

## High efficiency of energy utilization in 'cafeteria'- and force-fed rats kept at 29°

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(Received 18 March 1983 - Accepted 29 November 1983)

1. Male, Sprague-Dawley (Charles-River) rats, of initial weight 272 g, were given a powdered stock diet (T1) *ad lib.*, force-fed a synthetic diet (T2) or offered a range of palatable foods in conjunction with the powdered stock diet (T3) or a similar diet supplemented with certain minerals and vitamins (T4).
2. Metabolizable energy (ME) intake (kJ/d) averaged 303, 453, 402 and 383 for T1, T2, T3 and T4 respectively and corresponding weight gains were 5.5, 6.9, 8.2 and 7.9 g/d and were significantly different ( $P < 0.001$ ).
3. The intakes of T3 and T4 rats ranged from 10 to 60% above the mean value for T1.
4. Crude protein (CP; nitrogen  $\times 6.25$ ) retentions were similar for T1, T3 and T4 rats and significantly lower ( $P < 0.01$ ) for T2 rats. Fat retentions were 1.1, 4.1, 2.9 and 2.4 g/d for T1 to T4 respectively ( $P < 0.001$ ).
5. The energy contents of the gain (MJ/kg) were 12.7, 26.0, 16.7 and 14.9 for T1 to T4 respectively ( $P < 0.001$ ) and energy retentions (kJ/d) were 70, 179, 139 and 117 respectively ( $P < 0.001$ ).
6. A linear regression of energy retention (ER) on ME yielded a slope of 0.78 and a mean energy requirement for zero balance of 510 kJ/kg body-weight<sup>0.75</sup>.
7. These results are in conflict with reports of 'diet-induced thermogenesis' in 'cafeteria'-fed rats.

The laboratory rat has been used extensively as an experimental model of obesity in man. Hyperphagia can be achieved by feeding high-fat diets (Mickelsen *et al.* 1955) by gastric intubation (Cohn & Joseph, 1959) or by offering a varied diet (Scalafani & Springer, 1976). With the former two methods the efficiency of energy utilization is consistently high (Schemmel *et al.* 1972; McCracken & McNiven, 1983). However, Rothwell & Stock (1979) reported that 'cafeteria'-fed rats, i.e. those offered a varied diet, consumed more than twice as much energy as control rats given a pelleted stock diet but that most of the extra energy consumed was liberated as heat. This phenomenon, designated 'diet-induced thermogenesis', has since been reported in various strains of rats (Rothwell & Stock, 1982) and in mice (Trayhurn *et al.* 1982) although Armitage *et al.* (1981) did not observe any significant alteration in the efficiency of utilization of energy by 'cafeteria'-fed rats. Most studies have been conducted at 24°, i.e. below the zone of thermoneutrality (Swift, 1944) but Andrews & Donne (1982) reported a large increase in the oxygen consumption of 'cafeteria'-fed rats at 30°.

One difficulty with 'cafeteria'-feeding is that many of the palatable foods used are poor sources of minerals and vitamins, and it seemed probable that the varied diet was deficient in some important nutrients. On the other hand, the fat content of a varied diet is much higher than that of the stock diet with which it has been compared in previous studies.

The aims of the present experiment were: (1) to determine the effect of 'cafeteria'-feeding on energy intake and expenditure of rats in a thermoneutral environment, (2) to compare the efficiency of energy utilization of rats given a varied diet or force-fed a synthetic diet of equivalent nutrient content and (3) to determine the effect on energy utilization of improving the nutrient balance of the varied diet.

## EXPERIMENTAL

Thirty male, Sprague-Dawley rats, obtained from Charles-River, Kent, were acclimatized to their new environment for at least 1 week prior to the start of the experiment. They were weighed daily and, on the 7th day, allocated to one of six weight blocks (five rats per block). When a block reached the mean initial weight of 272 g, each rat was randomly allocated to one of four dietary regimens (Table 1) or slaughtered for initial carcass composition. All animals were housed individually in wire metabolism cages. Room temperature was  $29 \pm 1^\circ$  and the light-dark cycle was: light, 08.00–18.00 and 20.00–22.00 hours; dark, 18.00–20.00 and 22.00–08.00 hours. This arrangement was to facilitate tube-feeding. The cages for T1 and T2 rats were placed over plastic containers charged with 0.1 l sulphuric acid (0.1 M). Faeces and urine were collected together for 7-d periods and subsequently freeze-dried. The cages for T3 and T4 rats were placed over solid trays lined with absorbent paper to facilitate separation of feed and faeces.

