METODOLOGY FOR CALORIMETRIC DETERMINATION OF URINE

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ABSTRACT

This paper describes the development of a method for direct determination of the urinary energy content through a bomb calorimeter. Two mililiters of urine were dried under 100ºC for 7 hours followed by a dessecador for an adittional period of 15 hours. Four hundred mg of mineral oil were added to the samples as spike. Sacarose solutions in different concentrations (30, 60 and 90%) were used to do recovery tests. The recovery rates were 98%, 81% and 95 % respectively, with a coefficient of variation of 5% between samples (n=40). The results showed that the method here described to measure urinary energy content has good reliability and reproducibility and can be applied in other studies.

KEY WORDS: direct calorimetry; urine; method

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INTRODUCTION

Energetic value for foods and diets are usually calculated by factorial methods based in the approximate composition of the sum of the caloric value for proteins, lipids and carbohydrates and by means of general factors of energy conversion, that is, 4 kcal/g protein, 9 Kcal/g lipid and 4 Kcal/g carbohydrate (LIVESEY, 1991).

The amount of energy available in diets used to maintain good health and performance is of greatest importance in nutrition (LIVESEY, 1995). This available energy is known as metabolizable energy. Factorial methods are not satisfactory to determine the real available energy in foods rich in fibers nor when a fine precision is required, when direct calorimetry should be used (MILLER; AYNE, 1995). The metabolizable energy can be calculated by the subtraction of the value of the loss of energy of foods while ingested due to inability of animals to oxidize the complementary nitrogen. (MILLER; PAYNE, 1995). Energetic loss through urine is only significant for proteins, since the product of its oxidation, urea, still contains an appreciable amount of energy (MILLER; JUDD, 1984).

The methods used to determine the contents in macronutrients, micronutrients and the energetic value for foods in nutritional tables differ and, sometimes, the content in carbohydrate is determined by the difference between the total amount and the remaining macronutrients, and not directly. When such macronutrient is calculated by the difference in relation to the remaining, it includes the fibers in the food, which is multiplied by the factor to the calculation of the energy, leading to a metabolizable value to a non-digestable component (MILLER and JUDD, 1984). These latter authors compare the values of metabolizable energy of foods rich in fibers found in nutritional tables and by means of direct calorimetry, as can be seen in TABLE 1.

TABLE 1 – Energetic content in foods according to tables (factorial data) and according to the assessment of the metabolizable energy.

Foods	Energy referred in the nutritional tables (Kcal/100 g of food)	Metabolizable energy (Kcal/100 g of food)
Frozen beans (12 g of fibers/100 g)	41	34
Spinach (6.3 g/100 g)	30	24
Coconut (13.6 g/100 g)	351	294
Nut (14.3 g/100 g)	565	502
Olives (4.4 g/100 g)	103	84
Dried damascus (24 g/100 g)	182	171

* Adapted from Miller and Judd (1984).

Direct calorimetry is a method in which the energetic value of foods is determined by their direct burn in a combustion chamber in presence of pure oxygen and the heat produced by the combustion is than directly measured in relation to the used pattern (MILLLER and JUDD, 1984). Thus, the expression combustion heat, such as the one measured in the calorimetric bomb, indicates the heat released by the combustion of all carbon and hydrogen in presence of oxygen to form carbon dioxide and water, including the heat yielded by he oxidation of other elements such as sulfur that may be present in the sample.

In order to determine the value of metabolizable energy present in the diets both the determination of the calories in the foods that of the energy that is being eliminated in stools and urine are necessary (MILLER; JUDD, 1984).

Although there are formules that use the amount of nitrogen in the urine to determine the eliminated calories, the technique of direct calorimetry is more precise once it directly assesses the amount of energy present in urine using the calorimetric bomb.

MATERIALS AND METHODS

Collection of urine

Samples of urine from adult, male Wistar rats weighting circa 180g from the Central Biotery of USP at Ribeirão Preto were used. Animals were kept in metallic metabolic cages with a funnel in the floor with gall wool inside for retention of insoluble material, aiming to collect urine without residues round the clock. The urine samples were collected in probes with three drops of HCl 6N and immediately frozen at -20ºC until use.

