The rapid determination of carcass fat by the Foss-Let specific gravity technique

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1. Carcass fat was determined by extraction with tetrachloroethylene and measurement of the solvent's change in density. The results were comparable in precision to those of a reference method; the new method extracted storage lipid but little structural lipid.
2. The technique is simple, rapid and appropriate for many nutritional studies.

The standard methods for determining fat, such as extraction in the Soxhlet apparatus and gravimetric estimation, tend to be time-consuming, and use inflammable organic solvents. A technique which overcomes these difficulties has been developed by Foss Electric Ltd for the food and agricultural industries (Usher, Green & Smith, 1973). Fat is extracted into tetrachloroethylene (TCE), and the resulting fall in the specific gravity of the solvent is measured in a magnetic float cell. The technique has been evaluated with foodstuffs (Usher *et al.* 1973; Egberg, Potter & Honold, 1974; Pettinati & Swift, 1975). The deviation from the reference methods was rarely more han 1% fat content, and the reproducibility was comparable.

The present study assessed the usefulness of the procedure for the analysis of laboratory animal carcasses. Eleven mouse carcasses (Aston Strain) were freeze-dried minced and assayed in duplicate. Three of the carcasses were of obese (obob) mice. The precision and accuracy of the technique were determined by comparing the results with a gravimetric reference method applied to the same carcasses.

METHODS AND MATERIALS

Chemicals

Tetrachloroethylene (reagent grade) and ammonium sulphate were obtained from BDH Chemicals Ltd, Poole, Dorset. Organic solvents used for the reference method of fat extraction and for chromatography were from May & Baker Ltd, Dagenham. Essex, and Silica Gel was obtained from E. Merck AG, Darmstadt, West Germany (Kiesel Gel GF_{254} Typ 60).

Extraction of fat with TCE

Weighed samples of 2–3 g freeze-dried carcass were extracted with 30 ml TCE at room temperature using a standard blade homogenizer (MSE Ltd, Crawley, Sussex) at 1000 – 2000 rev/min for 2 min. (A dry sample is necessary to avoid emulsification). The extracts were then filtered through Whatman 541 paper into a Buchner flask.

The carcass residue was recovered, rehomogenized with a further 30 ml solvent, and filtered. The two filtrates were transferred to a 100 ml volumetric flask. A further 30 ml of TCE was taken to rinse the apparatus and then added to the volumetric flask. The three combined filtrates were made up to volume, shaken, and allowed to stand. Any solid matter which collected on the surface was removed by aspiration with a Pasteur pipette.

Foss-Let estimation

The specific gravity of the extracts was measured in the Foss-Let 15310 instrument (Foss Electric U.K. Ltd, The Chantry, Bishopthorpe, York YO2 1QF).

To calculate the percentage of fat in the sample, the manufacturer supplies a conversion table, the validity of which has been verified by Usher *et al.* (1973) and by ourselves.

Reference method

Weighed samples of 2–3 g carcass were analysed by a method based on that of Southgate (1971). Each sample was extracted with 3 volumes of 50 ml of a mixture of chloroform and methanol (2:1, v/v). The procedure was essentially the same as that for the TCE extraction. Solvent was removed from the pooled filtrates using a rotary evaporator. The crude fat extract which remained was then taken up by shaking with two 50 ml volumes of boiling petroleum ether (boiling range 40–60°). The petroleum ether was transferred to a volumetric flask, made up to 100 ml, shaken, and allowed to stand until clear. Samples (15 ml) were removed and left to evaporate at room temperature in tared foil dishes which were then reweighed.

Thin-layer chromatography

In order to assess the specificity of the TCE extraction procedure the extracts were subjected to thin-layer chromatography.

Glass plates (20 cm × 10 cm) were spread with a 250 μm thick layer of silica gel and activated by heating in an oven at 110° for 1 h. The following lean carcass extracts were applied: (i) whole dry carcass in TCE, (ii) whole dry carcass extracts made by the reference method, (iii) extracts made by the reference method from the residue left after TCE extraction. Each plate was spotted with the three extracts from a single carcass; all three spots in a set contained amounts of lipid corresponding to the content of similar weights of dry carcass. The extracts of whole dry carcass were applied in volumes containing about 1 mg lipid. The plates were developed in a solvent system of chloroform–methanol–water (14:6:1, by vol; Muldner, Wherrett & Cumings, 1962); this procedure yields a one-dimensional separation by class, in which the polar lipids are less mobile. The separated lipids were visualized on the plate by spraying with 4 M-aqueous ammonium sulphate (Smith, 1969) and heating in an oven at 160° for 1 h.

