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DETERMINATION OF ENERGY CONTENT OF FEEDS

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INTRODUCTION

In nutrition studies it is often necessary to know the calorific value of the feed, faeces and flesh. These values are necessary to compute the energy budget and to determine the efficiencies of absorption and conversion. In nutrition requirement studies it is essential that the test diets be isocaloric. Therefore determining the energy content of feeds and its components is one of the basic techniques used in nutrition studies.

UNIT OF MEASUREMENTS

Generally the energy content is expressed in terms of calorie (cal) or kilogram calorie (Kcal). The calorie is defined in terms of heat units as the heat required to raise the temperature of one gram of water at 15°C by 1°C. The usefulness of calorie is due to the fact that all forms of energy can be converted into calories, while they cannot be wholly transferred into any other forms of energy. However, in the international unit system SI (System International) which has been adopted by many countries, instead of calorie, the joule (j) a unit of mechanical energy has been introduced. The direct link between mechanical energy and heat energy is expressed in joule's law, namely that the equivalent of

one calorie in units of mechanical energy is  $4.187 \times 10^7$  ergs or 4.187 joules (Grodwinski et al., 1975). A list of energetic equivalents and conversion factors are given below.

1 kilo calorie (Kcal, C)	= 1000 gram calorie or calorie (cal, C)
1 Kcal	= 3.968 British thermal units (Btu) at 60°F
1 Kcal	= 4187 joules
1 joule	= $2.388 \times 10^{-4}$ cal
1 Btu	= 0.252 Kcal at 15°C

It is best to express the energy content of the sample in terms of ash free dry weight, for ash is often responsible for 10-15% of the dry weight. In general, calorie value (ash free) should occur between a lower limit set by glucose (3.74 Kcal/g) and an upper limit determined by the value of oils and fatty acids (9.37 Kcal/g). Not all substances fall within this range for example glycine (2.08 Kcal/g), uric acid (0.91 Kcal/g) etc., (Paine, 1971).

#### METHODS OF DETERMINING ENERGY CONTENT

There are four common methods for determining the energy content of materials.

I. Component analysis: Lipid, protein, and carbohydrate are the energy nutrients. If their quantitative values are known, calorific value can be calculated by applying appropriate caloric conversion factors. Most of the conversion factors (Table 1) given in literature closely agree with each other.

Table 1 - Calorific value (KJ/g)

<u>Protein</u>	<u>Lipid</u>	<u>Carbohydrate</u>	
23.7 (5.65)	39.6 (9.41)	17.2 (4.1)	Brody (1945)
23.4	39.2	17.2	Cho (1973)
23.6	39.5	17.2	Jobling (1983)

\* (The values given within brackets are in Kcal/g from which the values in KJ/g have been calculated on the basis of

$$1 \text{ joule} = 2.388 \times 10^{-4} \text{ Kcal}$$

Usually the energy content of pellets comprising a mixture of the three basic nutrients is usually 20-25 KJ/g.

The draw back in component analysis is that it needs results out of three analytical procedures before computing the energy value in contrast with the less time consuming bomb calorimeter method. The errors in estimating each of the components is reflected in the final energy value. The conversion factor for each component has been based on Brody's (1945) work on mammals. Brett and Groves (1979) have pointed out that though 39.5 KJ/g is appropriate for the saturated fats of mammals, a more appropriate figure for the highly unsaturated fats associated with fish may be 36.2 KJ/g.

The advantage of component analysis is that unlike other studies involving energetics, in nutrition the proximates composition of the feed is always worked out and therefore it is easier to calculate the energy value. Also, in laboratories where a bomb calorimeter is not available, the component analysis is the next best method to get an accurate value of energy content.

## II. Wet oxidation:

The principle of this method is that the sample material is heated with an oxidising agent so that the sample is oxidised. By titration, the remaining oxidising agent is determined, from which the amount of oxygen consumed by the sample material could be determined. The sample calorie value is calculated by multiplying the oxygen consumed (mg) x 3.38 cal. Various oxidising agents like dichromate and potassium iodate have been used. The spectrophotometric method of determining organic oxidisable matter as carbon can also be used.

The draw backs in this method are that it is still not certain whether the wet oxidation technique for all the oxidisable carbon (Paine, 1971). The method assumes that all the carbon present is as carbohydrate. Therefore, the amount of nitrogen in the sample, greater is the deviation of, the wet oxidation value from that of bomb calorimetry.

## III. Thermo chemical method:

Theoretically it is possible to calculate the enthalpy of any given substance from known heats of formation. If the percentage composition of constituent elements are known, then a balanced empirical equation could be developed representing the oxidation reaction of a bomb.

