Energy losses associated with oven-drying and the preparation of rat carcasses for analysis

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(*Received* 14 *May* 1976 – *Accepted* 27 *May* 1976)

1. The effect of oven-drying on the heat of combustion of rat carcasses was studied and found to produce losses of 10% of the original energy content. Oven-drying also produced a small loss of nitrogen.

2. A method of carcass preparation not involving oven-drying was tested and found to produce suitably homogeneous samples for analysis without any loss of energy.

In many animal experiments it is necessary to estimate changes in the total body content of the major constituents such as water, fat and protein as well as energy. It has been the practice in many laboratories to estimate body water by oven-drying carcasses and then grinding the dried material before taking samples for further analysis. Marked changes in the appearance of animal tissues occur after heat treatment but no mention is ever made of the possible effects this might have on subsequent analyses. The assumption that these effects were negligible became suspect when values obtained for the heat of combustion of fat extracted from oven-dried rat carcasses were found to be considerably lower than expected. Additional problems associated with this method of sample preparation are that it is often difficult to obtain homogeneous samples and it is a time-consuming process.

In this paper a series of experiments are described that were designed to determine the effect of oven-drying on the heat of combustion and nitrogen content of rat carcasses and other materials. Having found significant losses, a method of carcass preparation not involving oven-drying was tested.

METHODS

Effects of oven-drying

The materials studied were glycerol trioleate, a synthetic rat diet ((g/kg) 100 fat 200 protein, 600 starch) and a freeze-dried homogenate of a fresh rat carcass. Approximately 1 g samples were weighed into nickel-bomb crucibles and heated in a hot-air oven at 105°. The samples were heated for periods that would normally be required to produce constant weight. Thus, glycerol trioleate was dried for 18 h, the diet for 48 h and the rat carcass for 64 h. Some samples were also heated at lower temperatures. Control samples were weighed out at the same time and left at room temperature in a desiccator.

The samples weighed into crucibles were used for the determination of heat combustion using the ballistic bomb calorimeter (Miller & Payne, 1959). Deter-

minations on control and treated samples were made successively in a random order. The calorimeter was calibrated using sucrose as the thermochemical standard.

Samples of rat carcass were also weighed into Kjeldahl flasks and left at 20 or 105° for 64 h before estimating N content by the Kjeldahl procedure.

In addition to these experiments with preweighed amounts of freeze-dried rat carcass, the heat of combustion of rat carcasses that had been oven-dried intact was compared with that of carcasses prepared without oven-drying.

Preparation of carcasses without oven-drying

The method of preparation adopted was based on the techniques described by Mickelsen & Anderson (1959) and Hartsook & Hershberger (1963). Frozen carcasses were chopped into *c.* 30 mm cubes, placed in beakers and heated for 15 min at 103 kN/m² (gauge). The autoclaved carcass was transferred, using water (1:1, v/w), to a preweighed Waring blendor jar. With the lid in position, the blendor speed was slowly increased using a rheostat on the electrical supply to the mixer. This was done to prevent excessive bumping and to avoid material sticking to the lid of the jar. The carcasses were homogenized at high speed for a total of 10 min, stopping occasionally to prevent overheating of the material. Then the total weight of homogenate was determined and this weight used when calculating total carcass contents. The particle size of the homogenate was then further reduced using a Silverson emulsifier (Silverson Machines Ltd, Chesham, Bucks.). The emulsification was done in the blendor jar and the mixture was then sampled while mixing using a syringe attached to a length of wide-bore polyethylene tubing. Samples for the determination of dry matter content and heat of combustion were transferred to preweighed nickel bomb crucibles, re-weighed and then deep-frozen before freeze-drying in a glass desiccator evacuated to less than 13 N/m², using phosphorous pentoxide as the desiccant. Samples usually reached constant weight after 24–36 h and could then be ignited in the bomb calorimeter.

Samples for N determination were transferred directly into preweighed Kjeldahl flasks, and were either digested immediately or deep-frozen until required. The same procedure was used for sampling into preweighed specimen tubes for long-term storage.

The effects of the various treatments and procedures were compared using Student's *t* test for unmatched values, and all probabilities given are two-tailed.

RESULTS AND DISCUSSION

Effects of oven-drying

The results of the bomb calorimetry of the oven-dried materials are given in Table 1. There were small but variable losses of dry matter resulting from the heat treatment, but these were not sufficient to explain the decreases in the heat of combustion. The results were therefore expressed as kJ/g original material, and in this way losses due to volatilization or oxidation, or both, are combined. It was quite apparent that there was a loss of energy from all three materials as a result of

Table 1. *The effect of oven-drying on the heat of combustion of glycerol trioleate, a synthetic rat diet and a freeze-dried homogenate of fresh rat carcass, and on the nitrogen content of the rat carcass*

(Mean values with their standard errors)

	Treatment		No. of determinations	Heat of combustion (kJ/g)*		Statistical significance of difference from control value
	Temperature (deg)	Duration of treatment (h)		Mean	SE	
Glycerol trioleate	20	18 (control)	6	39·8	0·3	—
	44	18	6	39·3	0·4	NS
	105	18	6	37·0	0·4	$P < 0·001$
	105	64	6	36·1	0·4	$P < 0·001$
Rat carcass	20	64 (control)	10	24·1	0·3	—
	72	64	7	22·1	0·4	$P < 0·005$
	105	64	10	21·7	0·2	$P < 0·001$
Diet	20	48 (control)	6	20·9	0·1	—
	70	48	6	20·2	0·3	$P < 0·05$
	105	48	6	19·6	0·2	$P < 0·005$
				N content (mg/g)*		
				Mean	SE	
Rat carcass	20	64 (control)	6	1·73	0·003	—
	105	64	6	1·68	0·001	$P < 0·005$

NS, not significant.

