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## **Female and Male Wistar Rats (*Rattus norvegicus*) Discriminate Diets According to Energetic Quantity**

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The food choice of animals is influenced by several factors including the quantity and nutrients available. It is not known, however, whether, faced with alternatives that present the same amount of food, with similar flavor and obtained with the same response cost, rats would discriminate between diets with different energetic quantities. The aim was to verify whether female and male Wistar rats (*Rattus norvegicus*) discriminate between three types of food that differ in their energetic content (whether or not they prefer one) and whether the flavor could affect the choice between two diets with equal energetic quantities. Twelve Wistar rats (six of each sex) underwent tests of choice between pairs of diets of different energetic values. After the tests, the animals had at their disposal, in the home cage, two diets with the same energetic content, which differed in flavor (one contained sucrose) - Flavor test. The consumption of each diet was measured for five consecutive days. All the subjects demonstrated a preference for the more energetic alternative, regardless of the combination of diets presented. In the Flavor test the animals did not show significant preference for any diet, that is, the consumption of both the sweet and non-sweet diet were statistically equal for all subjects. It was concluded that the animals, regardless of sex, discriminated between the diets with different energetic values and that the flavor did not seem to be a determinant variable in the food choice. Keywords: Energetic value discrimination, Food choice, Food flavor, Sex difference, Animal model, Rats.

*Keywords:* energetic value discrimination, food choice, food flavor, sex difference, animal model, rats

In addition to the building (proteins and lipids) and regulator (minerals and vitamins) nutrients, animals need energy, mainly derived from carbohydrates and lipids, to ensure their survival and reproduction (Pyke, Pulliam, & Charnov, 1977; Zucoloto, 2008). The choice of food involves foraging patterns that prioritize a positive energetic balance, i.e., the amount of calories consumed is greater than the amount of calories expended in the maintenance and reproduction of the organisms (Meire & Ervynck, 1986; Schoener, 1971). The theory of optimal foraging (MacArthur & Pianka, 1966) postulates that the animal's food choice is directed toward the greatest possible accumulation and consumption of energy per unit of time, thus maximizing energy gains.

When animals have different food alternatives available (foraging sites, different types or quantities of food), specific characteristics of the situation control their choice and preference (Dunlap, Papaj, & Dornhaus, 2017; Harel et al., 2016). These factors include the quantity of food available in each alternative and its probability of being obtained (Baum & Rachlin, 1969; Catania, 1963a, 1963b; Herrnstein, 1961, 1970; Mazur, 1988; Neuringer, 1967; Vaughan, 1985), as well as the energy balance, which is the relationship between the amount of calories consumed and the amount spent on the maintenance and reproduction of the animals (Meire & Ervynck, 1986; Schoener, 1971).

The energetic quantity, however, is not the only factor controlling the animals' food choice. The amount of nutrients available in the food consumed is as important as the energetic value of it. Studies have shown that animals are able to balance the intake of macronutrients in situations where these are available separately for consumption (self-selection) (Hall, Jackson, Vondran, Vachina, & Jewell et al., 2018; Hall, Vondran, Vachina, & Jewell, 2018; Silva, Kitagawa, & Vázquez, 2016). The experiments by Richter, Holt

and Barelare (1938) performed with rats made use of a procedure (self-selection) in which macro and micro nutrients were provided in pure form inside homing cages. Seeing that self-selection made possible to freely choose the daily proportion of each nutrient that would be ingested by the subjects, they demonstrated an ability to adapt their nutritional needs according to the demand, thus maintaining growth and reproduction at normal levels. Subsequent studies with the same species confirmed the findings of Richter et al. in different conditions, such as physical activity vs. inactivity (Collier, Leshner, & Squibb, 1969), the light/dark cycle phase (Shor-Posner et al., 1991) and the comparison between males, females and their offspring (Jean, Fromentin, Tomé, & Larue-Achagiotis, 2002). The work on self-selection indicates that food choice refers not only to the discriminability of the quantity or availability (probability) of food, but also to the discrimination of aspects related to pre and postingestive signals.

