarized in Table 2.

All parameters were higher in pressed honey ($p < 0.05$), which is due to the greater quantity of pollen in pressed honey. A high correlation was found between pollen content and total carbohydrates ($r = 0.965$), total lipids ($r = 0.862$), total proteins ($r = 0.977$), total flavonoids ($r = 0.980$), and ascorbic acid ($r = 0.852$). Pollen is a source of protein (da Silva et al., 2016), lipids (Almeida-Muradian et al., 2013), carbohydrates, flavonoids, and ascorbic acid (Komosinska-Vashev, Olczyk, Kazmierczak, Mencner, & Olczyk, 2015). It is important to point out that all honey samples analyzed in this study were produced in the same apiary using standard handling procedures and that the bees collected resources from the same area. Thus, the differences observed are due to the process of honey extraction.

Carbohydrates in honey ranged from 682.34–745.13 and 822.65–838.95 g kg⁻¹ in centrifuged and pressed honey, respectively. Sugar composition depends mainly on the honey's botanical (the types of flowers used by the bees) and geographical origins and is affected by climate, processing, and storage (Escuredo et al., 2014; Tornuk et al., 2013). This study provides evidence that the extraction process can increase the sugar content of honey. This may be due to the increased pollen content of pressed honey, because pollen has an average of 30.8% carbohydrates (Komosinska-Vashev et al., 2015). The sugars in honey contribute to energy value, hygroscopicity, viscosity, and granulation (Kamal & Klein, 2011). All honey samples evaluated fall within the range of standards required for commercialization established by the European Regulations of Quality (European Union, 2002).

Lipids in honey samples ranged from 0.83–0.89 and 1.04–1.31% in centrifuged and pressed honey, respectively. The lipid content of honey samples in this study was greater than that reported by Almeida-Muradian et al. (2013), who found 0.37 to 0.39% lipids

Table 2

Nutritional parameter analyses of wild honey samples extracted by centrifugation and pressed processes.

Process	Total carbohydrates (g kg ⁻¹)	Total lipids (%)	Total proteins (%)	Total flavonoids (mg QE kg ⁻¹)	Ascorbic acid (mg kg ⁻¹)
Centrifuged	698.41 ± 23.28b	0.85 ± 0.03b	0.15 ± 0.01b	3.08 ± 0.19b	252.57 ± 20.17b
Range	682.34–745.13	0.83–0.89	0.15–0.16	2.82–3.22	220.81–271.39
Pressed	831.35 ± 6.64a	1.13 ± 0.12a	0.24 ± 0.01a	7.96 ± 0.61a	310.24 ± 13.88a
Range	822.65–838.95	1.04–1.31	0.23–0.25	7.07–8.65	288.82–324.58

Data were expressed as average ± standard deviation based on three measurements ($n = 6$ honey samples/process). Different letters in the same column indicate a significant difference between values according to Tukey's test ($p < 0.05$).

Table 3

Macro-mineral (K, Ca, Mg, and Na) and micro-mineral (Fe, Zn, Cu, Li, Al, and Ni)* content (mg kg⁻¹) of wild honey samples extracted by centrifugation and pressed processes.

Process	Macro-minerals			Micro-minerals					Mean total mineral content
	K	Ca	Mg	Na	Fe	Zn	Cu	Li	
Centrifuged	967.72 ± 5.37b	264.11 ± 0.34b	86.26 ± 0.37b	9.70 ± 0.06b	7.55 ± 0.22b	1.42 ± 0.08b	0.42 ± 0.02a	N.D.	1337.26 ± 6.44b
Range	956.98–970.59	263.67–264.67	85.67–86.79	9.69–9.83	7.23–7.89	1.41–1.43	0.41–0.43	N.D.	–
Pressed	1038.53 ± 8.29a	326.90 ± 0.43a	96.60 ± 0.51a	20.24 ± 0.04a	18.55 ± 0.13a	1.72 ± 0.01a	0.42 ± 0.01a	0.30 ± 0.01	1503.27 ± 9.41a
Range	1026.57–1046.71	326.25–327.48	96.02–96.62	20.19–20.32	18.34–18.72	1.70–1.73	0.41–0.43	0.29–0.31	–

Data were expressed as average ± standard deviation based on three measurements ($n = 6$ honey samples/process). Different letters in the same column indicate a significant difference between values according to Tukey's test ($p < 0.05$). N.D., not detected.

