Energy balance and energy values of α-amylase (*EC* 3.2.1.1)-resistant maize and pea (*Pisum sativum*) starches in the rat

BY G. LIVESEY, I. R. DAVIES, J. C. BROWN, R. M. FAULKS AND S. SOUTHON

Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich NR4 7UA

(*Received 30 August 1988 – Accepted 17 November 1989*)

Apparent and partial digestible energy values for α-amylase (*EC* 3.2.1.1)-resistant, retrograde starches, isolated from cooked maize and pea starches (RMS and RPS respectively), were determined in male Wistar rats (about 180 g) during a 28–29 d balance period with ten animals per treatment. The starches were provided as supplements (100 g/kg diet) to a semi-synthetic basal diet (B), and their effects on the apparent digestibilities of nitrogen and fat, and on gains of live weight, fat and lean tissue were examined. Diet B alone was the control; sucrose (Su) and Solka-floc cellulose (SFC) were also examined for reference. Apparent digestibilities for Su, SFC, RMS and RPS were 1·0, 0·16, 0·98 and 0·89 respectively. Whereas the apparent digestibilities of gross energy, N and fat in the diet were unaffected by supplementation with Su, each was decreased by supplementation with SFC, RMS and RPS. Partial digestible energy values calculated from the intakes and faecal losses of energy in the basal and supplemented diets were 15, 12·4 and 0·8 kJ/g for RMS, RPS and SFC respectively. These values were smaller than corresponding apparent digestible energy values calculated from the apparent digestibility of the supplement and its gross energy value. Only the Su and starch supplements increased the intake of apparent digestible energy and the gain of live weight. Both starches and Su increased total energy (and fat) deposition to almost similar extents. It is concluded that the resistant starches contribute significant dietary energy, enhance growth and elevate fat deposition to extents almost similar to Su.

Resistant starch: Energy balance: Unavailable carbohydrate: Rat

Starch fractions resistant to hydrolysis with α-amylase (*EC* 3.2.1.1) are collectively called resistant starch (Englyst *et al.* 1982). The polysaccharides of native, granular starches are very resistant (Ring *et al.* 1988) and are dispersed by cooking to form visco-elastic pastes which gel on cooling (Miles *et al.* 1985). The dispersed polysaccharides are readily digested by α-amylase but subsequently re-associate or retrograde to form small aggregates (crystallities) which are held together by hydrogen bonding in highly ordered structures (Collinson, 1968). Retrogradation of amylose imparts resistance to α-amylase, and such starches are termed retrograde resistant starches. The amylopectin fraction of starch is solubilized less readily than the amylose fraction, explaining a predominance of amylose in retrograde resistant starches. When dissolved, however, amylopectin will retrograde to form aggregates but these may be almost completely degraded by α-amylase (Ring *et al.* 1988).

Retrograde resistant starch, in addition to escaping digestion in vitro partly escapes digestion in vivo, with some being fermented (Englyst & MacFarlane, 1986); Wyatt & Horn, 1988) and some appearing in faeces (Englyst & Cummings, 1985; Bjorck *et al.* 1986; Faulks *et al.* 1989). This led Bjorck *et al.* (1986) to recommend resistant starch (formed during the baking of bread) to be regarded as dietary fibre. Indeed, debate has arisen as to whether resistant starch should be included within the analytical value, for dietary fibre (Berry, 1986). It appears that current practices for the analysis of total dietary fibre may include appreciable amounts of resistant starch with some foods (Englyst *et al.* 1987). Including this fraction with dietary fibre would make the analysed fibre content of some

Table 1. *Compositions of the basal and supplemented diets**

	Basal diet
Casein (g)	168
DL-Methionine (g)	2
Sucrose (g)	692
Maize oil (g)	80
Mineral mix (g)†	40
Vitamin mix (g)‡	20
Total (g)	1002

* Supplemented diets (basal diet with the addition of one of the following: 100 g/kg basal diet): Su, additional sucrose (< 10 g water/kg); SFC, solka-floc cellulose (50 g water/kg); RMS, resistant maize starch (< 10 g water/kg); RPS, resistant pea (*Pisum sativum*) starch (< 10 g water/kg). For dietary treatment, see below.

† Contained (g/1002 g basal diet): $CaHPO_4$, 13·0; $CaCO_3$, 8·2; KCl, 7·03; Na_2HPO_4, 7·4; $MgSO_4.H_2O$, 4·0; $MnSO_4.H_2O$, 0·18; $ZnCO_3$, 0·01; $FeSO_4.H_2O$ 0·144; $CuSO_4$, 0·015; KIO_3, 0·001.

‡ Contained (mg/1002 g basal diet): nicotonic acid, 60; cyanocobalamin in mannitol (Glaxo), 50; calcium D-pantothenate, 40; thiamin hydrochloride, 10; riboflavin, 10; pteroylmonoglutamic acid, 5; D-biotin, 1; menadione, 1; Rovimix E-25 (Roche), 300; Rovimix A-500 (Roche), 25; Rovimix D_3-500 (Roche), 15; choline bitartrate, 1800.

foods open to manipulation by food technologists because the extent of retrogradation depends on the processing conditions (Berry, 1986; Ring *et al.* 1988). The controversy is twofold. First, are significant quantities of resistant starch analysed as dietary fibre in foods as eaten? Second, do the physiological effects and health consequences of such starches broadly resemble those suggested for dietary fibre? (See review by the Federation of American Societies for Experimental Biology (FASEB), 1987.)