One of the most important and most studied pre-ingestive signals is the flavor (i.e., taste, odor and texture – Touzani & Sclafani, 2007) of the food, perceived by the animals when it comes into contact with their gustatory apparatus. Richter et al. (1938) suggested that, in self-selection experiments, flavor plays an important role in the discrimination of the ingested macronutrient, since odor and taste are inseparable components of food. Oliva et al. (2017) suggested that the characteristic flavor of each food also serves as an indication as to its nutritional value, as with the sweet flavor, which indicates the presence of sugars (Montameyeur & Matsunami, 2002) and the characteristic flavor of the presence of lipids (Treesukosol & Moran, 2018). Rats of the *R. norvegicus* species, for example, have preference for solutions sweetened with sucrose or sucralose, compared to pure water (Sclafani & Ackroff, 2017). However, it must be considered that a specific flavor is only a sign of the presence of a specific nutrient if the relationship between them is stable over time (Myers, 2017) and therefore, this may not be the main control factor of the food choice.

When food is ingested it causes specific neural stimulation depending on the type of macronutrient or micronutrient present in its composition (Sclafani & Ackroff, 2016; Steinert, Feinle-Bisset, Geary, & Beglinger, 2013). This stimulation generates postingestive signals (Keast, 2017), which seem to be preponderant in the feeding choice of the animals, as shown by Araujo et al. (2008), Elizalde and Sclafani (1990), and Miller and Teates (1986). In these studies, the gustatory system of some species of rodents was blocked and, even under these conditions, the animals presented preference for the food with greater nutritional value. In these studies, however, the time required to establish this preference was higher among the animals whose gustatory system had been blocked, compared to the control animals, indicating that the palate plays a facilitating, however, not determinant role in the choice of foods to be consumed. Other similar evidence comes from the study by Sclafani and Ackroff (2017): although the rats in the study consumed a greater amount of sucralose and sucrose solutions compared to pure water consumption, the animals presented higher intake of sucrose solution (greater energy value) compared to the consumption of the sucralose solution.

These studies on food choice and self-selection indicate that animals are able to balance the intake of each macronutrient according to their daily needs, when food is continuously available, and that they also tend to prefer diets with higher energetic quantities. However, these studies do not clarify whether this pattern of food choice would remain in a situation in which animals needed to choose between diets that were energetically different as a supplementary energy source to the conventional diet.

In the situation proposed in the present study, Wistar rats were kept under food restriction (80% of the ad libitum weight) and had conventional food available on a controlled daily basis. In daily sessions conducted in specific apparatus the animals had access to caloric supplementation, being able to choose, in discrete trials, between two diets of similar appearance, differing only in terms of the energetic quantity. Considering that animals tend to choose alternatives with greater amounts of food because they are able to discriminate their energy needs, would these animals discriminate between alternatives in which the amount of food was equal but energetically different? Under these conditions, would the animals show a preference for the alternative

containing the diet with the highest energy amount? To investigate whether flavor would be a decisive indication in the choice of the animals, after the experiment of choice was concluded, the animals were submitted to a flavor test, having two diets of the same energy quantity available (*ad libitum*), but differing in relation to the flavor (i.e., one of them contained sugar).

The aims of this study were (a) to verify whether male and female Wistar rats discriminate between three types of diets that differ only in relation to energy quantity and are offered as caloric supplementation to the conventional diet and (b) to verify whether the subjects establish a preference for one of two diets that are calorically alike but differ in flavor (one with a sweet flavor).

## Method

### Subjects

Subjects were six female and six male naive Wistar rats (*Rattus norvegicus*). Rats were 60 days old and weighed on average 250 g (females) and 350 g (males) upon arrival. Between 60 and 70 days of age, all the animals were housed in couples of the same sex in polypropylene rat cages (41 x 34 x 24 cm) with metallic lid. The room was maintained at a controlled temperature ( $24^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ), with a daily light/dark cycle (12h/12h, with lights on from 6h to 18h). All experimental protocols were performed in the light phase. The animals had water and commercial chow (Nuvilab<sup>®</sup>) *ad libitum* until day 70, when the weight monitoring was initiated: all individuals were weighed every two days in order to establish the baseline (variation less than or equal to 10g/day over three consecutive days). After weight stabilization, the food restriction process was initiated in order to keep the animals at 80% of their *ad libitum* weight. This was determined by calculating 80% of their *ad libitum* weight measured just prior to food restriction (one week prior to the beginning of Exploratory training). To account for growth during periods of restriction, rats' target weights were adjusted upward by 5g (male) and 3g (female) per week, an adaptation from Orsini, Willis, Gilbert, Bizon and Setlow (2016). Throughout the procedure, the weight of the animals were monitored daily. They were kept in pairs throughout the entire accommodation period, being isolated for 1h/day for controlled access commercial chow.