* Values expressed per g original material; for details, see p. 305.

oven-drying. The only loss that was not significant was that for glycerol trioleate heated for 18 h at the relatively low temperature of 44°. Also given in Table 1 are values for N content of the rat carcass; heating produced a small (2·9%) but significant loss.

The energy losses for rat carcass heated at 105° for 64 h were one-tenth of the original energy content. Errors of this magnitude are outside the range normally accepted for energy balance experiments. Although many workers are prepared to tolerate errors up to 1%, Blaxter (1956) has stated that errors greater than ±0·5% are undesirable.

There is a possibility that heating small amounts of material in nickel crucibles promotes greater losses of energy than might occur when intact rat carcasses are oven-dried. This was studied using six rats which were oven-dried intact, ground using a coffee grinder and the heats of combustion of samples compared with those of six rat carcasses prepared without oven-drying. The rats in the two groups were litter-mates matched for sex and body-weight. The results given in Table 2 indicate that the average body-weight of the two groups was practically identical, and there was no significant difference in dry weight. The heats of combustion were, however, significantly lower as a result of oven-drying; equivalent to an 8% loss of energy/dry carcass. Estimates of total carcass energy from these values indicated an over-all loss of 11·3% resulting from oven-drying.

Table 2. *Comparison of two methods for the preparation of rat carcasses for bomb calorimetry**

(Mean values with their standard errors for six rats/group)

	Group A		Group B		Statistical significance of difference between group
	Mean	SE	Mean	SE	
Body-wt (g)	97	5	98	5	NS
Dry wt (g)	27·5	0·5	28·5	0·6	NS
Heat of combustion (kJ/g dry wt)	26·0	0·4	28·3	0·3	$P < 0·01$

Group A, whole rat carcass oven-dried before grinding; Group B, whole rat carcass homogenized before freeze-drying; NS, not significant.

* For details of procedures, see p. 307.

As a result of these experiments oven-drying was discontinued; the nature of the changes responsible for the energy losses found was not studied. An alternative method for carcass preparation was therefore developed which produced a wet homogeneous mixture suitable for the direct analysis of constituents. Bomb calorimetry, however, requires a dry sample and although the homogenate can be freeze-dried easily in bulk, there is a danger of losing material and increasing sampling errors when re-mixing the dried carcass. It was therefore decided to freeze-dry preweighed samples in the crucibles used for bomb calorimetry.

Before adopting the alternative method for routine preparations it was necessary to confirm that: (1) there were no losses of energy or systematic errors associated with the autoclaving and subsequent manipulations; (2) the final product was indeed homogeneous, and sampling errors were within acceptable limits.

In order to test the first condition it was necessary to measure the heat of combustion of a material before and after autoclaving, homogenizing and freeze-drying. It is not possible to estimate the heat of combustion of rat carcass before drying, and so for this particular test two diets were used since they could be combusted in their pretreated condition. The diets were essentially the same as that used for the oven-drying experiments, but contained two levels of fat, 150 and 300 g/kg (diets 1 and 2 respectively). The heats of combustion (kJ/g; mean $\pm$ SE, n 6) of the two diets were: diet 1: before treatment 20·2 $\pm$ 0·1, after treatment 20·1 $\pm$ 0·1; diet 2: before treatment 22·7 $\pm$ 0·1, after treatment 22·6 $\pm$ 0·1; the differences between values were not significant.

In Table 3 values for dry matter and heat of combustion are given for six rat carcasses prepared without oven-drying. The associated coefficients of variation gave some measure of the homogeneity of the preparations, although the values for heat of combustion were also influenced by the reproducibility of the ballistic bomb calorimeter. Coefficients of variation for estimates of homogenate dry weight ranged from 0·46 to 1·03 (mean 0·62), whilst the corresponding values for energy ranged from 0·53 to 1·33 (mean 0·82). It should be noted that these results were obtained using samples corresponding to less than 1 g dry matter (usually 0·6–0·8 g).

Table 3. *Dry matter contents and heats of combustion for freeze-dried homogenates of fresh rat carcass*

(Mean values and standard deviations for six estimations/homogenate)

Rat no.	Dry matter (g/kg homogenate)			Heat of combustion (kJ/kg homogenate)		
	Mean	SD	CV	Mean	SD	CV
4	417	2	0·48	4980	30	0·60
5	390	2	0·51	3740	20	0·53
6	409	3	0·73	6360	60	0·94
7	434	2	0·46	5930	40	0·67
17	404	2	0·50	4870	40	0·80
25	385	4	1·03	5980	80	1·32

As a result of these studies we conclude that the method not involving oven-drying does not result in any energy losses, either due to oxidation or to loss of material during the various processes. The resultant product is reasonably homogeneous and although the preparation time is still lengthy, this is compensated for by the reduction in sampling and other errors. Loss of moisture from the homogenate could be a source of error if it is left exposed and if it were re-mixed after storage. To avoid this, it is best to anticipate all analytical requirements and take weighed samples for storage when the homogenate is prepared.

I.A.M. is supported by the Wellcome Trust.

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