We showed previously that retrograde resistant starches produced from pea (*Pisum sativum* var. Scout) and Snoflake maize starch differ quantitatively in their utilization in the rat (Faulks *et al.* 1989). The material from both sources was partially available as carbohydrate, some was fermented and some entered the faeces. The resistant pea starch was the more resistant, both to enzymes and fermentation. The difference in resistance to fermentation in vivo was also evident for in vitro anerobic fermentation by large bowel micro-organisms of the rat and human being (Wyatt & Horn, 1988). The present work extends these studies by examination of the effects of retrograde resistant starches on energy exchange in the rat. Studies on their energy values and effects on energy and fat deposition are relevant to the debate on their possible similarity to dietary fibre in their physiological effects, with respect to both fibre analysis and the possible health consequences (Health and Welfare Canada, 1986; FASEB, 1987). Possibly, resistant starch may have a role in explaining those observations that led Cleave (1974) and Burkitt & Trowell (1975) to their 'dietary fibre hypothesis', and which implicate the lack of dietary fibre as contributing to obesity.

A note is appropriate here about use of terminology. The present paper discriminates between apparent digestibility, a commonly used term, and partial digestibility, a currently uncommonly used term. Apparent digestibility is the proportion of an ingested food substance unrecovered in faeces (Merrill & Watt, 1973). Partial digestibility is the difference in apparent digestible nutrient between two diets expressed as a proportion of the difference in that nutrient intake (Kleiber, 1975). Partial digestibility and apparent digestibility may be identical values, e.g. when the apparent digestibility is independent of the amount of the nutrient in food ingested and, in particular when concerned with energy, when the nutrient has no effects on the faecal losses of other nutrients. With unavailable carbohydrates, which may increase the faecal losses of protein and fat (Southgate & Durnin, 1970; Kelsay *et al.*

1978; Judd, 1982), it is useful to discriminate between apparent and partial digestibility as suggested in a recent workshop on energy and complex carbohydrates (Livesey, 1989*a*). A recent enquiry and analysis of the literature (Livesey, 1990) showed that apparent digestibility and apparent digestible energy values of complex unavailable carbohydrates are indeed different from the corresponding partial digestibility and partial digestible energy values, apparent values always being higher than the partial values. The current paper continues to make this important distinction, reporting both apparent and partial digestible energy values for the resistant starches and the reference substances sucrose and Solka-floc cellulose.

MATERIALS AND METHODS

Dietary supplements

The retrograde, porcine α-amylase-resistant starches were prepared from pea (var. Scout; RPS) and Snoflake maize-starch flour (Corn Products Co. (UK) Ltd, Manchester; RMS) as described previously (Faulks *et al.* 1989). Solka-floc cellulose (grade B92030; SFC) was obtained from Jorgensen and Wettre Ltd, London.

Animals, housing and diets

Sixty male Wistar rats (CFHB, Remote Wistar; Interfauna UK Ltd, Huntingdon), weighing about 100 g on arrival, were immediately allocated at random to one of six treatment groups of ten animals and placed singly into polypropylene cages with wire-mesh bottoms and tops. The rats were then kept in a single room at 20–22° with good air circulation and ventilation and with a 12 h light–12 h dark cycle. For the fist 13 d all animals received, *ad lib.*, water and a semi-synthetic diet free from non-starch polysaccharides and starch (diet B, Table 1). On day 14, after an overnight fast, one group of animals was killed by cervical dislocation after intraperitoneal injection of sodium pentobarbital (60 mg/ml; 2 ml/kg body-weight). The remaining groups, after the overnight fast, received the prescribed diets (Table 1) at a daily rate of 12 g basal diet per animal for the first 20 d and 14 g basal diet per animal thereafter, until the animals were killed on days 28 or 29. The intake of food and the spillage (usually $< 1\%$ of intake) was measured daily. When spillage occurred, an additional quantity of fresh food equal in weight to the spillage of the previous day was provided to ensure equality of intake of basal diet among the dietary groups over the longer period.

Energy and nutrient balances

The balance period was for 28 or 29 d. Values collected for each period were combined to give a '28–29 d balance'. At the beginning of the balance period one group of ten animals was killed, dissected and stored at −20° until analysis to permit assessment of energy and nutrient deposition by differential carcass composition; the remaining animals were killed on days 28 or 29, when on each day five animals from each dietary treatment group were taken at random. Samples of the diets (Table 1) and of the supplements were taken at the time of preparation and again after the end of the balance period. Faeces were collected daily before feeding and frozen at −20° until analysis. Urine was discarded daily with the paper trays.

Body composition

Anaesthetized rats (160 mg sodium pentobarbital/ml; 2 ml/kg body-weight intraperitoneally; Euthatal; May and Baker, Dagenham) were killed by cervical dislocation. The alimentary organs were dissected free and their contents removed: from the stomach and large intestine with a glass slide and from the small intestine by squeezing with forceps.

Both epididymal fat pads were also removed. The separated tissues and remaining carcass were weighed, freeze-dried for at least 10 d and reweighed to obtain wet weight, dry weight (>980 g dry matter/kg) and water contents for the organs and the whole body. These dry materials were recombined, cut into small pieces and ground in a coffee grinder before being sampled for the estimation of total body fat and lean dry mass.

