British Journal of Nutrition (1988), **60**, 605–617

Importance of body tissues as sources of nutrients for milk synthesis in the cow, using ^{13}C as a marker

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(*Received 11 January 1988 – Accepted 23 June 1988*)

1. The proportions of carbon in individual milk constituents derived from feed and body tissues in dairy cows, were estimated by taking advantage of the natural variations which occur in the ratio, $^{13}C:^{12}C$ present in C_3- and C_4-plant species.

2. Four cows, which had previously grazed C_3 plants (ryegrass (*Lolium* spp.) and white clover (*Trifolium repens*)), were accustomed to indoor feeding on a ration of C_3-plant material (cut pastures and barley meal). The ration was then changed abruptly to one of C_4-plant material (paspalum (*Paspalum dilatatum*) hay, maize silage and meal) for a period of 8 or 9 d in early and again in late lactation.

3. During early lactation it was estimated that 54% of the C in milk fat was derived from the body fat reserves of high genetic merit cows. Corresponding values for casein and lactose were 34 and 24% respectively, if it is assumed they were derived from body protein reserves. In contrast steam-volatile fatty acids in milk fat were almost entirely derived from dietary sources.

4. The proportional contribution of body-tissue C to individual milk constituents varied considerably between animals, possibly associated with genetic merit or the size of the body reserves available for mobilization.

5. In late lactation, when cows were close to energy and protein balance, contributions of body-tissue C to milk fat, casein and lactose ranged up to 19, 19 and 8% respectively.

6. Estimates of endogenous losses of C in faeces averaged 12 and 9% in early and late lactation respectively, and corresponding values for endogenous urinary C were 23 and 15%.

An important attribute of the high-producing dairy cow, particularly in early lactation, is its ability to draw on body reserves to support milk production when the intake of nutrients is insufficient to meet requirements. Cows in moderate condition at calving have been shown to produce at a higher level than those which are thin (e.g. Holmes *et al.* 1985), due presumably to a greater contribution of body-tissue reserves to milk production. In addition, cows of high, as compared with low, genetic merit not only mobilize more body tissue in early lactation but subsequently partition more dietary energy into milk production and less into live-weight gain (Grainger *et al.* 1985). While these differences may be related to observed differences in blood metabolites and hormones (Flux *et al.* 1984), much of the physiology involved remains unclear, in part because small changes in body-weight or tissue composition are difficult to measure in the live animal. Hence quantitative information is required on the extent and composition of changes in body tissue during lactation and the relative importance of diet and mobilized tissue as sources of energy or essential nutrients for the synthesis of milk.

An experimental approach to the problem was suggested by D. J. Minson (personal communication) and emanated from the work of Minson *et al.* (1975). They demonstrated that $^{13}C:^{12}C$ in cattle products reflected the ratio of the stable isotopes in the feed eaten. Thus hair and milk from cows fed on plants which fix carbon dioxide by the Calvin (C_3) pathway, had lower $^{13}C:^{12}C$ than did the same products from cows fed on plants which utilize the dicarboxylic acid or C_4 pathway. Other workers (e.g. DeNiro & Epstein, 1978; van der Merwe, 1982) have shown that the isotopic composition of the whole body of an animal is similar to that of its diet.

Minson *et al.* (1975) suggested that if cows which had previously grazed only temperate (C_3) species were suddenly changed to a diet comprising C_4 plants, the origin of C (feed *v* body tissues) could be estimated by measurements of the isotope concentrations in milk. A $^{13}C:^{12}C$ value identical to that for C_4 plants would show that all the C was derived directly from the diet, whereas a value similar to that for C_3 plants would imply that all the C was obtained from body tissues. Intermediate values for $^{13}C:^{12}C$ would indicate that C was obtained from both sources and the relative contributions could be calculated from simple proportions. It was therefore decided to investigate the potential of this approach as a means of identifying the quantitative importance of those nutrients used for the synthesis of milk which are derived from body tissues. The experiment also allowed estimation of endogenous losses of C in urine and faeces to be obtained for lactating cows at normal feeding levels. In order to assess the sensitivity of the technique, measurements were made at two stages of lactation on cows of two extremes in genetic merit, which would be expected to provide the physiological extremes of body tissue mobilization.