Preparation of samples for analysis

Samples were defrosted and homogenized. Two mL of urine were placed in metallic crucible and dried in aired oven at 100ºC for 7 hours. Then, samples were transferred to a dissecator containing sodium hydroxide pastille for humidity adsorption for circa 15 hours. All samples weighed circa 2 g in liquid state and were dried and in duplicate two folds. After dried, samples weighed circa 0.2 g.

Calorimetric determination in urine

For assessment of the energy present in the samples it was added circa 400 mg of pure mineral oil were added to the urine, weighed in an analytical scale. The mineral oil, which has a caloric value of 11000 calories, was used as a spike. The amount of oil was inserted in the calculation software of the equipment as well as the net weight of the sample to be quantified. These data were in the final calculation of the caloric value made by the PARR 1265 calorimetric bomb, which gives results in calories per gram. One calorie equals 4.1868 joules (J) and is defined as the energy necessary to increase in 1ºC the temperature of 1g of water at 15ºC.

Recuperation test

The concentrations of sugar used in the tests of recuperation were 30, 60 and 90%. Ten tests were performed for each used concentration.

Standard

Benzoic acid drops with caloric value of 6318 cal/g, were used as standard. Benzoic acid is used as a reference substance for calorimetric fuel because it burns entirely in the presence of oxygen. Besides that, it is not hygroscopic and is available in a highly pure form.

RESULTS

The calorimetric values for dry urine can be seen in TABLE 2.

The test for recuperation done by addition of a 30%, 60% and 90% saccarose solution to the dried urine was respectively 98%, 81% and 95%. The variation quotient was 5% (n=40).

TABLE 2 – Weight of the dried urine and caloric value obtained after the analysis by the calorimetric bomb.

Sample	Weight of the dried urine	Caloric value (cal/g)
1	0.1980	2249.26
1′	0.2006	2245.87
2	0.2041	2086.60
2′	0.2153	2050.41
3	0.2182	2081.60
3′	0.2143	2058.28
4	0.2054	2011.81
4′	0.2075	1982.39
5	0.2082	2010.37
5′	0.2188	1999.54
6	0.2318	1953.85
6′	0.2215	2035.06
7	0.2295	2112.53
7′	0.2250	2280.60
8	0.2132	2260.20
8′	0.2095	2228.52
9	0.2222	2218.82
9′	0.2034	2067.25
10	0.2008	2099.59
10′	0.1991	2090.24
11	0.2439	2266.08
11′	0.2423	2157.23
12	0.2361	2060.10
12′	0.2360	1949.18
13	0.2119	1991.52
13′	0.2144	2130.98
14	0.2109	2167.80
14′	0.2254	2061.00
15	0.2156	1921.35
15′	0.2255	1902.59
16	0.2364	1988.86
16′	0.2355	1914.88
17	0.2386	2166.14
17′	0.2379	2109.31
18	0.2362	1952.49
18′	0.2317	2135.34
19	0.2321	2169.78
19′	0.2168	2067.80
20	0.2297	1989.82
20′	0.2098	1949.07

* Mean calories per gram of dried urine sample: 2081.80
Samples were analyzed in duplicate, for example: 1 and 1’.

DISCUSSION AND CONCLUSION

The metabolizable energy in diets is determined by the difference between the calories in the food and the energy eliminated in stools and urine (MILLER; JUDD, 1984). Calories present in urine, generally speaking, are determined by indirect techniques, using formulae that take into consideration the existing nitrogen in the sample. A more precise determination could be done by the direct quantification of calories by means of a calorimetric bomb, which is not commonly reported in the literature. Probably, the processing of the urine sample for calorimetric analysis could be the reason for such limited use of the mentioned method. The caloric values for urine samples are usually low, being below the limits of sensibility of the calorimeter. Analyzes made in our laboratory for the determination of the caloric value of liquid urine can be reproduced and need further standardization of the methodology for urine drying that could guarantee reliability to them. To the analysis it was necessary to add mineral oil to the urine sample aiming to increase the caloric value in order to allow the detection of calories in the sample. The results reveal that the method for urine processing showed a good reproducibility and can be taken as reliable and adequate to the detection of the energy present in the sample.

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