Analysis

Food and faeces were freeze-dried to obtain the moisture contents of food and dry matter in faeces. The dry faecal pellets were ground to a homogeneous powder with a mortar and pestle. Dry food, supplements and faeces (0·5–1·0 g) were analysed for combustible energy in a Gallenkamp adiabatic bomb calorimeter using benzoic acid thermochemical standard. Nitrogen was analysed from about 1·0 g samples by the Kjeldahl method and fat was analysed from 1·0–2·0 g samples of tissue of 5–8 g faeces by refluxing for 90 min in a Soxtec system (Tecator; Hoganes, Sweden) with a 50 ml solvent mixture of dichloromethane (AnalaR)–methanol (AnalaR) (9:1, v/v) and weighing the quantity extracted after evaporation of solvent. Cellulose in the SFC diet and in faeces was determined after sulphuric acid hydrolysis and glucose determination by glucose oxidase (*EC* 1.1.3.4) as described in detail previously (Faulks *et al.* 1989). The same method was used to determine the starch content of feed and faeces for the RMS and RPS diets. Sucrose (Su) was analysed as fructose by high-performance liquid chromatography as described previously (Faulks *et al.* 1989).

Calculation of apparent and partial digestible energy values

Apparent digestibility is the proportion of a substance ingested that is not later recovered in the faeces. It is calculated (Merrill & Watt, 1973; Kleiber, 1975) as:

$$\text{apparent digestibility} = (I-F)/I,$$

where I is the quantity ingested and F is the quantity recovered from faeces. For N, fat, Su, RMS, RPS and SFC the quantities I and F were determined by chemical analysis of food and faeces using the methods described earlier; for gross dietary energy, bomb calorimetry was used as described earlier.

Apparent digestible energy values of the supplements were calculated from their determined heats of combustion multiplied by their apparent digestibilities (Merrill & Watt, 1973).

Partial digestible energy values of supplements (DE_s) were determined from the change in the balance of gross energy intake and gross faecal energy losses with increasing intake of the supplement (Kleiber, 1975). DE_s were calculated according to a formula which has small inherent errors and is preferred to some more commonly used methods of calculation (see Livesey, 1989*b*).

$$DE_s = \Delta H_s - ([(E_{tf}/M_{td}) - (E_{cf}/M_{cd})]/(M_s/M_{td})),$$

where ΔH_s is the heat of combustion of the supplement (kJ/g dry matter), E_{tf} and E_{cf} are the energies lost to faeces on diets with (test diet) and without (control diet) the supplement respectively, and M_{td}, M_{cd} and M_s are respectively the masses of basal diet eaten with the test and control diet and the mass of supplement eaten. All mass values were corrected for moisture.

Calculation of fat and lean dry mass deposition

The accumulation of body fat and dry lean mass in individual animals during the 28–29 d balance period was assessed from the differences in these quantities at the beginning and end of the balance period. Direct analysis of these quantities was possible for the end of the balance period. The initial composition of the experimental animals was derived by linear regression analysis from the initial live weight and the body composition of those animals killed at the beginning of the balance period. The accumulation of fat and of dry lean mass was expressed in terms of energy, taking 1 g fat to contain 39·5 kJ and 1 g dry lean matter to contain 20 kJ (equivalent to 24 kJ/g protein, 0 kJ/g ash; average 150 g ash/kg). The 20 kJ/g dry lean matter had been established by direct determination in a bomb calorimeter of samples from two whole bodies for each dietary group.

Additional calculations

Digestible energy intake is the difference between gross energy intake and loss to the faeces. Net energy deposited is the sum of the energies deposited as fat and dry lean mass. A combined value for energy expenditure and energy lost to urine is given as the difference between digestible energy intake and net energy deposited.

Changes in net energy deposition, in fat and lean mass deposition and in energy expenditure (+losses to urine) due to the supplements are the differences between these components with the unsupplemented diet (B) and the supplemented diets (Su, SFC, RCS and RPS). These values are expressed per unit weight of supplement ingested (kJ/g dry weight). The changes in energy expenditure (+loss to urine), expressed per unit weight of supplement, were calculated as the difference between the partial digestible energy values (kJ/g) and the net energy value (kJ/g).

Statistics

Analysis of variance was used. When the variate was expected to be time dependent (e.g. food and energy intake, energy accumulations and expenditure) two-way analysis was performed using the 28–29 d distinction as a blocking factor. Accumulation of body fat and energy and the estimate of energy expenditure (plus loss to urine) covaried with live weight at the start of the balance period, so analysis of covariance was used. The degrees of freedom (df) indicate the method of analysis, with fifty animals and five dietary treatments: 45 df with one-way analysis of variance, 44 df with two-way analysis of variance and 43 df with analysis of covariance. Least significant differences (LSD) between dietary treatments are at the $P < 0.05$ level. With digestibility values, the analysis of variance was performed on untransformed values unless mean values were close to a whole proportion when analysis was on the angular transformation, arc sine $\sqrt{x}$, appropriate for proportionate data to equalize variances. Unpaired Student's t test were used to compare apparent digestible energy with partial digestible energy values for each supplement. All statistical methods are those described by Mead & Curnow (1983).

RESULTS

Live-weight gains

Supplementing the basal diet with Su, RMS or RPS during the balance period significantly increased live-weight gain, whereas SFC had no effect (Table 2). During the 13 d of prefeeding with the control diet (B), there were no significant differences among dietary groups in live-weight gains.