MATERIALS AND METHODS

Animals and management

Four mature Friesian cows, two of high genetic merit (HBI) for production of milk fat (breeding index 126 and 127) and two of lower genetic merit (LBI; breeding index 105 and 107), which had calved in late August were used. They were grazed on C_3 pastures (predominantly ryegrasses (*Lolium* spp.) and white clover (*Trifolium repens*) except for two 3-week periods of stall-feeding commencing in weeks 5 and 35 of lactation.

Each stall-feeding period consisted of two 'feeding' periods. During the first 10–14 d the cows were fed twice daily on a C_3 diet comprising pasture and pelleted barley (*Hordeum vulgare*) meal in an approximately 3:1 (w/w; dry weight basis) mixture. Cows were then fasted for 24 h to encourage clearance of C_3 food residues and then transferred abruptly to a C_4 diet consisting of pelleted maize (*Zea mays*; 4–6 kg) meal, and maize silage (1 kg dry matter (DM)) together with paspalum (*Paspalum dilatatum*) hay *ad lib.*, for a further period of 8 or 9 d.

During the last 2 d of each of the stall-feeding periods the cows were fitted with harnesses which allowed the separate collection of total urine and faeces production. Cows were machine milked twice daily either at 06.00 and 15.30 hours or 08.30 and 16.30 hours; the latter regimen being used when the harnesses were fitted.

Sampling and analysis

Feeds and excreta. Samples of all the feeds used during the two experimental periods were oven-dried (80°) in order that the intakes of individual cows could be measured. Representative daily samples of faeces and urine were obtained during C_4 feeding and total collections made over the last 2 d. Faeces were oven-dried and urine samples analysed fresh or freeze-dried. The dried samples were ground (1 mm sieve), thoroughly mixed, and analysed for energy and nitrogen concentrations (for methods, see Grainger *et al.* 1985) so that balances could be estimated.

Samples of the feeds consumed and selected faecal samples were used to produce a neutral-detergent fibre (NDF) fraction (Goering & Van Soest, 1970) and also an in vitro 'indigestible fibre' fraction by digesting some of the NDF fraction with cellulase (Pfizer, I_2) for 24 h (Roughan & Holland, 1977).

Milk samples. Milk yields were measured for individual cows and composite daily samples analysed for fat, protein and lactose concentrations (Milkoscan; Foss). Skim milk and cream were prepared from 50 ml milk by centrifugation at 4° for 20 min at 1000 ***g***.

Fat. Fat was extracted from the cream by mixing 1 vol. cream with 1 vol. water and adding 2 vol. 15 M-sulphuric acid. The mixture was allowed to stand and separate into two phases. The aqueous phase was discarded following aspiration and the fat phase washed twice with hot distilled water. The fatty acid composition of milk fats produced by individual cows at the end of the C_4 feeding periods was analysed by gas–liquid chromatography.

Steam-volatile fatty acids: Approximately 1 g milk fat was weighed and then gently boiled for 1 h in a Buchii digestor with 20 ml 0·1 M-sodium hydroxide to saponify the triglycerides. The mixture was cooled and acidified with 25 ml 1 M-hydrochloric acid and the steam-volatile fatty acids collected by distillation in a Buchii apparatus. The distillate was titrated with 0·1 M-NaOH to pH 7·5. The sodium salts were weighed following evaporation of the water in an oven at 90°. The recovery of steam-volatile fatty acids, butyric and hexanoate, would be expected with this procedure and the fatty acid composition of the salts in one sample was C_4, 0·69; C_6, 0·25; and C_8, 0·02 on a molar basis.