The rats were weighed daily and approximate daily intakes of individual feeds were determined so that the nutrient intakes could be estimated. After 21 d the rats were killed by chloroform anaesthesia, undigested food residues were removed from the gastrointestinal tract and various organs, including liver, interscapular brown adipose tissue (IBAT), epididymal and kidney fat pads, were removed and weighed. The livers were stored separately at  $-20^\circ$  for subsequent analysis. The carcasses were prepared for analysis as described by McCracken & McNiven (1983). Samples of milled freeze-dried material were analysed for crude protein (CP; nitrogen  $\times 6.25$ ) by the macro-Kjeldahl method, for ash by ignition in a muffle furnace at  $500^\circ$  for 8 h and for ether extract by the Soxhlet method ( $40-60^\circ$  BP; petroleum ether). Carcass energy retention (ER) was calculated from protein and fat retention using the factors 23.8 and 39.3 MJ/kg respectively (Brouwer, 1965). The livers were analysed by the same method after freeze-drying and milling. Glycogen was calculated by difference.

*Diets*

The compositions of the three synthetic diets are shown in Table 2. The palatable foods used to form the varied diet were:

Bacon	Cream crackers	Battenburg cake
Corned beef	Hovis crackers	Swiss roll
Luncheon meat	Digestive biscuits	Sponge cake
Breakfast strips	Rich-tea biscuits	Fairy cake
Pork sausage	Ginger-thins	Ginger cake
Beef sausage	Shortbread	Sponge fingers
Chopped ham & pork	Cracottes	Popcorn
Cheese	Milk chocolate	Cheese wotsits
Crisps	Milky Way	Pasta

Some of these were found not to be accepted by the rats and were offered only once. Four foods were offered daily, two at 09.30 hours and two at 20.30 hours. A meat course was offered each evening and a regimen as similar as possible to that of Rothwell & Stock (1979) was followed. The food given to the force-fed animals was mixed to a slurry with tepid water immediately prior to administration. At each feeding time, representative samples were taken for dry matter (DM) determinations ( $100^\circ$  in a forced-draught oven for 24 h). Initially the tube-fed animals were given 6 ml, containing approximately 0.75 g DM/ml, three times daily at 09.00, 16.00 and 21.00 hours. This was increased to 30 ml/d over the first week.

Table 1. *Experimental treatments*

Treatment	Diet	Dietary regimen
T1	1	Powdered stock diet <i>ad lib.</i>
T2	2	Force-fed, three times daily
T3	1	Powdered stock plus four palatable foods daily
T4	3	Powdered stock plus four palatable foods daily
T5	—	Starting controls

Table 2. *Composition and analysis of synthetic diets (g/kg) given alone or in combination with palatable foods\**

Diet...	1	2	3
Casein	180	180	180
Sucrose	250	250	250
Starch	480	428	467
Maize oil	30	100	30
Dunn's salt†	50	35	50
Vitamin mixture‡	10	7	20
Methionine	—	—	3
Crude protein (nitrogen × 6.25) (g/kg DM)	163	170	163
Gross energy (MJ/kg DM)	18.1	19.9	18.2
Metabolizable energy (MJ/kg DM)§	17.2	19.3	17.3

\* See Table 1.

† Contained (g/kg): calcium orthophosphate 434.0, potassium chloride 271.0, disodium hydrogen phosphate 114.2, magnesium sulphate 87.0, sodium chloride 54.5, ferric citrate 38.0, calcium iodide 1.1, manganese sulphate 0.20, sodium fluoride 0.01.

‡ Contained (g/kg): retinol 3.44, cholecalciferol 0.05,  $\alpha$ -tocopheryl acetate 10, choline 100, inositol 10, *p*-aminobenzoic acid 5, niacin 4, menadione 1, pyridoxine 0.50, thiamin 0.50, riboflavin 0.32, folic acid 0.10, biotin 0.02, cyanocobalamin 0.002.

§ Determined directly for diets 1 and 2 and calculated as 0.96 digestible energy for diet 3.