Food intake and faecal bulking with dry matter

As planned, there were no statistically significant differences in the intake of fresh basal diet (Table 3). The moisture content of the diets given was low, as expected for a mainly Su diet. Dry matter loss to faeces was not affected by Su (Table 3). This loss was elevated ($P < 0.01$)

Table 2. *Influence of the dietary supplements on live weights and live-weight gains (g) in rats*

(Mean values)

Dietary treatment*	Live wt Day −14	Live wt Day 0	Live wt Days 28–29	Live-wt gains† During prebalance period	Live-wt gains† During balance period
B	98[a]	181[a]	257[a]	83[a]	76[a]
Su	101[a]	191[a]	283[b]	90[a]	91[b]
SFC	102[a]	188[a]	271[c]	85[a]	82[a]
RMS	93[a]	177[b]	282[bc]	85[a]	104[c]
RPS	102[a]	188[a]	284[b]	85[a]	96[bc]
LSD	9	13	13	10	9
(df)	(45)	(45)	(45)	(45)	(44)

B, basal diet; Su, B+sucrose; SFC, B+Solka-floc cellulose; RMS, B+resistant maize starch; RPS, B+resistant pea (*Pisum sativum*) starch; LSD, least significant difference between treatments in vertical columns at $P < 0.05$.

[a–c] Mean values in a vertical column with different superscript letters were significantly different ($P < 0.05$).

* For details, see p. 469.

† No significant covariation with live weights at the beginning of each feeding period.

four times with SFC and 1·3 and 1·9 times with RMS and RPS respectively. Dry matter lost to faeces expressed as a percentage of dry weight of supplements Su, SFC, RMS and RPS ingested were 0, 97, 9 and 27 respectively (Table 3; faecal bulking).

Energy intake, faecal energy losses and the digestible energy values of the supplements

Supplementing the basal diet with Su, SFC, RMS and RPS increased the determined gross energy intake by 9·2, 8·9, 9·6 and 9·0% respectively (Table 3). With Su this increased gross energy intake was without effect on faecal energy losses, whereas the losses were increased 4·0-, 1·4- and 2·1-fold with SFC, RMS and RPS respectively. The calculated partial digestible energy values for the resistant starches (15·3 kJ/g RMS and 12·4 kJ/g RPS; Table 3) were significantly less than that for Su (16·5 kJ/g) and much higher than that for SFC (0·8 kJ/g) (Table 3). The resistant starches, therefore, added substantial amounts to digestible energy intake.

For SFC the additional dry matter in faeces was calculated to have a heat of combustion of 17·1 kJ/g (Table 3), similar to the determined heat of combustion for the SFC supplement of 17·3 kJ/g. This similarity is consistent with the additional faecal dry matter, due to SFC supplementation, being mostly carbohydrate. With RMS and RPS the calculated heats of combustion of the additional faecal dry matter were 23·7 and 20·6 kJ/g respectively, both significantly higher ($P < 0.01$) than the determined heats of combustion for these supplements, 17·5 and 17·4 kJ/g respectively. These additional energy losses to faeces, therefore, seem to include material additional to carbohydrate.

Apparent digestibility of nutrients and the apparent digestible energy value of the supplements

A decrease in the apparent digestibility of dietary gross energy was observed with SFC, RMS and RPS; this was accompanied, though not paralleled, by a decrease in the apparent digestibility of N and, to a lesser extent, fat (Table 4). Su was without effect on any of these variables. The apparent digestibilities of the supplements determined from the chemical

Table 3. *Food intake and faecal bulking during the balance period of 28–29 d and the partial digestible energy values of supplements, and associated variables in rats*

(Mean values)

Dietary treatment*	Intake of basal diet (g dry wt)	Intake of supplement (g dry wt)	Dietary moisture (g/kg)	Faecal dry matter (g)	Faecal bulking (g/g dry supplement)	Gross energy intake (kJ)	Faecal energy loss (kJ)	Partial digestible energy value of supplement (kJ/g)†	Apparent heat of combustion of additional faeces (kJ/g)	Heat of combustion of the supplement (kJ/g)
B	336[a]	0[a]	2·0	10·7[a]	—	6306[a]	176[a]	—	—	—
Su	337[a]	34·4[b]	1·8	10·8[a]	0·0[a]	6889[b]	178[a]	16·5[a]	—	16·5[a]
SFC	336[a]	32·6[c]	2·0	42·3[b]	0·97[b]	6871[b]	712[b]	0·8[b]	17·1[a]	17·3[b]
RMS	337[a]	34·5[b]	2·2	14·1[c]	0·09[c]	6922[b]	251[c]	15·3[c]	23·7[b]	17·5[b]
RPS	335[a]	34·4[b]	2·7	20·0[d]	0·27[d]	6875[b]	364[d]	12·4[d]	20·6[b]	17·4[b]
LSD	8	0·8	—	1·6	0·06	143	29	0·9	3·0	0·3
(df)	(44)	(44)	—	(44)	(45)	(44)	(44)	(45)	(45)	(3)

B, basal diet; Su, B+sucrose; SFC, B+Solka-floc cellulose; RMS, B+resistant maize starch; RPS, B+resistant pea (*Pisum sativum*) starch; LSD, the least significant difference between dietary treatments at $P < 0·05$.

[a–d] Mean values in a column with different superscript letters were significantly different ($P < 0·05$).

* For details, see p. 469.