The study was conducted according to the Brazilian bioethics in animal experiments resolutions, through analysis and approval (authorization 16.5.676.59.9) of the Ethics Committee on Animals Use of the University of São Paulo - Ribeirão Preto.

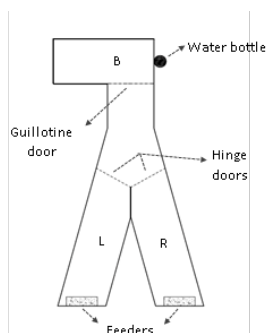
### Equipment

A modified Y-maze (Figure 1), with closed arms, 50cm in height and 15cm wide was used. In compartment B there was a guillotine door that was opened manually by the experimenter and gave access to the arm of choice in Y. On the outside of this compartment a plastic water bottle was attached, which provided water at will throughout the session. On each side of the bifurcation (alternatives L and R - left and right) there was a non-return hinge door and at the end of each arm a metal feeder (4 x 6 x 1.5 cm), suspended at a height of 3cm.

### Diets

For the first part of the study (Preference Tests), three diets that differed in energy amount (D, D1 and D2) were designed. In the Flavor Test, two other diets were tested, one containing sucrose (S) and one not (N). Table 1 shows the composition and energetic quantity in 100g of each of the diets. Maizena<sup>®</sup> cornstarch and União<sup>®</sup> refined sugar were used as the carbohydrate source; Sadia<sup>®</sup> animal lard were used as the lipid source; Kasvi<sup>®</sup> Agar K25-611001 were used as the agar source.

**Figure 1.** A Modified Y-Maze



*Note:* Trials began in Compartment B, with the guillotine door closed, and ended after the animal consumed all the food pellets contained in the chosen alternative (R or L).

**Table 1**

*Nutritional Composition of the Different Types of Diets and their Respective Energy Values in 100g*

Nutrition Information (100 g portion)					
Food	Lipids	Carbohydrate (Cornstarch)	Carbohydrate (Sucrose)	Agar	Energy
D	10 g	20 g	15 g	55 g	230 kcal
D1	10 g	45 g	15 g	30 g	330 kcal
D2	10 g	70 g	15 g	5 g	430 kcal
S	–	50 g	30 g	20 g	320 kcal
N	–	80 g	–	20 g	320 kcal

*Note:* The feeds D, D1 and D2 were tested in the first part of the study; Diets S and N were tested in the second part of the study.

### Pelletization of the Diets

The diets used were designed and produced weekly by the first author of this study and each type of diet was stored separately in glass jars with airtight lid, which were kept in a cabinet in the anteroom of the experimental room.

A rectangular mold of 252 cm<sup>2</sup> containing 375 circular, 1-cm (diameter) compartments was used for the diets used in the first part of this study. The ingredients were mixed in a plastic container until the mixture had a consistency of granulated flour. Water was then added until the dough reached a sandy consistency, which was placed in the rectangular mold so that it acquired the pellet shape. After removal from the mold, the pellets were placed in an oven at a temperature between 60°C and 70°C for approximately 90 min or until they became firm. At the end of the process, each pellet weighed approximately 27 mg ( $\pm 2$  mg).

Diets A and N went through the same pelletizing process; however, the pellets were molded manually in a cylindrical format, with each pellet being approximately 10 g.

### Procedure

The preference test was divided into nine phases: Exploratory Training, Diet Test I, Inversion of Alternatives I, Baseline I, Diet Test II, Inversion II, Baseline II, Diet Test III, and Inversion III. The aim of this sequence was to allow the animals to be exposed to different comparisons between the diets and/or changes in the position in which they were presented. This would verify whether the animals had a preference for the consumption of one of them and, in the inversions, whether the preference shown in the tests was related to the composition of the diet or the position in which it was presented. In the baseline phases, the animals were exposed to

diets of the same energetic composition as preparation for the next test. After passing through this experimental sequence, all the animals were exposed to the second part of the study, the flavor test, in which it was tested whether the animals would establish preference for consuming one of two diets with different flavor but equal energetic quantities over five days. Each phase will be described in sequence.