#### *Calculation of nutrient composition of varied diets*

Palatable foods were weighed individually and refusals and spillage separated daily and weighed so that the consumption of each item could be estimated. Refusals of meats and foods of low DM content were dried at 100° for 24 h before conversion of refused food back to a fresh matter basis. Each food was analysed for DM content and gross energy (GE). Diets 1 and 3 were weighed daily and replenished when necessary.

The vitamin contents of the synthetic diets were calculated from the dietary ingredients. Proximate constituents, energy and minerals were determined. The nutrient composition of the total diet consumed by each T3 and T4 rat was calculated from the weights of the individual food items consumed, DM and energy values obtained by direct analysis and values for other nutrients taken from food tables (Paul & Southgate, 1978).

#### *Measurement of energy balance*

At the end of the experiment, the three weekly samples of freeze-dried excreta for each rat in T1 and T2 were bulked and energy determined by adiabatic bomb calorimetry. The metabolizable energy (ME) intake was determined by subtraction of the excreta energy from the GE consumed. For T3 and T4 the GE offered was calculated from the total weight of each food item and its measured GE content. The digested energy (DE) consumed was

Table 3. *Metabolizable energy (ME) intake (kJ/d), gains of weight, crude protein (CP; nitrogen  $\times$  6.25), fat (g/d) and energy (kJ/d) and energy content of gain (MJ/kg) and gross efficiency for rats given diet 1 ad lib. (T1), force-fed diet 2 (T2) or offered a varied diet in combination with diet 1 (T3) or diet 3 (T4) for 21 d†*

(Mean values and standard error of the difference for six rats, 13 df)

Treatment...	T1	T2	T3	T4	SED	Statistical significance
ME intake (kJ/d)	303	453	402	383	21.8	***
Wt gain (g/d)	5.5	6.9	8.2	7.9	0.65	**
CP gain (g/d)	1.04	0.65	1.11	1.14	0.106	**
Fat gain (g/d)	1.12	4.09	2.86	2.43	0.411	***
Energy gain (kJ/d)	70	178	139	117	11.71	***
Energy content of gain (MJ/kg)	12.7	26.0	16.7	14.9	1.46	***
Gross efficiency of energy gain	0.23	0.39	0.34	0.31	0.019	***

\*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

† For details of diets and treatments, see Tables 1 and 2.

obtained by subtracting the energy content of the combined food refusals, spilt food and faeces for the 21-d period from the GE offered. ME was estimated as 0.96 DE (Agricultural Research Council, 1981). Heat production was calculated as the difference between ME intake and ER.

The results were subjected to analysis of variance, an iterative procedure being used to estimate missing plots which occurred due to the premature deaths of two T2 rats.

#### RESULTS

The ME intake of the rats offered a varied diet (T3 and T4) ranged from 334 to 478 kJ/d, an increase of between 10 and 60% over the mean T1 intake. The mean increases in ME intake for T2, T3 and T4 over T1 were, respectively, 50, 33 and 26% (Table 3) and were significant ( $P < 0.001$ ). Weight gain was lowest for T1 and highest for T3 and T4, the difference being significant ( $P < 0.01$ ). Carcass CP gain was similar for T1, T3 and T4 and significantly lower ( $P < 0.01$ ) for T2. Carcass fat gain was 265, 157 and 117% higher for T2, T3 and T4 rats respectively than for the controls ( $P < 0.001$ ). As a consequence of the large differences in the proportions of CP and fat in the carcass gain, there were large differences in the energy content of the gain. The mean energy content of the gain of T2 rats (26.0 MJ/kg) was significantly greater ( $P < 0.001$ ) than that of the other three treatments and more than twice that of T1 rats. Carcass energy gain was significantly increased by giving a varied diet or by force-feeding ( $P < 0.001$ ). Gross efficiency of energy gain was lowest for T1 and significantly higher for T3 and T4 ( $P < 0.001$ ) and for T2 ( $P < 0.001$ ).

When the results were expressed per unit metabolic body-weight ( $\text{kg } W^{0.75}$ ) the calculated mean heat productions (Table 4) of T1, T3 and T4 rats were similar and significantly lower ( $P < 0.05$ ) than for T2 rats. Because of the range of ME intake which occurred in T1, T3 and T4, it was possible to plot a regression of ER on ME intake (Fig. 1). The pooled results yielded the equation

$$\text{ER (kJ/d per kg } W^{0.75}) = 0.78 \text{ ME} - 395 \quad (r 0.95).$$

Applying the pooled slope to the individual treatments yielded estimates of the energy requirement for zero balance of 505, 508, 476 and 502 for T1, T2, T3 and T4 respectively.