† Calculated from the balance of energy intake and loss to faeces by formula, see p. 470.

Table 4. *Influence of supplements on the apparent digestibilities of energy, nitrogen and fat, the apparent digestibility of supplements, and the apparent and partial digestible energy values of the supplements in rats*

(Mean values)

Dietary treatment†	Apparent digestibility of gross energy‡	Apparent digestibility of nitrogen‡	Apparent digestibility of fat‡	Apparent digestibility of supplement‡¶		Apparent digestible energy value of supplement§ (kJ/g dry wt)	Partial digestible energy value of supplement‖ (kJ/g dry wt)
				Means	Radians		
B	0·973[a]	0·941[a]	0·936[a]	—	—	—	—
Su	0·974[a]	0·941[a]	0·935[a]	1·000[a]	1·53	16·5[a]	16·5[a]
SFC	0·896[b]	0·925[b]	0·926[b]	0·162[b]	0·40	2·8[b]	0·8[b]*
RMS	0·964[c]	0·915[c]	0·926[b]	0·983[c]	1·44	17·2[c]	15·3[c]*
RPS	0·947[d]	0·890[d]	0·927[b]	0·890[d]	1·24	15·6[d]	12·4[d]*
LSD (45 df)	0·004	0·006	0·008	—	0·06	0·6	0·9

B, basal diet; Su, B + sucrose; SFC, B + Solka-floc cellulose, RMS, B + resistant maize starch; RPS, B + resistant pea (*Pisum sativum*) starch; LSD, least significant difference at $P < 0.05$.

[a-d] Mean values in a vertical column with different superscript letters were significantly different ($P < 0.05$).

* Significant difference (Student's t test) between apparent and partial digestible energy values: $P < 0.05$.

† For details, see p. 469.

‡ Calculated from the balance of intake (I) and faecal loss (F) and equals $(I-F)/I$ (see p. 470).

§ Calculated from the balance of intake and faecal loss of supplement and equals $\Delta H_s \times (I-F)/I$ where ΔH_s is the heat of combustion of the supplement (see p. 470).

‖ Calculated from the change in gross energy intake (ΔIE) and faecal energy loss (ΔFE) and is equivalent to $\Delta H_s \times (\Delta IE - \Delta FE)/\Delta IE$ but calculated more accurately by an alternative formula (see p. 470).

¶ Values for apparent digestibility of the supplements were transformed to the angular form (arc sine $\sqrt{x}$) to equalize variances, the need for which increases as values approach unity for comparison between dietary supplements.

analysis of food and faeces (Table 4) indicated Su was completely utilized, whereas more than 80% of SFC appeared in the faeces. Statistically significant amounts of RMS (2% of intake) and RPS (11% of intake) also appeared in the faeces. The apparent digestible energy value for each complex carbohydrate was significantly ($P < 0.05$, unpaired Student's t test) higher than the corresponding partial digestible energy value (Table 4). This is consistent with the additional losses to the faeces of protein, and to a lesser extent fat, on dietary supplementation with these materials.

Body composition

By the end of the balance period the mean values (Table 5) for whole body (excluding digesta) wet weight, dry weight and dry lean mass were higher ($P < 0.05$) in animals fed on diets supplemented with Su, RMS or RPS than in animals fed on the unsupplemented, basal diet (B). The absolute weight of water was also higher in animals fed on these supplements though the difference was significant only with the resistant starches. There were no significant differences among any dietary groups when water was expressed relative to wet body-weight.

Table 5 shows that animals fed on supplements providing substantial additional digestible energy (Su, RMS, RPS; Tables 3 and 4) had more body fat, in absolute terms, compared both with animals fed on SFC, which provided little digestible energy (Tables 3 and 4), and with animals fed on diet B which was unsupplemented. Similar observations were made when fat was expressed as a proportion of dry lean matter (Table 5). The dry weight of the epididymal fat pads tended to be higher for Su, RMS and RPS than for SFC or diet B alone, the effect being statistically significant for RPS.

Energy and nutrient deposition

The deposition of energy and fat, and the expenditure of energy (+losses to urine) each covaried significantly with live body-weight at the start of the balance period; by contrast, lean tissue deposition showed no covariance. The coefficients of covariance, after accounting for treatment effects, were −14, −13, +17 and −1 kJ/g live weight respectively (Table 6). This shows, not unexpectedly, that on the fixed amounts of food provided, the larger animals expended more energy than the smaller animals in the dietary groups and that the energy deposited in the body as fat was consequently less in the larger animals than in the smaller animals. The means and LSD in Table 6 for energy and fat deposition, and energy expenditure are values obtained after accounting for these covariances.

The additional digestible energy intake due to supplementing with Su, RMS and RPS (Table 6) was associated with more fat and more energy deposition than with SFC and diet B.

Lean dry mass deposition was higher for the supplemented diets than for diet B, and was significantly higher with RCS than with the other supplements.

Energy provided in the diets but not recovered in the faeces or in the body was designated 'energy expenditure plus losses to urine' (Table 6). This value was significantly elevated by Su and RMS compared with diet B alone whereas SFC and RPS were without statistically significant effect.