* Al and Ni were not detected.

in wild honey, and by Escuredo et al. (2013), who found mean values of 0.1%.

The protein contents ranged from 0.15–0.16 and 0.23–0.25% in centrifuged and pressed honey, respectively. The presence of proteins in honey is attributed to secretions from the salivary glands and pharynx of bees and to vegetal sources (nectar and pollen) (da Silva et al., 2016; Sak-Bosnar & Sakac, 2012). The protein contents in this study differ from those of Almeida-Muradian et al. (2013), who found $0.49 \pm 0.01\%$ total protein in wild honey samples collected in the northeast of Brazil.

Total flavonoids in honey ranged from 2.82–3.22 and 7.07–8.65 mg QE kg⁻¹ in centrifuged and pressed honey, respectively. The phenolic content of honey is affected by floral sources (Can et al., 2015), and the results of this study demonstrate that the extraction process also can affect phenolic content. Phenolic compounds act as antioxidants due their ability to inhibit lipid peroxidation and lipoxygenase activity *in vitro* (Gómez-Estaca, Lopez-de-Dicastillo, Hernandez-Munoz, Catala, & Gavara, 2014). The total flavonoid values obtained here are less than those reported by Can et al. (2015) in different types of honey derived from Turkish flora. The values obtained in pressed honey are within the range obtained by Escuredo et al. (2013).

Ascorbic acid, another important antioxidant, was found in honey samples at levels ranging from 220.81–271.39 and 288.82–324.58 mg kg⁻¹ in centrifuged and pressed honey, respectively. These values are higher than those reported by Ferreira, Aires, Barreira, and Estevinho (2009) and Ciulu et al. (2011). Ascorbic acid is essential in the biosynthesis of collagen, carnitine, and neurotransmitters. Low serum levels of vitamin C may have serious health implications for humans and are particularly relevant to the onset and progression of degenerative diseases such as cancer and cardiovascular disease (Li & Schellhorn, 2007).

The consumption of honey with greater nutrient content can help defend against oxidative stress, primarily because of the presence of antioxidants such as ascorbic acid and flavonoids (Devasagayam et al., 2004).

3.3. Mineral content

The macro- and micro-mineral contents of centrifuged and pressed honeys are summarized in Table 3.

The macro-mineral K, Ca, Mg, and Na contents in pressed honey samples exceeded those in centrifuged samples by 7, 23, 12, and 108% ($p < 0.05$), respectively. The micro-minerals Fe and Zn were 45 and 21% higher ($p < 0.05$), respectively, in pressed honey as well. The micro-mineral Li was detected only in pressed honey, and the Cu content did not differ ($p > 0.05$) between honey extraction processes. Ni and Al were not detected in any samples. Our results indicate that the extraction process influences the mineral content of honey produced in the same geographical region, which correlates with the greater pollen content of pressed honey. A high correlation was found between pollen content and total minerals ($r = 0.992$).

The macro- and micro-mineral levels in honey are related to the floral source and can vary widely among honeys of different origins (Chua, Abdul-Rahaman, Sarmidi, & Aziz, 2012). Mineral content is an important consequence of the geographical origin of honey, and the types of plants and soil can influence the mineral composition (de Alda-Garcilope, Gallego-Picó, Bravo-Yagüe, Garcinuño-Martínez, & Fernández-Hernando, 2012; Karabagias et al., 2014).

Increased mineral content leads to darker and more strongly flavoured honeys (Escuredo et al., 2013; Karabagias et al., 2014), which are attractive features especially considering the health benefits of consuming foods rich in minerals.