Casein. Casein was precipitated by titrating 150 ml skim milk to pH 4·6 with 0·1 M-HCl. After centrifugation the whey was decanted and retained, and the casein was washed twice with approximately 100 ml distilled water (pH 4·6). The washed casein was dried in an oven at 90°.

Lactose. Approximately 75 ml acid–whey was boiled for 1 h to precipitate the whey proteins which were removed by centrifugation. The clarified whey was evaporated to 3 ml and lactose allowed to crystallize out over 48 h at 4°. The supernatant fraction was decanted following centrifugation and the crystals recovered and dried at 90°.

^{13}C:^{12}C. Subsamples (3–5 mg) of most of the animal or plant tissues collected, and especially those obtained during the last 2–3 d of each feeding period, were analysed for the natural isotope ratio, ^{13}C:^{12}C present (for methods, see Rounick *et al.* 1982). Results are expressed as a difference ($\delta^{13}C$) from the ratio for the number of atoms of ^{13}C:the number of atoms of ^{12}C for a carbonate standard (Belemnite; Pee Dee) where:

$$\delta^{13}C‰ = \frac{^{13}C:^{12}C \text{ of sample} - ^{13}C:^{12}C \text{ of standard}}{^{13}C:^{12}C \text{ of standard}} \times 1000.$$

In these terms, C_4 plants have mean $\delta^{13}C$ values of $-12·5$ and C_3 plants, which discriminate against ^{13}C, a mean value of $-26·5$ (van der Merwe, 1982). Duplicates of individual samples differed by less than 0·2 ‰.

Body tissue precursors for milk synthesis

The $\delta^{13}C$ values in milk during C_4 feeding depend on the proportional contributions obtained from body tissues (C_3 label) and diet (C_4 label). Hence estimates of the proportion (x) of C in individual milk components derived from body tissues were calculated, after rearranging and solving for x in the following equation:

$$M_4 = xR_3 + (1-x)D_4,$$

where M_4, D_4 and R_3 are the $\delta^{13}C$ values for the milk component, the diet and body tissue reserves respectively. The values used for R_3 were those obtained from subcutaneous body fat ($-30·1$) and 'fat-free' muscle ($-25·4$) obtained following diethyl ether extraction of the appropriate tissue samples taken from M. longissimus (region of last rib) from two 15-month-old Friesian bulls fed on C_3 pastures. Differences in $\delta^{13}C$ values between the same tissues from the two bulls were negligible.

Endogenous faecal and urinary losses

The average proportion of C in the faeces of the cows which was derived from body tissues was estimated from the $\delta^{13}C$ value of the faeces obtained during the C_4 period (F_4) by making the following assumptions: (1) that the $\delta^{13}C$ value of body tissues (R_3) were the same as those taken from two pasture-fed bulls; (2) the $\delta^{13}C$ value of 'endogenous free' faeces (F_0) during the C_4 feeding was the same as that for the indigestible-fibre fraction obtained from the in vitro digestion of the feeds. The endogenous proportion (x) of faecal C was calculated using the equation:

$$F_4 = xR_3 + (1-x)F_0.$$

An alternative estimate was also made using the assumption that the $\delta^{13}C$ value for endogenous free faeces during C_4 feeding was the same value as that for the NDF, obtained from the faeces of cow no. 171.

The proportion of C in the urine of individual cows derived from body tissues, was estimated from the $\delta^{13}C$ values of the urine obtained during the last 2 d of the C_4-feeding period (U_4) by making the following assumptions: (1) that the $\delta^{13}C$ value of body fat and protein reserves (R_3) were $-30{\cdot}1$ and $-25{\cdot}4$ respectively (as stated previously); (2) that the $\delta^{13}C$ value for the 'endogenous free' urine (U_0) during C_4 feeding was the same as that for the feed. The endogenous proportion (x) of urinary C was calculated using the equation:

$$U_4 = xR_3 + (1-x)U_0$$

RESULTS

Intakes and production levels

Metabolizable energy (ME) intakes, production of milk solids and estimated energy and N balances for pairs of HBI and LBI cows are summarized in Table 1.