It is useful to partition the supplementary energy from feeding these food materials into fractions of the supplementary intake which appear to be used, deposited and expended. These are expressed in Table 6 in terms of kJ/g supplement eaten. It appears that with Su about 36% of the additional digestible energy is expended, the remainder being deposited in the body, mostly as fat. With SFC little additional digestible energy was provided and there were no effects on energy deposition and expenditure. RMS, which provided 93% as

Table 5. *Empty body composition of rats fed on the basal and supplemented diets for 28–29 d*

(Mean values)

Dietary treatment*	Wet wt (g)	Dry wt (g)	Water (g)	Water (g/g wet wt)	Dry lean mass (g)	Fat (g)	Fat (g/g dry wt lean mass)	Fat pads† (g)
B	254^{a}	91^{a}	163^{a}	0·64^{a}	62^{a}	28^{a}	0·45^{a}	2·2^{a}
Su	277^{bc}	101^{b}	175^{b}	0·63^{a}	67^{b}	34^{b}	0·51^{b}	2·5^{ab}
SFC	267^{b}	93^{a}	173^{ab}	0·65^{a}	66^{ab}	27^{a}	0·41^{a}	2·2^{a}
RMS	279^{c}	99^{b}	179^{b}	0·64^{a}	67^{b}	32^{ab}	0·48^{c}	2·4^{ab}
RPS	280^{bc}	102^{b}	178^{b}	0·64^{a}	67^{b}	35^{b}	0·52^{bc}	2·8^{b}
LSD	11	4	12	0·03	5	4	0·02	0·4
(df)	(44)	(44)	(44)	(45)	(44)	(44)	(45)	(44)

B, basal diet; Su, B + sucrose; SFC, B + Solka-floc cellulose; RMS, B + resistant maize starch; RPS, B + resistant pea (*Pisum sativum*) starch; LSD, the least significant difference at $P < 0·05$.

$^{a-c}$ Mean values in a column with different superscript letters were significantly different ($P < 0·05$).

* For details, see p. 469.

† Sum for both epididymal fat pads.

Table 6. *Energy intake, deposition and expenditure in rats fed on the basal and supplemented diets over the balance period of 28–29 d*

(Mean values)

Dietary treatment*	Digestible energy intake (kJ)	Energy deposition (kJ)	Fat deposition (kJ)	Lean tissue deposition (kJ)	Energy expenditure and losses to urine (kJ)	Partial digestible energy value (kJ/g dry supplement)	Change in energy deposited (net energy value) (kJ/g dry supplement)	Change in fat deposition (kJ/g dry supplement)	Change in lean tissue deposition (kJ/g dry supplement)	Change in energy expenditure (+losses to urine) (kJ/g dry supplement)
B	6134^{a}	722^{a}	229^{a}	504^{a}	5388^{a}	—	—	—	—	—
Su	6711^{b}	1084^{b}	523^{b}	554^{b}	5603^{b}	16·5^{a}	10·5^{a}	8·5^{a}	1·4^{a}	6·0^{a}
SFC	6158^{a}	786^{a}	235^{a}	547^{b}	5359^{a}	0·8^{b}	2·0^{b}	0·2^{b}	1·3^{a}	−1·2^{b}
RMS	6671^{b}	995^{c}	388^{c}	617^{c}	5698^{c}	15·3^{c}	7·9^{c}	4·6^{c}	3·2^{b}	7·4^{a}
RPS	6511^{d}	1138^{b}	577^{b}	562^{b}	5383^{a}	12·4^{d}	12·1^{a}	10·1^{a}	1·7^{a}	0·3^{b}
LSD	51	80	97	34	87	0·9	2·3	2·8	1·0	2·5
(df)	(44)	(43)	(43)	(44)	(43)	(45)	(43)	(43)	(44)	(43)
Coeff. cov. (kJ/g live wt)	—	−14	−13	−1	+17	—	—	—	—	—
F ratio†	—	44	26	NS	52	—	—	—	—	—

B, basal diet; Su, B+sucrose; SFC, B+Solka-floc cellulose; RMS, B+resistant maize starch; RPS, B+resistant pea (*Pisum sativum*) starch; LSD, least significant difference between dietary treatments at $P \leqslant 0{\cdot}05$; Coeff. cov., the coefficient of the covariance, i.e. the slope of the regression line for residual difference from means for the variate of interest, after correction for treatment effects, plotted *v.* residual differences of live weight of animals at the start of the balance period.

[a-d] Mean values in a column with different superscript letters were significantly different ($P < 0{\cdot}05$).

* For details, see p. 469.

† *F* ratios > 7 indicate significant covariance.

much partial digestible energy as the supplementary Su, also significantly elevated energy expenditure. However, as much energy appeared to be deposited within the fat and lean tissue together as appeared to be expended. With RPS, which provided 75% as much partial digestible energy as Su, most appeared to be deposited as fat.

DISCUSSION

When attempting to partition small increases in gross energy intake into energy losses, deposition and expenditure, it is necessary to perform experiments meticulously to obtain accurate and precise information. The experimental design adopted usually permits a coefficient of variation for the estimation of apparent digestibility of dietary gross energy of about 0·5% (Livesey & Davies, 1988). In this work, values were 0·3, 0·2, 0·5, 0·3 and 0·8 for diets B, Su, SFC, RMS and RPS respectively. This enabled precise estimates to be made for the partial digestible energy values of these supplements. However, high precision does not mean that accuracy has been achieved. To help achieve this, use was made of a calculation procedure for partial digestible energy values which involves only small magnification of measurement errors (Livesey, 1989*b*). Consistent with the attainment of accurate values are the expected high value for Su (16·5 kJ/g, equal to 100 (SE 1) % of its heat of combustion) and the low value for SFC (0·8 kJ/g, equal to 5 (SE 2) % of its heat of combustion). The latter energy value is expected to fall between 0 and 2 kJ/g from our previous studies in the rat (Davies *et al.* 1987; Harley *et al.* 1989). A value of −0·7 kJ/g for the partial digestible energy value of SFC in man has been estimated (Harley *et al.* 1989), which is similar to that obtained in the present study with the rat.