In the present work, K was the most abundant element in honey in agreement with studies performed by Alqarni, Owayss, and

Mahmoud (2012). Macro- and micro-minerals promote fundamental functions in biological systems including maintenance of normal physiological responses, induction of the overall metabolism, stimulation of the circulatory and reproductive systems, and catalysis of various biochemical reactions (Alqarni et al., 2012).

3.4. Absolute pollen count

To explain the differences found in the physicochemical parameters and nutritional and mineral contents of centrifuged and pressed honey, we analyzed the pollen content of the honey samples.

The absolute pollen counts per 10 g of honey equaled 8.640 ± 1.440 and 48.720 ± 3.840 pollen grains in centrifuged and pressed honey, respectively. Honey extracted by press processing exhibited approximately 5.6 times more pollen grains ($p < 0.05$) than honey extracted by centrifugation. Based on the classification of Louveaux, Maurizio, and Vorwohl (1978), samples extracted by centrifugation and press processing can be classified as Group I (<20,000 pollen grains) and Group II (25,000–60,000 pollen grains) honeys, respectively. Thus, our results provide new insight into the pollen composition of honey samples and evidence that the extraction process directly affects the pollen content of honey.

Pollen is important for honey bee nutrition and is a greatly valued product in natural medicine, because of its medical and nutritional applications (Komosinska-Vashev et al., 2015). Bee pollen, being a mixture of collected floral pollens, varies widely in composition (Campos et al., 2008) and is composed of many chemical substances including proteins, amino acids, carbohydrates, lipids, fatty acids, phenolic compounds, enzymes, coenzymes, vitamins, and minerals (Komosinska-Vashev et al., 2015).

Strong and positive correlations were found between pollen content and physicochemical parameters, nutritional and mineral contents of honey samples. Our results provide information about the principal physicochemical parameters, nutritional properties, and macro- and micro-mineral contents that are changed by the process of honey extraction; and that the higher levels of pollen content of pressed honey explain the differences observed in the current study.

Our results provide evidence that honey extracted by the pressed process can yield a product with higher nutritional value, greater macro- and micro-mineral content, and physicochemical parameters within the standards of commercialization. Honey extraction by pressed processing can produce a differentiated product for sale in specific markets, thereby increasing beekeepers' income and enhancing consumers' health.

It is important to consider that all combs are lost in the extraction of honey by pressed processing and that bees must rebuild combs to store honey. This problem is avoided in centrifugation, where the combs can be returned to colonies for honey storage by bees. Thus, honey extracted by pressed processing should command a higher price in its commercialization.

4. Conclusion

This study characterized and compared wild honey samples extracted by centrifugation and pressing process. Our results demonstrate that physicochemical parameters of honey samples (moisture, pH, total acidity, ash, dry matter, and qualitative absence of hydroxymethylfurfural) were higher in pressed honey. The honey extracted by press processing has higher nutrient (total carbohydrates, total lipids, total proteins, total flavonoids, and ascorbic acid) and mineral (K, Ca, Mg, Na Fe, Zn, and Li) contents. Our results demonstrated that the quantity of pollen in pressed honey samples was 5.6-fold higher than in centrifuged samples.

Strong and positive correlations were found between pollen content and physicochemical parameters, nutritional and mineral contents of honey samples. Our results provide evidence that honey extracted by the pressed process can yield a product with higher nutritional value, greater macro- and micro-mineral content, and physicochemical parameters within the quality standards established by the European Union of commercialization.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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S.M. Kadri Animal Scientist; PhD in Animal Sciences, São Paulo State University, College of Medicine Veterinary and Animal Sciences, Botucatu, São Paulo, Brazil.

R. Zaluski Biologist; PhD Candidate in Animal Production, São Paulo State University, College of Medicine Veterinary and Animal Sciences, Botucatu, São Paulo, Brazil.

R.O. Orsi Biologist; PhD (Animal Production) and Professor, São Paulo State University, College of Medicine Veterinary and Animal Sciences, Botucatu, São Paulo, Brazil.