### ***Exploratory Training***

In this phase, the subjects learned to pass through the hinge doors of the labyrinth to come in contact with the amount of food pellets available at the end of L and R alternatives (four pellets of the Type D diet in each alternative). One session was performed with 20 forced trials, 10 in each arm (the first 10 in the L arm and the other 10 in the R arm). In each trial, only the door of one of the arms was unlocked, allowing the passage of the animals. Each trial began with the opening of the door of Compartment B and ended after the consumption of all the pellets available in the compartment feeder. The interval between trials was 20 s and started after the subject was reintroduced into Compartment B. The session ended after 20 trials or 30 min. If the animal was still for 10 min or did not complete the 20 trials, the session was terminated and restarted the next day.

### ***Diets Tests***

In this phase, the establishment of a preference for diets with different energetic values was tested. The diets were tested in pairs: D×D1, D×D2, and D1×D2. The order of presentation of the pairs was counterbalanced among the subjects. In each session, there were 36 trials, the first 6 of forced choice (3 for each arm) and the others of free choice. Each trial was started with the animal in Compartment B with the guillotine door closed; after 20 s, the door was opened for the passage of the animal and closed in sequence. The trial was ended when the animal consumed all the food pellets contained in the feeder of the chosen alternative. In each arm, there was one type of diet (D, D1, or D2, depending on the pair tested) that remained the same until the test was finished. There were four pellets in each feeder, all of the same type of diet.

It was considered that a preference had been established when the percentage of choosing one of the alternatives was equal to or greater than 80% of the trials of one session for three consecutive sessions (calculation of binomial distribution, considering a 95% confidence interval). If this criterion was not reached in 15 sessions, it was considered that the animal had not established a preference between the diets, and the phase was terminated.

Three diet tests were performed. In Diet Test I, half of the animals (three males and three females) started with the D×D1 test, and the others were exposed to the D×D2 test. In Diet Test II, these sequences were inverted so that all the animals performed the two tests (D×D1 and D×D2). In Diet Test III, all the animals were exposed to the D1×D2 test.

### ***Inversions of Alternatives***

Phases of inversion of the position of the diets (L and R) were conducted shortly after each diet test in order to verify whether the preference observed in the tests was related to the discrimination of energy values or to a simple preference for position. The number of trials and the general procedures were similar to the diet test.

### ***Baseline***

In this phase, a type of washout between inversion and a new diet test was performed. Each session had 36 trials; however, in both alternatives there were four pellets of Diet D. This phase had a fixed duration of five sessions. In the next phase, a diet test, the most energetic diet was placed on the arm that was least preferred in baseline. For this, the percentage of choices in each of the alternatives at the end of the five sessions was calculated. If the percentages of choice were equal between the alternatives, a draw between the sides was carried out for the allocation of the most energetic diet. Two baselines (I and II) were conducted after Inversion I and Inversion II. After the completion of all diet tests and their respective inversion phases, the flavor test was carried out.

### ***Flavor Test***

In this phase, the animals had access to the conventional food for one week after the end of Inversion III, so that their weights returned to the patterns without food restriction (100% of the ad libitum weight). The subjects were then individually housed and only had access to Diets S (with sucrose) and N (without sucrose) at the same time, both with the same energy amount, for five consecutive days. For this, the food compartment of the metal cover of the home cage had to be divided into two independent parts. One of the parts contained Diet S and the other, Diet N. The males were provided with a daily portion of 30 g of Diet S and 30 g of Diet N (60 g in total) and the females with 20 g of Diet S and 20 g of Diet N (40 g in total). Every 24 hr, the amount of food remaining in the cage of each animal was weighed and replenished with the amount required to complete the total amount determined for each sex.

## Data Analysis

For the diet tests and inversions, we calculated the median, quartiles, and the maximum and minimum values of the number of sessions for the conditions D×D1, D×D2, and D1×D2. These calculations were performed for both female and male animals. The median was chosen as the measure of central tendency since our data did not follow a standard distribution (analysis of data distribution and Shapiro-Wilk test).