Table 4. Metabolizable energy (ME) intake, energy retention (ER) and calculated heat production (HP; kJ/d per kg body-weight<sup>0.75</sup>) of rats given diet 1 ad lib. (T1), force-fed diet 2 (T2) or offered a varied diet in combination with diet 1 (T3) or diet 3 (T4) for 21 d†

(Mean values and standard error of the difference for six rats, 13 df)

Treatment...	T1	T2	T3	T4	SED	Statistical significance
ME intake	719	1030	858	827	34.1	***
ER	165	405	294	252	31.5	***
HP	553	625	564	575	21.3	*

\* $P < 0.05$ , \*\*\* $P < 0.001$ .

† For details of diets and treatments, see Tables 1 and 2.

Table 5. Weight (g) of interscapular brown adipose tissue (IBAT) and weights (g/kg carcass) of IBAT, kidney and epididymal fat pads of starting controls (T5), rats given diet 1 ad lib. (T1), force-fed diet 2 (T2) or offered a varied diet in combination with diet 1 (T3) or diet 3 (T4) for 21 d†

(Mean values and standard error of the difference for six rats, 18 df)

Treatment...	T1	T2	T3	T4	T5	SED	Statistical significance
IBAT (g)	0.33	0.62	0.49	0.42	0.26	0.094	**
IBAT (g/kg)	0.88	1.58	1.14	0.99	1.00	0.205	*
Kidney fat (g/kg)	23.9	37.0	34.8	32.5	10.6	2.33	***
Epididymal fat (g/kg)	16.4	27.7	25.3	24.5	11.4	2.51	***

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

† For details of diets and treatments, see Tables 1 and 2.

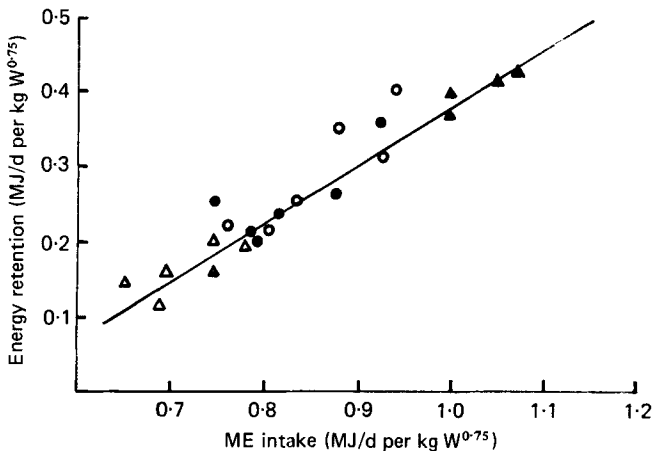


Fig. 1. Linear regression of energy retention (ER; MJ/d per kg body-weight<sup>0.75</sup> ( $W^{0.75}$ )) on ME intake (ME; MJ/d per kg  $W^{0.75}$ ) for rats given diet 1 ad lib. ( $\Delta$ ), force-fed diet 2 ( $\blacktriangle$ ) or offered a varied diet in combination with diet 1 ( $\circ$ ) or diet 3 ( $\bullet$ ) for 21 d. For details of diets and treatments, see Tables 1 and 2.

$$ER = 0.78 ME - 395, \quad r = 0.95.$$

Table 6. *Weight (g) of liver, and dry matter (DM), crude protein (CP; nitrogen  $\times$  6.25), fat and glycogen contents of liver (g) of rats given diet 1 ad lib. (T1), force-fed diet 2 (T2) or offered a varied diet in combination with diet 1 (T3) or diet 3 (T4) for 21 d†*

(Mean values and standard error of the difference for six rats, 13 df)

Treatment...	T1	T2	T3	T4	SED	Statistical significance
Liver wt (g)	16.4	18.5	19.0	18.3	2.21	NS
DM (g/liver)	4.4	7.2	5.4	5.7	0.90	**
CP (g/liver)	2.9	3.2	3.0	3.2	0.33	NS
Fat (g/liver)	0.5	3.2	1.1	1.3	0.86	**
Glycogen (g/liver)	1.0	0.7	1.1	1.0	0.22	NS

NS, not significant; \*\* $P < 0.01$ .