In early lactation during the C_3-feeding period the HBI cows produced more fat, protein and lactose than the LBI animals so that the HBI animals were in greater energy deficit than the LBI cows (-57 *v.* -23 MJ ME/d). Production of milk and milk solids was reduced in all cows on the C_4 diet, reflecting the reduced energy and protein intakes. Energy balance remained more negative for the HBI than the LBI cows (-26 *v.* -12 MJ ME/d). Both groups retained approximately 20 g N/cow per d over the last 2 d of the C_4-feeding period. Unfortunately one LBI cow, after 2 d on C_4 feeding, was returned to grazing due to lameness so that the values obtained during early lactation were from three cows.

In late lactation the production of milk and milk solids was similar for both breeding-index groups during C_3 feeding and decreased during fasting. Following the change to C_4 feeds all cows were able to maintain similar but lower yields to those obtained previously. At the end of this period all cows were close to energy balance and were retaining 20–34 g N/cow per d.

$\delta^{13}C$ values for diets

The $\delta^{13}C$ values for the feeds are presented in Tables 2 and 3 and show the expected differences between the C_3 (barley meal and pasture) and C_4 plants (paspalum hay, maize meal and silage). Within the C_4-plant grouping all feeds had similar values ($-11{\cdot}3$ to $-11{\cdot}7$ ‰) but for the C_3 feeds the barley meal was about 3 units more enriched in ^{13}C than the pasture (-26 *v.* -29 ‰).

Table 1. *Mean daily intakes, energy and protein balances, and production values for pairs of high (HBI) and low genetic merit (LBI) cows fed on C_3 and C_4 diets*, at two stages of lactation*

	Early lactation		Late lactation	
	C_3	C_4	C_3	C_4
HBI cows				
Energy intake (MJ ME)	155	124	150	116
Crude protein† in diet (g/kg DM)	176	132	189	131
Proportion of concentrates in diet	0·23	0·43	0·25	0·29
Milk yield (l/cow)	24·1	17·2	12·9	9·7
Milk fat yield (kg/cow)	1·23 (0·66)	0·70	0·61 (0·55)	0·48
Protein yield (kg/cow)	0·87	0·49	0·43	0·34
Lactose yield (kg/cow)	1·22	0·83	0·58	0·42
Energy balance‡ (MJ ME/d)	−57	−26	+10	−6
N balance (g/d)	—	+21	—	+20
LBI cows				
Energy intake (MJ ME)	127	118§	157	119
Crude protein† in diet (g/kg DM)	175	127	191	128
Proportion of concentrates in diet	0·29	0·48	0·23	0·29
Milk yield (l/cow)	25·0	20·1	13·5	11·6
Milk fat yield (kg/cow)	0·73 (0·56)	0·56	0·56 (0·38)	0·46
Protein yield (kg/cow)	0·77	0·53	0·46	0·40
Lactose yield (kg/cow)	1·15	0·92	0·63	0·54
Energy balance‡ (MJ ME/d)	−23	−12	+27	+1
N balance (g/d)	—	+20	—	+34

ME, Metabolizable energy; DM, dry matter.

* For details, see p. 606.

† Nitrogen × 6·25.

‡ Energy balance = ME intake − (ME requirements for milk + maintenance) using Agricultural Research Council (1980) standards.

§ One cow only.

Figures in parentheses indicate yield during 24 h fast.

The values in Table 2 also indicate the extent to which major constituents of feeds (e.g. cell-wall material, NDF) have different $\delta^{13}C$ values and hence the degree to which the absorbed nutrients may differ from the mean dietary values.

Constituents soluble in neutral detergent (1-NDF) are virtually 100% digested whereas the 'indigestible fibre' portion (in vitro) should be the major constituent of the faeces. The various fractions, with the exception of the indigestible fibre of maize silage and the NDF and 'indigestible fibre' of the barley meal, were all within 0·7 ‰ of the original sample. It is therefore unlikely that the average $\delta^{13}C$ values of 'digested' nutrients would have differed markedly from those in the whole diets.