The non-parametrical Friedman test was employed to verify if there was a difference in the number of sessions between conditions ( $p < .05$ , confidence interval = 95%). Kendall's  $W$  coefficient of concordance was calculated to determine the effect size. The scores from this coefficient were used to classify the conditions as trivial ( $< 0.1$ ), small (0.1-0.3), intermediate (0.3-0.5), and high ( $> 0.5$ ).

To verify if there was a difference between female and male animals for the number of sessions ( $p < .05$ , confidence interval = 95%), the Mann-Whitney non-parametrical test was employed. The medians of the total number of sessions of each subject were used, disregarding the conditions. Moreover, we calculated the Rank-Biserial Correlation ( $r_b$ ) to analyze the size of the effect, which was then classified as trivial ( $< 0.1$ ), small (0.1-0.3), intermediate (0.3-0.5), and high ( $> 0.5$ ).

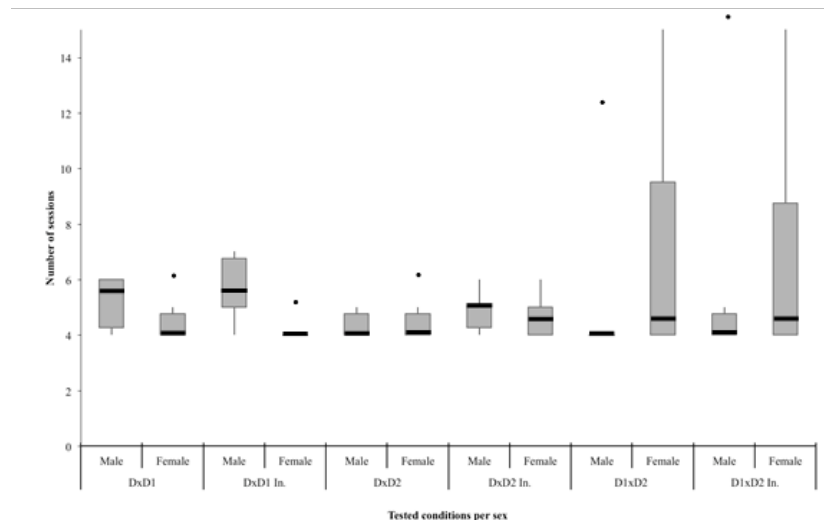
For the flavor test, we calculated the average and standard deviation of the consumption (g) for the sweet and nonsweet diets in the five consumption test sessions for female and male animals. The mixed model ANOVA was used to verify if there was a difference in the consumption ( $p < .05$ , confidence interval = 95%) between diets, groups, sessions, and interactions between these variables. The mixed model ANOVA was only performed after verifying the normality (analysis of data distribution and Shapiro-Wilk test), homoscedasticity (Levene's test), and sphericity (Mauchly's test) of the data. The Greenhouse-Geisser correction for sphericity was performed to calculate the  $p$ -value derived from the comparison between the sessions and their interactions with other variables. For the consumption, we calculated the size effect ( $\omega^2$ ) for each comparison of the mixed model ANOVA. The value of the effect size ( $\omega^2$ ) was classified as trivial ( $< 0.01$ ), small (0.01-0.06), intermediate (0.06-0.14), or high ( $> 0.14$ ).

## Results

In the first step of our study, all animals established their preference for the most energetic diet, independent of the presented combination. Figure 1 shows the number of sessions that female and male animals took to reach the criteria of preference for each diet test and inversion.

### Figure 2

Number of Sessions to Establish the Preference for one of the Diets on the Diet Test and its Respective Inversion for Females and Males



Note. The horizontal lines represent the median, the vertical bars indicate the interquartile intervals, the vertical lines indicate maximum and minimum, and the dots show the discrepant data.

Because the number of sessions to reach the preference is a discrete variable, we did not verify any approximation for normality through graphical, numerical, and statistical investigations (Shapiro-Wilk). For this reason, we opted to conduct a nonparametric analysis to calculate the median and quartiles (75<sup>th</sup> and 25<sup>th</sup>) for the diet test (D×D1, D×D2, and D1×D2) and inversion, separated by female and male animals with maximum and minimum (limit calculated using Maximum Limit = P75 + 1.5 × Interquartile Interval and Minimum Limit = P25 - 1.5 × Interquartile Interval, which allowed us to exclude the discrepant data).