The present energy balance values and energy values need putting further into context; they are representative of the whole 28–29 d balance period. We showed previously that during this time the appearance of material in faeces was delayed, partly at least due to time taken to traverse the alimentary tract, and some adaptation to utilization of SFC, RPS and RMS also occurs (Faulks *et al.* 1989). However, all this is virtually complete within the first 3 d, i.e. within the first 10% of the balance period, for SFC and RMS, and a slow continuing adaptation occurs beyond that time for RPS. Hence, while accurate and precise digestible energy values are given for these materials over the 28–29 d balance period, it is considered that for SFC the balance of large bowel retention and time to adaptation resulted in a value which is a marginal overestimate (due to retention), and that for RMS and RPS the values are marginal underestimates of what would have been expected for more fully adapted animals.

While both RMS and RPS decreased the digestibility of protein and, to a lesser extent, fat (Table 4), the effects are quantitatively small by comparison with certain observations made with fibre and whole foods in humans (Southgate & Durnin, 1970; Kelsay *et al.* 1978; Judd, 1982). The effects of these resistant starches on the digestibility of protein (N) and fat are, however, still quantitatively significant, making the partial digestible energy values less than the apparent digestible energy values. However, it appears that the partial digestible energy value of fibre in conventional foods is not an absolute value and depends on its contribution to gross energy intake (Livesey, 1990). Values as high as approximately 10 kJ/g (2·4 kcal/g) have been calculated when fibre contributes 10–14% of gross energy in human diets (Livesey, 1990). The supply of additional energy from RMS and RPS (partial digestible energy values of 15 and 12·4 kJ/g respectively) is still higher than ever observed for fibre in humans, though equivalent to the highest values obtained for fibre isolates; for example with the soluble fibres, guar gum (10 kJ/g; Davies *et al.* 1987) and gum arabic (14 kJ/g; Harley *et al.* 1989) at similar levels in the diet of rats. Whether with resistant starches, or even with non-starch polysaccharides used as supplements, the partial digestible energy value is dependent on its level of incorporation remains to be investigated.

Whereas Su increased fat and energy deposition, SFC had no effect. Each resistant starch behaved more like Su than cellulose with respect to energy retention in the body (Table 6). These observations confirm a supply of the digestible energy from Su, RMS and RPS (Table 4) and suggest a lack of any pharmacological effect to decrease fat deposition, as observed with guar gum in one experiment (Davies *et al.* 1987). Thus, energy from these resistant starches was conserved very efficiently.

Examination of the LSD in Table 6 shows that variation in energy deposition is mostly due to variation in fat rather than lean tissue deposition (ratio 3:1). Moreover, fat, not lean tissue deposition covaried with live weights of these animals at the start of the balance period (Table 6). It was changes in fat deposition more than changes in lean tissue deposition that generally accounted for the difference in energy retained in the body among the supplement treatments. It is possible that the lower fat deposition in RMS-fed compared with Su- and RPS-fed animals (Table 6) was due to the higher value for lean tissue gain (Table 6). Whether the last effect, which was significant, was really due to RCS supplementation needs confirmation.

That dietary fibre may aid weight loss in subjects is now commonly considered but unproven (Health and Welfare Canada, 1986; FASEB, 1987). This problem arises in discussions about the dietary fibre hypothesis (Cleave, 1974; Burkitt & Trowell, 1975) as it applies to obesity in Western populations. The link between the abundance of fibre or absence of Su and the relative absence of obesity may depend more on the presence of starch and the relative absence of dietary fat (Southgate, 1987). However, a possible contributory role of resistant starch needs consideration. It is concluded that α-amylase-resistant starches RMS and RPS, when given as supplements, each enhance growth and energy deposition in the rat to an extent almost similar to Su. Their apparent digestibility and partial digestible energy values are relatively high and they cause only relatively small losses to faeces of protein and fat by comparison with dietary fibre in some human studies. Therefore, the present observations do not support the suggestion that resistant starch, rather than refined Su, has a preventive or other role in the development of obesity.

REFERENCES

Berry, C. S. (1986). Resistant starch: formation and measurement of maize starch that survives exhaustive digestion with amylolytic enzymes during determination of dietary fibre. *Journal of Cereal Science* **4**, 301–314.

Bjorck, I., Nyman, M., Pedersen, B., Siljestrom, M., Asp, N. G. & Eggum, B. D. (1986). On the digestibility of starch in wheat bread-studies *in vivo*. *Journal of Cereal Science* **4**, 1–11.

Burkitt, D. P. & Trowell, A. C. (1975). *Refined Carbohydrate Foods and Disease, Some Implications of Dietary Fibre*. London: Academic Press.

Cleave, T. C. (1974). *The Saccharine Disease*. Bristol: John Wright and Sons Ltd.

Collinson, R. (1968). Swelling and gelatinisation of starch. In *Starch and its Derivatives*, pp. 194–201 [J. A. Radley, editor]. London: Chapman & Hall.