$\delta^{13}C$ values for milk, tissues and excreta

The mean $\delta^{13}C$ values for major constituents of milk from the cows, are shown in Fig. 1. The values varied between individual milk constituents, with diet and also with stage of lactation. Individual cow differences, as indicated by the size of the standard errors, were greatest in early lactation, especially for milk fat. The mean $\delta^{13}C$ values of milk components and excreta over the last 2 d of C_3 and C_4 feeding for pairs of cows, grouped according to breeding index, are presented in Table 3.

Table 2. *$\delta^{13}C$ (‰) values for feeds and faeces (in cow no. 171) and some of their chemical constituents in C_3- and C_4-feeding periods* during early and late lactation*

	Original sample	Neutral-detergent-fibre fraction	Indigestible-fibre fraction
Roughages			
Spring pasture (early lactation)	−29·3	−29·0	−29·4
Autumn pasture (late lactation)	−28·6	−27·9	−28·7
Paspalum dilatatum hay	−11·5	−11·7	−12·0
Maize silage	−11·7	−11·8	−13·5
Concentrates			
Barley meal	−25·3	−26·5	−26·4
Maize meal	−11·6	−11·3	−11·3
Faeces (cow no. 171)			
C_3 feeding: early lactation	−30·3	−29·7	−29·9
late lactation	−29·4	−28·7	−28·5
C_4 feeding: early lactation	−14·2	−12·6	−12·8
late lactation	−13·8	−12·7	−13·0

$\delta^{13}C$, Difference from the value for no. of atoms of ^{13}C: that of ^{12}C for a carbonate standard (see p. 605).
* For details, see p. 606.

Table 3. *$\delta^{13}C$ (‰) values for diets, faeces, urine and milk constituents for pairs of high (HBI) and low genetic merit (LBI) cows fed on C_3 feeds, fasted for 24 h or fed C_4 feeds at two stages of lactation*

	Early lactation			Late lactation		
	C_3 diet	Fasted	C_4 diet	C_3 diet	Fasted	C_4 diet
HBI cows						
Diet	−28·6	—	−11·4	−27·8	—	−11·6
Faeces	−30·2	—	−14·0	−29·4	—	−13·6
Urine	−27·6	—	−15·2	−26·3	—	−13·8
Milk fat	−30·8	−30·9	−21·4	−30·0	−30·6	−14·8
Casein	−27·1	−25·7	−16·2	−25·8	−27·1	−14·0
Lactose	−29·0	−29·9	−14·7	−27·5	−28·5	−12·7
Fatty acids C_{4-8}	−28·2	−24·1	−11·8	−25·2	−26·0	−12·0
LBI cows						
Diet	−28·4	—	−11·4	−27·8	—	−11·6
Faeces	−30·2	—	−13·7	−29·6	—	−13·6
Urine	−27·9	—	−15·1	−26·2	—	−14·3
Milk fat	−29·7	−30·6	−19·4	−29·5	−30·5	−15·1
Casein	−26·7	−27·8	−14·9	−25·8	−26·7	−13·5
Lactose	−28·9	−29·8	−12·9	−27·2	−28·3	−12·6
Fatty acids C_{4-8}	−28·5	−25·4	−11·0	−25·1	−25·3	−13·3

$\delta^{13}C$, Difference from the value for no. of atoms of ^{13}C: that of ^{12}C for a carbonate standard (see p. 605).
* For details of C_3 and C_4 feeds, see p. 606.
See Fig. 1 for between-cow variation.