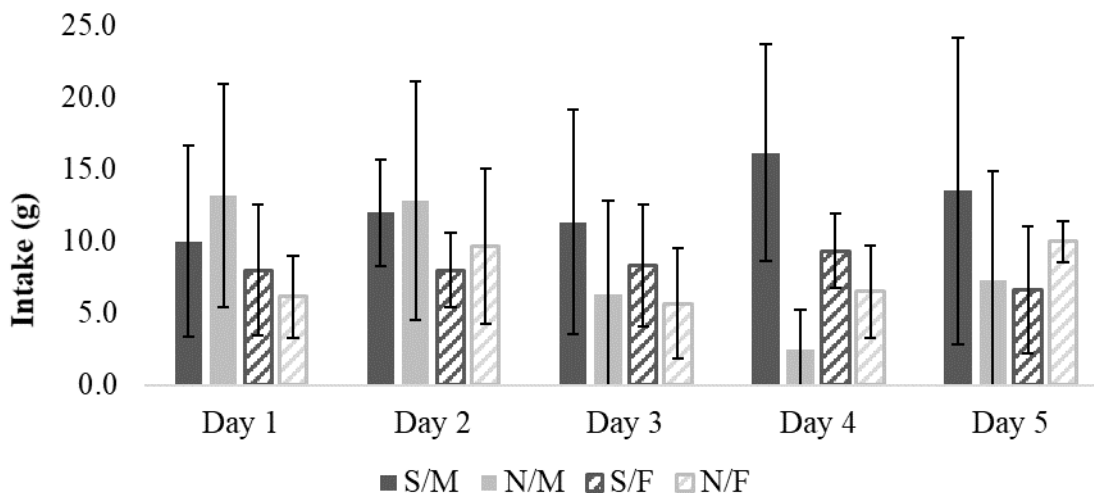
When we compared the conditions of choice and the sequence in which the pairs were presented, we did not observe any statistical difference in the number of necessary sessions to reach the preference criteria [ $\chi^2(5) = 2.33, p = 0.80, \text{Kendall's } W = 0.24$ ]. This finding was similar for both sexes. Moreover, we also did not observe statistical differences when we compared sex independently of the combination of the presented diets ( $W = 17.00, p = 0.93, r_b = -0.06$ ).

The median number of sessions varied between four and six for groups and tested conditions. Figure 2 shows that, in condition D1×D2 (diet test and inversion), females exhibited a higher interquartile interval than in the other conditions. The interquartile interval for females was also higher than the ones obtained for males in all conditions.

Figure 3 shows the consumption in grams of the diets with (S) and without sucrose (N) by females and males over five days, during the flavor test.

**Figure 3**

Mean Consumption, in grams, of Diets with (S) and without Sucrose (N) of Males (M) and Females (F) over the Five Days of the Flavor Test



We did not observe (Figure 3) differences in the consumption of the diets when we considered the interactions between sex, sessions, and diet type [ $F(1, 10) = 0.85, p = 0.38, \omega^2 < 0.01$ ], the interaction between sessions and sex [ $F(2.14, 21.35) = 0.64, p = 0.55, \omega^2 < 0.01$ ], the interaction between diet types and sex [ $F(1, 10) = 0.85, p = 0.38, \omega^2 < 0.01$ ], the interaction between diet types and sessions [ $F(2.598, 25.982) = 2.99; p = 0.055; \omega^2 = 0.13$ ], between sessions [ $F(2.135, 21.345) = 1.5; p = 0.24; \omega^2 = 0.033$ ], and between diet types



[ $F(1, 10) = 1.33$ ;  $p = 0.28$ ;  $\omega^2 = 0.024$ ]. However, there was a significant difference in the total proportion of consumed diet between sex [ $F(1, 10) = 8.67$ ,  $p < 0.05$ ,  $\omega^2 = 0.39$ ], because females naturally feed less than males.