The above method is more of theoretical interest than of practical utility.

## IV. Bomb calorimetry:

The principle of all bomb calorimetry is that a known weight of sample is ignited electrically and burned in an excess of oxygen in the bomb. The oxidation is explosive and complete so that the whole heat output is instantaneous.

The heat output is estimated by directly measuring the rise in temperature of water surrounding the bomb or by means of a thermocouple and a potentiometer. Comparing this rise with that obtained when sample of known calorific value is burnt, the calorific value of the sample material can be determined. There are different types of calorimeters and they can be grouped into two categories - (1) whether the heat loss from the calorimeter during test is eliminated or not and (2) based on the sample weight that need to be used.

Adiabatic and non-adiabatic calorimeters: In adiabatic bomb calorimeters the bomb is placed in a calorimeter vessel containing a fixed quantity of water. The calorimeter vessel is surrounded by an outer jacket and there are provisions to add hot or cold water to keep the jacket temperature equivalent to the calorimeter. In non-adiabatic calorimeter this provision is lacking and therefore the temperature rise in the calorimeter vessel has to be corrected for radiation.

Micro, Semi micro and Macro calorimeter: Based on the range of sample size that can be used the bomb calorimeter can be grouped into micro with a sample range 1-10 mg, semi-micro with 10-100 mg and macro with 200 mg to 1.5 g. The minimum and maximum sample size in each of these categories varies depending on the make of the bomb calorimeter. Phillipson micro-bomb calorimeter, the semi micro Parr calorimeter and the macro Gallenkamp calorimeter are the commonly used bomb calorimeters.

Basic features of a bomb calorimeter: All types of bomb calorimeters have the following features namely (i) Bomb (ii) Oxygen supply (iii) firing assembly (iv) temperature sensing and recording device.

(i) Bomb: This is made of thick stainless steel and the capacity depends on the type of bomb calorimeter. It consists of two halves, both of which can be screwed together with a teflon or rubber 'O' ring washer in between them. One part of the bomb is hollow and to the other part two ignition circuit terminals and sample holder are attached. Of the two terminals one is insulated with which sample holder is attached, and the other connected to the bomb. At the time of loading the sample a platinum fuse wire is attached connecting the terminals. The platinum fuse wire is bent in such a way it is in intimate contact with the sample. In some instruments instead of pt fuse wire a cotton wig used. There is an inlet for oxygen which can be closed and an outlet controlled by a needle valve. The oxygen pressure inside the bomb is read through a gauge. The sample is placed in a platinum crucible and kept over the sample holder of the bomb.

(ii) Oxygen supply: The oxygen is supplied to the bomb from an oxygen cylinder by means of reducer and two gauges measure the oxygen pressure inside the bomb and the oxygen cylinder.

(iii) Firing assembly: It consists of batteries, condenser, resistors, charging and firing circuit, timer and operating switches. The whole thing is mounted on a control board to which are attached other electrical connections.

(iv) Temperature sensing and recording device: This consists of either a sensitive thermometer (with an accuracy of  $0.0002^{\circ}\text{C}$ ) or a thermocouple. In bomb calorimeters with thermometer the bomb is immersed in a fixed weighted amount of water which is constantly stirred during the test. The temperature rise in the bomb is passed to the water and its rise in water is recorded. In those calorimeters with the thermocouple, it is attached directly to the body of the bomb.

It is advantageous over the thermometer in that it takes lesser time and a permanent record can be made by attaching the thermocouple to a potentiometric strip chart recorder. High precision spot galvanometer or a potentiometer too can also be used.

#### Sample preparation

The sample for the sake of uniformity need to be dried in a hot air oven-(55-80°C) or a freeze drier. Drying rapidly as far as possible is essential because of the progressive decomposition of fats to fatty acids with different calorific values. The next step is grinding the sample to a fine powder and an aliquote of the powdered material is-made into a pellet in a pellet press. It is also advisable to dry the pellet until a constant weight is attained. The criteria governing sample size is that the total heat released by the sample should fall within the limited temperature range over which each bomb calorimeter is linear. For example in Gallenkamp bomb calorimeter, the recommended heat release is 4.0 Kcal. This gives a sample range of about 0.4 g for fat to about 1.5 g for a material such as urea. If the sample is difficult to ignite or not adequate enough, a known amount of standard material usually benzoic acid has to be mixed to give the recommended total heat release. All materials which have low bulk density and high surface area must first be compacted in order to reduce their rate of combustion and to prevent incomplete combustion. If needed few drops of water with a wetting agent can be added and allowed to soak in before igniting the sample.