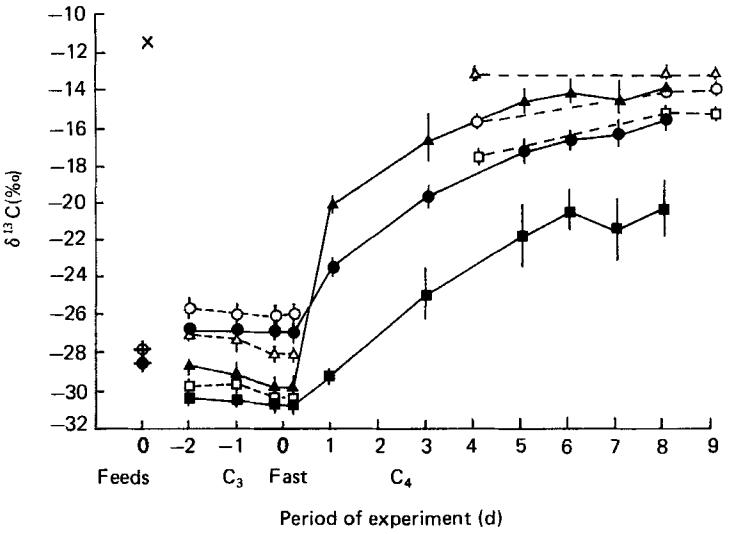

Fig. 1. Mean changes in $\delta^{13}C$ (‰) for major milk constituents in four cows fed on C_3 and C_4 diets at two stages of lactation (for details of diets, see p. 606). (□), Milk fat; (○), casein; (△), lactose during early (■, ●, ▲) and late (□, ○, △) lactation. Values are means with their standard errors represented by vertical bars; (+), C_3 feeds; (×), C_4 feeds. $\delta^{13}C$ is the difference from the value for no. of atoms of ^{13}C: that of ^{12}C for a carbonate standard (see p. 607).

C_3 feeding. During the C_3-feeding period, the milk fat (−29·5 to −30·8) and, to a lesser extent, the lactose were more depleted in ^{13}C compared with the feed, whereas the casein was enriched (−25·8 to −27·1). Faeces were depleted (−29·4 to −30·2) in ^{13}C but urine enriched (−26·3 to −27·9). Body-tissue samples were not taken from the cows but samples of subcutaneous fat and 'fat-free' muscle from two Friesian bulls fed on pasture provided mean $\delta^{13}C$ ‰ values of −30·1 and −25·4 respectively. There was no marked breeding-index effect, but there was a small consistent stage of lactation effect in that values for the diet and each of the milk solids in late lactation were not quite as depleted in ^{13}C as in early lactation (about 1 ‰ unit difference).

Fasting for 24 h resulted in depletion of $\delta^{13}C$ values for milk fat, casein and lactose produced, with the exception of the casein from HBI cows in early lactation. The steam-volatile fatty acids in milk fat, following fasting, were enriched during early but depleted during late lactation for both breeding-index groups.

C_4 feeding. Following the change to C_4 feeding the $\delta^{13}C$ values in most cases had stabilized at their new levels by 5–7 d (Fig. 1). Relative to the C_4 feed all the milk solids were more depleted in $\delta^{13}C$ early in lactation than late in lactation, and the largest difference existed for milk fat. Body fat and protein reserves, particularly in early lactation, were clearly contributing C to milk synthesis and excretion products.

Precursor pools from body tissues

Estimates of the proportion of the C in individual milk components derived from body reserves for pairs of HBI and LBI cows at two stages of lactation are given in Table 4. The values vary, depending to some extent on whether body fat or protein is assumed to be the main source of C, as the $\delta^{13}C$ ‰ for tissue fat and protein differed. Assuming that the majority of the tissue C contribution to milk fat is derived from body fat and that for casein and lactose came from body protein, then the findings indicate a greater proportion of the

Table 4. *Estimates of the proportion of carbon in milk fat, casein, lactose and short-chain fatty acids in milk fat derived from body tissue fat or protein* in high (HBI) and low genetic merit (LBI) cows in early and late lactation*