## Discussion

When exposed to the comparisons of energetically different diets in the diet tests and inversions, all animals established a preference for the more energetic diet, regardless of the combination, order, and position presented in the apparatus. In the flavor tests, animals of both sexes consumed similar amounts of both diets, without establishing a preference for one of them, which indicates that the presence of sucrose does not seem to have played a decisive role in the consumption of the two diets.

In the first session of the diet test, the animals chose the diets alternately, which was similar to the foraging pattern, in which the available alternatives were evaluated before the establishment of a preference (Harel et al., 2016; Schoener, 1971). From the second session of the diet test, however, most animals started to establish a preference for the more energetic alternative, a preference maintained until the established criterion was achieved. These results can be interpreted in three ways: preference for the position (right-left) in which the diets were placed in the apparatus, preference for their energetic difference, or flavor preference.

Some studies have reported that rats, in discrimination trials involving position, can establish a preference for an alternative due to its spatial location. In the study by Beeler et al. (2012), for example, mice established a preference for the position (right or left) when two water bottles were available, regardless of the solution contained in each (sugar or sweetener). The authors argued that the position would be a more easily discriminable stimulus compared to caloric composition. In the present study, when the spatial location of the diets was inverted in the apparatus, initially the animals maintained their choice of the position where the most energetic diet had been in the previous phase. This persistence, however, was not maintained for more than two sessions, with the animals relocating their choices to the more energetic diet alternative. These results clearly indicate that the choice was not made due to the position, and this suggests that the animals were under the control of specific characteristics of the diets, such as the flavor and/or amount of calories.

A second explanation could be the possible variation in flavor among the D, D1, and D2 diets. A fact established in the literature is that rats (*R. norvegicus*) develop a preference for foods with high concentrations of fat (Cartoni et al., 2010; Fukuwatari et al., 2003; Pittman et al., 2006; Takeda et al., 2001) and sugar (Ackroff & Sclafani, 2011; Wojnicki et al., 2007). For this reason, these macronutrients were kept constant in the diets of the present experiments. However, the higher proportions of cornstarch contained in D1 and D2 may have altered the flavor of these foods, causing a preference due to flavor and not necessarily for their energetic quantity.

Sensitivity to different flavors has been shown to be evolutionarily important to animals because the flavor has a protective function of not allowing the ingestion of harmful food, as well as a selective function to determine which foods to ingest depending on the nutritional need of the animal (González et al., 2014). In the flavor test the animals did not show significant preference for any diet, that is the consumption of both the S and N diets were statistically equal for all subjects. This may suggest that only the sweet flavor was not a determining factor to promote a preference for the Diet S, but rather the fact that the diets have the same caloric amount. It is possible that sensitivity to postingestive nutritional effects may have counterbalanced the effect of the flavor as an indication of the most energetic food.

The hypothesis above is consistent with the findings of Sclafani and Ackroff (2017). Authors added an artificial grape flavor to a solution containing sucrose (sugar) and an artificial cherry flavor to a solution

containing sucralose (sweetener) and offered to mice, in order to verify whether the animals would continue to choose the flavors combined with sucrose and sucralose even when those components were removed, leaving only flavored water. In general, the consumption of the sucrose solution was higher in comparison to the solution containing sucralose. The animals also continued choosing the solution in which the artificial flavor was paired with sucrose, even when only pure water artificially flavored with the trained flavor was made available to the animals, which was not observed regarding the flavor paired with the sucralose solution. The authors concluded that the higher intake of the sucrose solution and the fact that only the paired artificial flavors to it were effective for conditioning, compared to the sucralose, was due to the postingestive energetic feedback provided only by sugar, since the ingestion of sugar guarantees energy to the animal, whereas sweetener does not.

The results of the Flavor test of the present study did not support the hypothesis that the choice of the animals for the most energetic diet occurred as a function of flavor, confirming the findings of other studies (Ackroff & Sclafani, 2014; Davis & Smith, 1990; Sclafani, Fanizza, & Azzara, 1999; Weingarten & Kulikovsky, 1989; Zukerman et al., 2013). Even though it is not possible to determine the role played by flavor in the discriminative performance of animals, it is reasonable to presume that the energetic difference of the diets made available in the first phase of the present study was, in fact, the determining factor for the animals' choice.