† For details of diets and treatments, see Tables 1 and 2.

The weight of the IBAT increased on all four treatments compared with the starting controls (Table 5). When expressed in proportion to carcass weight, there were no significant differences between T1, T3, T4 and T5 but the amount of IBAT of T2 rats was significantly higher ( $P < 0.05$ ). Kidney and epididymal fat depots increased more rapidly than IBAT, and kidney more rapidly than epididymal fat. Within both depots, weight increased in the order T5 < T1 < T4 < T3 < T2 ( $P < 0.001$ ).

Liver weight (Table 6) was not significantly affected by treatment but liver DM was significantly higher in 'cafeteria'- and force-fed rats ( $P < 0.01$ ) than in controls. The increased DM was almost entirely due to fat which increased in the order T1 < T3 < T4 < T2 ( $P < 0.01$ ).

#### DISCUSSION

Although hyperphagia occurred in both groups offered a varied diet the extent of the response was somewhat less than had been expected from the reports of Rothwell & Stock (1979). Consequently, the energy intake of the force-fed rats was higher than the mean values for T3 and T4 rats. The intake of stock diet by T3 and T4 rats was extremely low, approximately 10% ME intake, and this had two consequences for the interpretation of the results. First, the fat content was higher, the values being 107, 258 and 230 g/kg respectively for diets T2, T3 and T4 and the contents of calcium and phosphorus were lower in 'cafeteria' diets compared with the force-fed diet (Table 7). Also, the vitamin B content of the T3 diet was lower than that for T2. Second, the low stock intake resulted in the mean mineral and vitamin compositions of the total diet consumed by T4 rats being only slightly higher than that of the total diet consumed by T3 rats, and less than (US) National Research Council (1978) requirements. Thus the third objective of the experiment was not completely achieved. It is not clear whether the failure to observe any difference in energy utilization between T3 and T4 was due to the fact that both diets were deficient or that the deficiencies observed were not affecting the efficiency of energy utilization. The low Ca intakes of T3 and T4 rats are particularly disturbing and would have important implications in any long-term studies using a 'cafeteria'-feeding regimen.

The wide range of response of T3 and T4 rats to the varied diet is notable. Because of individual food preferences the proportion of fat (g/kg) in the total diet varied considerably (214–287 for T3 and 193–276 for T4 rats) and the extent of hyperphagia varied from 10

Table 7. Nutrient intakes (per MJ metabolizable energy) of rats given diet 1 ad lib. (T1), force-fed diet 2 (T2) or offered a varied diet in combination with diet 1 (T3) or diet 3 (T4) for 21 d\* and the estimated requirements for growing rats ((US) National Research Council, 1978)

(Mean values for six rats)

Treatment...	T1	T2	T3	T4	(US) National Research Council (1978)
Crude protein (nitrogen $\times$ 6.25) (g)	9.5†	8.8†	9.2	9.1	—
Lysine (g)	1.06	0.95	0.61	0.61	0.44
Methionine/cystine (g)	0.48	0.43	0.35	0.35	0.38
Calcium (g)	0.36†	0.24†	0.09	0.10	0.31
Phosphorus (g)	0.43†	0.30†	0.16	0.18	0.25
Thiamin (mg)	0.31	0.18	0.12	0.20	0.25
Riboflavin (mg)	0.19	0.14	0.10	0.17	0.19
Pyridoxine (mg)	0.31	0.18	0.10	0.18	0.38

\* For details of diets and treatments, see Tables 1 and 2.

† Determined directly; all other values calculated from ingredients using food tables (Paul & Southgate, 1978).

to 60% of the control value. Large differences in response have also been noted by Armitage *et al.* (1981) and are of interest in relation to possible hormonal or sensory factors affecting regulation of energy intake.

The differences in body composition which occurred are of interest for several reasons. First, it is clear that the 'cafeteria' regimen did not result in protein deficiency. It seems probable in fact that the T3 and T4 rats were achieving close to the maximum protein deposition rate. The calculated mean CP intakes (g/d) for T1, T2, T3 and T4 were 3.0, 3.7, 3.7 and 3.4 respectively. The intakes (g/d) of lysine and methionine/cystine were 0.32, 0.43, 0.25 and 0.22, and 0.15, 0.19, 0.15 and 0.13 and the mean CP retentions (g/d) for T1, T2, T3 and T4 were 1.04, 0.65, 1.11 and 1.14 respectively.