	Early lactation				Late lactation			
Body-tissue source...	Fat		Protein		Fat		Protein	
Breeding index...	HBI	LBI	HBI	LBI	HBI	LBI	HBI	LBI
Milk constituent								
Milk fat	0·54	0·43	—	—	0·17	0·19	—	—
Casein	—	—	0·34	0·25	—	—	0·19	0·14
Lactose	—	—	0·24	0·11	—	—	0·08	0·07
Fatty acids C_{4-8}	0·02	0·02	0·03	0·03	0·02	0·09	0·03	0·12

Differences between individual HBI cows were 0·17, 0·08, 0·12, 0·02 for milk fat, casein, lactose and C_{4-8} fatty acids in early lactation and maximum differences between cows, within breeding index groups, were 0·04, 0·06, 0·04, 0·03 respectively in late lactation.

* Assumes milk fat C is derived from body fat and casein and lactose comes from body protein. C_{4-8} fatty acids are assumed to come from either body fat or protein.

Table 5. *Estimates of the amounts (g/d) of milk constituents synthesized from carbon derived from body tissues* in high (HBI) and low genetic merit (LBI) cows at two stages of lactation*

	Early lactation			Late lactation		
	HBI	LBI	HBI:LBI	HBI	LBI	HBI:LBI
Milk fat	381	241	1·6	82	88	0·9
Casein	167	133	1·3	65	55	1·2
Lactose	198	101	2·0	34	38	0·9

* Assumes milk fat C is derived from body fat and casein and lactose comes from body protein.

precursor pool for fat, casein and lactose synthesis was derived from body tissues in early than in late lactation. Furthermore, early in lactation there were considerable differences between cows in the proportions of these constituents derived from body tissues. The contribution of body tissues to the precursor pool for C_{4-8} fatty acids was less than 5% except in the LBI cows late in lactation where it was 12%.

Estimates of the actual amounts (g/d) of individual milk solids synthesized from C released from body tissues are provided in Table 5. In early lactation, while the HBI cows produced 25% more milk fat during C_4 feeding than the LBI cow (Table 1), the body tissue contributions to milk fat, casein and lactose yields were respectively 60, 30 and 100% higher in the HBI cows. However, as it was noted that the HBI cows were in slightly better body condition at calving than the LBI cows, the relative size of body reserves rather than genotype may have been the dominant factor which influenced the proportion of milk solids derived from body tissue.

Endogenous faecal and urinary losses

The proportions of faecal and urinary C estimated to have originated from body reserves are given in Tables 6 and 7. Between 6 and 14% of the C in faeces and 20–27% of the C

Table 6. *Estimates of the proportion of faecal carbon derived from body fat or protein in high (HBI) and low genetic merit (LBI) cows at two stages of lactation*

	Early lactation		Late lactation	
Body-tissue source...	Fat	Protein	Fat	Protein
Estimate of endogenous free $\delta^{13}C$:				
From indigestible feed (in vitro)	0·10	0·14	0·09	0·13
From neutral-detergent fibre value from faeces (cow no. 171)	0·09	0·13	0·06	0·09

$\delta^{13}C$, Difference from the value for no. of atoms of ^{13}C: that of ^{12}C for a carbonate standard (see p. 607).

Table 7. *Estimates of the proportion of urinary C derived from body fat or protein in high (HBI) and low genetic merit (LBI) cows at two stages of lactation*

	Early lactation		Late lactation	
Breeding index	Fat	Protein	Fat	Protein
HBI	0·20	0·27	0·12	0·16
LBI	0·20	0·26	0·14	0·19

in the urine were found to be derived from endogenous sources in early lactation. By late lactation, endogenous losses in urine had fallen to 12–19%. There were no consistent differences between breeding-index groups.

DISCUSSION

Sources of precursors for milk synthesis

The switch from a diet of C_3 to one of C_4 plant material induced marked changes in the $\delta^{13}C$ values of the milk solids (Fig. 1). The rate of change for individual milk constituents and the plateau values reached provide quantitative information on the sources of precursors for milk synthesis.