Considering that food choice is an essential activity for the maintenance and reproduction of organisms, it is very important that animals are able to constantly evaluate the environment in which they are searching for food, so that they can maximize their choices among the alternatives available (Charron & Cabanac, 2004; Foo et al., 2016; Palminteri, Powell, & Peres, 2016; Pyke et al., 1977; Radtke, 2011; Trujano & Orduña, 2015; Tyson et al., 2016; Yáñez, et al., 2017). In the experimental situation proposed here, by concentrating their choices on the alternative that made the more energetic diet available, the rats demonstrated that they were able to remain under the control of postingestive signals, indicators of the presence of calories originating from the different diets (Weingarten & Kulikovsky, 1989). This pattern was verified despite the consumption of these diets occurring only in the experimental sessions and as a supplement of the conventional diet offered, indicating that the maximization of the energetic consumption was maintained even when the chosen diet was not the main source of energy.

Two exceptions were verified in relation to the establishment of a preference for the most energetic diet, both (one male and one female) in Inversion III, in which Diets D1 and D2 were presented. The two animals distributed their choices over both alternatives, not establishing a clear preference pattern for a specific diet, even though they preferred the more energetic (D2) in Diets Test III. It is possible that for these two individuals the energy difference between the diets had not been discriminated or had not been important from the point of view of maintaining their body functions. Perhaps the energy supplementation obtained from the two diets was similar for these individuals, resulting in both being indifferent to the consumption. The performance of these animals may suggest that the establishment of preference for the more energetic diet may have a limit, from which the addition of calories to one of the diets does not make it more advantageous compared to the diets with fewer calories. Future studies should investigate this possibility.

Another important point to consider is that there was no difference in the performance of males and females in any of the study phases. It is known that the sexual dimorphism of *R. norvegicus* (Asarian & Geary, 2013), hormonal regulation (Varma et al., 1999) and sensitivity to fats and sugars (Sinclair et al., 2017) are factors that act on differentiating the amount of food ingested between the sexes. However, all the animals in this study chose the more energetic diet when performing the diet test and inversion and established this preference in an equal number of sessions. Also, no differences were observed between the sexes regarding the consumption of Diets S and N. One possible explanation for this would be that, because the experimental diets

were incomplete, lacking protein, vitamins, and minerals in their composition, they had an energy supply function only. Knowing that the required amount of nutrients varies according to the sex, the animals were ingesting, in the first stage of the study, a diet that would energetically supplement the amount of conventional chow available to them daily. However, in the second stage, access to the food was restricted only to the experimental diets, so that the animals had to ensure the ingestion of some of the essential nutrients (carbohydrates, lipids, and fibers), due to the lack of others such as proteins, vitamins, and minerals.

The results discussed so far must be weighed against some limitations. The number of subjects used may not have reflected the behavioral pattern common to the species in the situation studied, as indicated by the difficulty that two animals had in establishing a clear preference for one of the diets when D1 and D2 competed with each other. The replication of this study with an enlarged sample might show greater variability of discrimination patterns, especially among the more energetic diets, suggesting other analysis possibilities. Another limitation refers to the fixed number of five days in the flavor test, which may not have been sufficient to establish a clear pattern of dietary choice. In the present study, five days of flavor testing were defined because the diets tested did not contain all the necessary nutrients for the maintenance of the animals, which could lead to a condition of malnutrition. Although it is possible that the extension of the length of time of the flavor test could better portray the consumption of diets differing only in terms of flavor, this could also make the analysis difficult because of the deterioration of the nutritional condition of the subjects due to the ingestion of a diet lacking in various nutrients. Despite these limitations, the results obtained contribute to the comprehension of the food choice of the species studied.

The results support the hypothesis that the rats studied were sensitive to the amount of energy present in food and that characteristics such as appearance, quantity, and probability are not the only indicators that guide food choice. Even the characteristic flavor of sucrose added to one of the diets was not decisive in the choice of nutritionally equal diets for the subjects studied. The flavor may contribute at first, serving as an indicator of the presence of nutrients; however, the determining variable in maintaining the consumption of a food is its nutritional value. The results showed that the animals were sensitive to the energetic quantity of the diets in such a way that they were able to evaluate which was the most advantageous, even as a supplement to the conventional diet.

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