Second, the force-fed diet also supplied adequate amounts of protein and amino acids but CP retention was severely reduced. Similar effects in force-fed rats have been reported previously (Cohn & Joseph, 1963; McCracken, 1975) but the mechanism has not been established. Another side-effect of force-feeding is the lipid accumulation in the liver which has also been reported in previous experiments (Denton *et al.* 1950; Sidransky & Baba, 1960). For these two reasons it is considered that force-feeding may not be a satisfactory means of achieving hyperphagia in young animals.

Third, there were large differences in fat content (and hence energy content) of the weight gain. Although the mean daily gain of T3 and T4 rats was only 45% higher than that of T1 rats, daily energy retention was increased 100%. The effect was even greater with T2 rats where daily gain was increased by 25% and energy retention by 176%. This emphasizes the dangers inherent in estimating energy retention from weight gain (see also McCracken & McNiven, 1983).

Despite the differences in diet composition the efficiency of energy utilization was uniformly high. Because of the wide range of intakes achieved, it was considered acceptable to plot a linear regression of ER on ME intake. Statistical analysis confirmed that a single slope yielded as good a fit to the figures as individual slopes. The mean intercept for zero energy balance of 510 kJ/kg  $W^{0.75}$  is higher than that observed by McCracken & McNiven (1983) with adult female rats, and higher than that obtained by regression by Pullar & Webster (1977). If a lower value for maintenance heat production (e.g. 400 kJ/kg  $W^{0.75}$ ) is applied to the individual treatments the partial efficiency of energy utilization ( $k$ ) for T1,

T2, T3 and T4 respectively becomes 0.52, 0.64, 0.64 and 0.59, indicating a higher efficiency of utilization for 'cafeteria'-fed rats than for controls. The results are clearly in conflict with the reports of increased oxygen consumption (Andrews & Donne, 1982) and with results obtained at 24° by Rothwell & Stock (1979). However, they are in agreement with those of Armitage *et al.* (1981) and Bestley *et al.* (1982).

The *k* value determined by regression (0.78) is somewhat lower than that obtained by McCracken & McNiven (1983) with adult rats (0.88). This is probably attributable to the higher energy cost of protein synthesis (Pullar & Webster, 1977) compared with the relatively low cost of direct fat incorporation in the adult rat.

The difference in response to 'cafeteria'-feeding observed in the present experiment and that reported by Rothwell & Stock (1979) is not easy to explain. Hervey & Tobin (1982*a*) have already drawn attention to some of the errors which arose in the Rothwell & Stock (1979) experiment. However, one has to assume large errors in estimation of the energy intake of the 'cafeteria'-fed animals in addition to the obvious error in the calculated intake of the controls. It is unlikely that the difference in environmental temperature will provide an explanation.

IBAT has been implicated in cold-induced thermogenesis (Foster & Frydman, 1978) in the rat and a similar role in so-called 'diet'-induced thermogenesis has been suggested (Rothwell & Stock, 1979; Tulp *et al.* 1982). The present results show that large increases in IBAT weight result from force-feeding or from offering a varied diet without any increase in heat production. The appearance of the IBAT of T3, T4 and, more especially, T2 rats was paler than that of T1 rats and this was probably due to increased deposition of triglyceride. This conclusion is in agreement with the results of Hervey & Tobin (1982*b*). The weight increases of the kidney and epididymal fat depots in force-fed and 'cafeteria'-fed rats were relatively larger than in IBAT. In the 'cafeteria'-fed rats these two depots accounted for approximately 30% of the extra fat stored.

It is clear that further studies are required to elucidate the cause of the large differences in response obtained in different laboratories. The present results are consistent with results obtained in rats made hyperphagic by other methods (McCracken & McNiven, 1983) and with the known effects of high-fat diets on the efficiency of energy utilization in other mammals (Vanschoubroek, 1966; Boyd, 1978). It seems probable, therefore, that the results of Rothwell & Stock (1979), Andrews & Donne (1982) and Trayhurn *et al.* (1982) arise from one or more experimental artefacts and are unlikely to be of relevance to the study of human obesity or to the efficiency of energy utilization of farm animals.

H. G. B. acknowledges the award of a post-graduate research studentship by the Department of Agriculture, Northern Ireland.

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