Davies, I. R., Johnson, I. T. & Livesey, G. (1987). Food energy values of dietary fibre components and decreased deposition of body fat. *International Journal of Obesity* **11**, Suppl. 1, 101–105.

Englyst, H. N. & Cummings, J. H. (1985). Digestion of the polysaccharides of some cereal foods in the human small intestine. *American Journal of Clinical Nutrition* **42**, 778–787.

Englyst, H. N. & MacFarlane, G. T. (1986). Breakdown of resistant starch and readily digestible starch by human gut bacteria. *Journal of the Science of Food and Agriculture* **37**, 699–706.

Englyst, H. N., Trowell, H., Southgate, D. A. T. & Cummings, J. H. (1987). Dietary fibre and resistant starch. *American Journal of Clinical Nutrition* **46**, 873–876.

Englyst, H. N., Wiggins, H. S. & Cummings, J. H. (1982). Determination of the non-starch polysaccharides in plant foods by gas-liquid chromatography of the constituent sugars as alditol acetates. *Analyst* **107**, 307–318.

Faulks, R. M., Southon, S. & Livesey, G. (1989). Utilization of α-amylase (*EC* 3.2.1.1)-resistant maize and pea (*Pisum sativum*) starch in the rat. *British Journal of Nutrition* **61**, 291–300.

Federation of American Societies for Experimental Biology (1987). *Physiological Effects and Health consequences of Dietary Fiber* [S. M. Pilch, editor]. Maryland, USA: Life Science Research Office.

Harley, L. J., Davies, I. R. & Livesey, G. (1989). Digestible energy value of gums in the rat – data on gum arabic. *Food Additives and Contaminants* **6**, 13–20.

Health and Welfare Canada (1986). *Report of the Expert Advisory Committee on Dietary Fibre*. Canada: Department of Health.

Judd, P. A. (1982). The effects of high intakes of barley on gastro-intestinal function and apparent digestibilities of dry matter, nitrogen and fat in human volunteers. *Journal of Plant Foods* **4**, 79–88.

Kelsay, J. L., Behall, K. M. & Prather, E. S. (1978). Effect of fibre from fruit and vegetables on metabolic responses of human subjects. I. Bowel transit time, number of defactions, faecal weight, urinary excretion of energy and nitrogen and apparent digestibilities of energy, nitrogen and fat. *American Journal of Clinical Nutrition* **31**, 1149–1153.

Kleiber, M. (1975). *The Fire of Life: An Introduction to Animal Energetics*, pp. 259–271. Huntington, New York: Robert E. Krieger Publishing Co.

Livesey, G. (1989*a*). Complex carbohydrates and energy, In *Nutrient Availability: Chemical and Biological Aspects*, pp. 385–387 [R. Fenwick, I. T. Johnson and D. A. T. Southgate, editors]. Cambridge: Royal Society of Chemistry.

Livesey, G. (1989b). Procedures for calculating the digestible and metabolizable energy value of food components making a small contribution to dietary intake. *Journal of the Science of Food and Agriculture* **48**, 475–481.

Livesey, G. (1990). Food energy values of unavailable carbohydrates and diets; an enquiry and analysis. *American Journal of Clinical Nutrition* **51**, 617–637.

Livesey, G. & Davies, I. R. (1988). Caloric value of fibre and guar gum. In *Low-calorie Products*, pp. 223–244 [G. Birch and M. Lindley, editors]. London: Applied Science Publishers.

Mead, R. & Curnow, R. N. (1983). *Statistical Methods in Agriculture and Experimental Biology*. London and New York: Chapman & Hall.

Merrill, A. L. & Watt, B. K. (1973). Energy values of foods: basis and derivation. *Agriculture Handbook* no. 74. Washington DC: United States Department of Agriculture.

Miles, M. J., Morris, V. J. & Ring, S. G. (1985). The roles of amylose and amylo-pectin in the gelation and retrogradation of starch. *Carbohydrate Research* **135**, 257–69.

Nyman, M., Asp, N. G., Cummings, J. & Wiggins, H. (1986). Fermentation of dietary fibre in the intestinal tract: comparison between man and rat. *British Journal of Nutrition* **55**, 487–496.

Paul, A. A. & Southgate, D. A. T. (1978). *McCance & Widdowson's The Composition of Foods*. London: H.M. Stationery Office.

Ring, S. G., Gee, J. M., Whittam, M., Orford, P. & Johnson, I. T. (1988). Resistant starch: its chemical form in food stuffs and effect on digestibility *in vitro*. *Food Chemistry* **28**, 97–109.

Southgate, D. A. T. (1987). Minerals, trace elements and potential hazards. *American Journal of Clinical Nutrition* **45**, 1256–1266.

Southgate, D. A. T. & Durnin, J. V. G. A. (1970). Calorie conversion factors: an experimental reassessment of the factors used in the calculation of the energy values of human diets. *British Journal of Nutrition* **24**, 517–535.

Stevens, J., Levitsky, D. A., Van Soest, P. J., Robertson, J. B., Kalkwarf, H. L. & Roe, D. A. (1987) Effect of psyllium gum and wheat bran on spontaneous energy intake. *American Journal of Clinical Nutrition* **46**, 812–817.

Wyatt, G. & Horn, N. (1988). Fermentation of resistant food starches by human and rat intestinal bacteria. *Journal of the Science of Food and Agriculture* **44**, 981–988.

Printed in Great Britain