Calibration: Since 1934 benzoic acid - 6.32 Kcal/g ( $C_6H_5COOH$ ) has been the international standard used in thermochemistry and all bomb calorimeters are calibrated against it. It is obtainable from the National Bureau of Standards, Washington DC 20234 as standard sample 391. The



purpose of calibration is to check whether there is direct dependences between the amount of substances burned and the thermometer recorded readings and to obtain calorific equivalent for one recorder division. The first step involves burning benzoic acid samples of different weight to find the range over which the measurement is linear. The second step involves burning 10 or more benzoic acid samples falling within the linear range of the calorimeter. From these recordings calorific equivalents for one recorder divisions is calculated.

#### Precautions

1. Weighing and temperature measurement errors should be avoided especially when handling low sample weights.
2. The sample material should be homogenous and this can be achieved by grinding and mixing. If the aliquote taken for making the pellet is not representative of the sample, it can cause serious errors especially in microbomb calorimeter.
3. A correction for the constant heat gain due to the firing current and firing cotton or fuse wire should be carried out.
4. In those calorimeters with thermometer, it is advisable to tap the thermometer gently before and during each reading so that adhesion between the mercury column and surrounding glass is minimised. After each run a stem correction to correct for the error due to differential expansion of thermometer's mercury column which is exposed to both water and air need to be carried out.
5. A radiation correction for the heat loss or gain during the temperature rise due to combustion of sample has to be carried out.

6. Another source of error is the formation of acids like nitric and sulphuric following combustion. An acid correction can be estimated by assuming that all the acid is  $\text{HNO}_3$  and titrating with 0.0725 N sodium carbonate. (At this normality 1 ml titrate is equivalent to 1 cal).
7. Incomplete combustion can result in an underestimate of calorific value. This happens mainly due to rapid admission of oxygen to the bomb which can blow part of the sample away. Other reasons are loose or too densely compacted pellet or low oxygen pressure.
8. Material with high ash content due to endothermic reactions within the bomb do not give an accurate value.
9. A violent combustion due to not compacted pellet or higher oxygen pressure or oxygen leak in the bomb during firing can lead to hot gases escaping past the sealing ring and cause irreparable damage to the bomb.

Ballistic bomb calorimeter: In ascertaining the calorific value of feeds and other non homogenous materials it is difficult to obtain a truly representative single sample. In such cases, the average of a number of less accurate results is superior to a single very accurately determined one. In Ballistic bomb calorimeter which is a macro-nonadiabatic type, replicate testing can be done very fast (upto 10 results per hour). Therefore, for estimating energy content of feeds, it is the ideal bomb calorimeter.

Procedure:

1. The crucible with the weighed sample is kept on the support pillar in the base of the bomb. One end of a 50 mm length of firing cotton is inserted between the coils of the firing wire and the other end dropped into centre of sample in the crucible.

2. After checking that the bomb sealing ring is in position in the groove, raise the knurled locking ring and engage the thread-of-the bomb body with that of the ring. Lower the bomb body and grasping it firmly with one hand screw the sealing ring.
3. Plug the thermo couple into the hole in the top of bomb body.
4. Close the pressure release valve in the bomb and open the valve in the front panel of the control box about  $\frac{1}{4}$  turn. Allow the pressure to rise to 25 atmo. and close the valve.
5. By means of galvo zero knob on the control box, bring the light spot index on the galvanometer to zero and check for 30 sec. for any drift due to temperature variation.
6. Stand back from bomb and press the firing button. After 10-15 sec. increasing deflections on the pressure gauge and then in the galvanometer will indicate that the firing has been successful.
7. Note the maximum deflection of the galvanometer.
8. Release the gases from the bomb through the pressure release valve.
9. Carry out the above procedure for estimating the correction for constant heat gain and for calibration with 0.7 g of benzoic acid.

Calculation of results:

Mass of benzoic acid	=	$W_1$ gram
Calorific value of benzoic acid	=	6.32 Kcal/g
Heat release from benzoic acid	=	$W_1 \times 6.32$
Galvanometer deflection without sample	=	$X_1$

Deflection with benzoic-acid = X2  
Deflection only due to benzoic acid = X2 - X1 = X3  
Calibration constant =  $\frac{6.32 \times W1}{X3}$   
= Y1

The mean of 10 calibration constants is calculated  
= Y2

Mass of sample = W2 gm  
Galvanometer deflection with sample = X4  
Deflection only due to sample = X4 - X1  
Heat release from sample = X5 x Y2  
Calorific value of sample =  $\frac{X5 \times Y2}{W2}$

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