RESULTS

Table 1 gives the results for both methods of determination. The mean difference ($\pm$ SEM) between duplicates was $3{\cdot}1 \pm 0{\cdot}9\%$ and $2{\cdot}2 \pm 0{\cdot}9\%$ for the Foss-Let and reference methods respectively. The Foss-Let procedure consistently gave a lower

Table 1. *Results for determination of fat in eleven dried mouse carcasses by Foss-Let and reference methods*

(Each result is the mean of two determinations. The average difference* between duplicates was 3·1 ± 0·9‡ and 2·2 ± 0·9‡ % for the Foss-Let and reference methods respectively)

	Fat content (g/kg)		
Carcass	Foss-Let	Reference method	% difference
1	371	387§	4·2
2	386	386§	0·0
3	434	464§	6·7
4	378	475	22·8
5	298	367	20·8
6	358	416	15·0
7	374	451	18·7
8	348	399	13·7
9†	727	753	3·5
10†	839	877	4·4
11†	855	873	2·1

* % differences calculated as $\frac{\text{difference between results}}{\text{mean of results}} \times 100$ (± SEM).

† Carcass from an obese mouse.

‡ Mean ± SEM.

§ Petroleum ether extraction at room temperature.

result, the average deficit for the eight lean carcasses being 12·7% of the fat content. This difference suggested that the two methods, when conducted in full, might extract different types of lipid particularly since the difference was much smaller in the case of the three obob carcasses (3·3% of the fat content) which have a much higher ratio of storage to structural lipid. This possibility was tested by qualitative analysis of the three types of extract with thin-layer chromatography.

Plate 1 shows a typical separation. The reference method extracts both neutral and structural lipids, while TCE extracts much neutral but little polar lipid. TCE also extracts a lipid which migrates just behind triglyceride in a position corresponding to cholesterol ester. The reference method when applied to the residue remaining after extraction with TCE, resulted in the removal of polar lipid but only trace amounts of triglyceride.

DISCUSSION

The quantitative analyses show that the Foss-Let method is almost as precise as the reference method, but it gives a lower value. When the material extracted by the reference method from the carcass residue remaining after extraction with TCE was determined gravimetrically, the values obtained accounted for the difference between the two methods of carcass analysis. Further extractions with TCE did not, however, increase the value obtained by the Foss-Let procedure. It therefore seemed probable that the reference method resulted in the extraction of lipids which were not extractable by TCE.

This was confirmed by separating the material obtained from the Foss-Let and reference procedures by thin-layer chromatography. Although TCE readily extracted triglyceride and other neutral lipids, it removed little of the polar structural lipid that was extracted by the reference method. The application of the reference method to material which had previously been extracted with TCE resulted in the removal of the structural lipid. The low values for carcass fat obtained with the Foss-Let procedure are therefore clearly due to the inability of TCE to extract polar lipids.

Other work using the Foss-Let procedure has shown that the method gives lower results when materials rich in structural lipids are being assayed. Pettinati & Swift (1975) found a deficit with meat samples containing less than 7·5% fat and Usher *et al.* (1973) reported reduced values with dried egg and milk powder. We have ourselves found that TCE extracts only half as much lipid as our reference method from rat brain, a tissue which contains abundant structural lipid but little triglyceride.

Conversely, obese mouse carcasses, which contain a higher proportion of storage to structural lipid, show a smaller deficit than lean carcasses (Table 1). For most work, and particularly in studies on obesity, measurement of storage lipid is more relevant than that of total lipid. The Foss-Let procedure is therefore highly appropriate. With its short analytical time (about 10 min) it should prove to be a powerful tool in carcass analysis.

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EXPLANATION OF PLATE

Separation by thin-layer chromatography of three lipid extracts of lean mouse carcass. 1 = Extract of whole dry carcass made by reference method. 2 = Extract of whole dry carcass in tetrachloroethylene. 3 = Extract made by reference method from residue left after tetrachloroethylene extraction. The solvent system was chloroform–methanol–water (14:6:1, by vol.), and the separation was visualized by spraying with 4 M-aqueous ammonium sulphate and heating at 160° for 1 h.

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