The dependence of the mammary gland on the feed as an immediate source of precursors for lactose synthesis is highlighted by the rapid rise in the $\delta^{13}C$ value for lactose following the change of diet. It was calculated that 35–40% of the precursors for lactose synthesis were derived from the C_4 feed during day 1. It is probable that propionic acid was the major component of these precursors. While the intake of the new diet was poor on the first day, following a 24 h fast, it is nevertheless of interest that estimates of the contribution of propionic acid to glucose turnover in the cow range from 30 to 50% (Waghorn & Baldwin, 1984).

In contrast, less than 10% of the C in milk fat and 15% in casein were derived from the C_4 feed on day 1. The slower increases in $\delta^{13}C$ values for fat and casein may partly reflect a delay as precursors (long-chain fatty acids (LCFA) and amino acids) pass from the rumen and are absorbed further down the alimentary tract. In addition, slower passage of fat than lactose through the secretory cells and down the duct system of the mammary gland may also have contributed to the difference in rate of change of the $\delta^{13}C$ values of the two components but the most likely explanation is that an exceptionally large amount of body tissue C was incorporated into milk fat (and probably casein) during day 1.

After 5 d the $\delta^{13}C$ values remained relatively constant (Fig. 1). The means of the values during the last 2 d of C_4 feeding were used to calculate the proportion of the C in each of the components of milk derived directly from the feed and indirectly from body reserves (Table 4). It is essential to emphasize that these are estimates of the proportion by origin of C in the various precursor pools and they cannot be used to estimate net loss of C from the tissues. The estimates of milk solids produced from precursors originating in the tissues (Table 5) represent minimum values for the amount of precursor released. Metabolites released from body tissue may also be re-incorporated into tissues or catabolized as a source of energy.

The finding that a large proportion (0·43–0·54) of the C in milk fat was derived from body tissues, especially in early lactation, is consistent with the large body-weight losses which often occur in early lactation, and with the knowledge that the C_{18} and at least part of the C_{16} fatty acids of milk fat are thought to be derived from plasma lipids (Moore & Christie, 1981), which have an endogenous as well as a dietary origin.

The small contribution of endogenous C to the synthesis of C_4–C_8 fatty acids (Table 4) contrasts with those estimates of the contribution of endogenous fatty acids to the total acetate flux in the body which have ranged up to 50% (Costa *et al.* 1976). Although it needs to be confirmed that this applies to the C_{10}–C_{14} fatty acids, the present findings indicate that products of β-oxidation of fatty acids in the body are not readily available for milk fat synthesis. This conclusion means that most of the endogenous C in milk fat (Table 4) must be in the C_{16}–C_{18} fatty acid fraction which made up nearly 80% of the total. The mean fatty acid compositions (weight %) of the milk fats during C_4 feeding were: C_{4-14}, 22·3 and 22·0; C_{16}, 30·0 and 40·1; C_{18}, 11·4 and 12·4; $C_{18:1-3}$, 36·3 and 25·4 in early and late lactation respectively.

The contribution of body-tissue C to casein and lactose synthesis was surprisingly large (up to 0·34) and may have important implications. If it is assumed that most of this C must have been derived from body protein, it can be calculated from the values in Table 5 that on average the cows must have been incorporating into milk solids the C from about as much body protein as body fat. Because the net amount of body protein mobilized in early lactation is thought to be much less than the amount of fat mobilized (Oldham, 1984), it is possible that the size of 'available' protein reserves could limit milk production. Indeed, Ørskov *et al.* (1981) have shown that more milk may be produced at the expense of body tissue by increasing the amino acid supply to cows already in negative energy balance.

Stage of lactation and genetic effects

The proportion of fat, casein and lactose synthesized from C originating in the tissue was up to threefold greater in early than in late lactation (Table 4). The energy intake, however, of the cows only varied between 116 and 124 MJ ME/d (Table 1) so the amount of precursors entering the appropriate pools from the feed would be similar between the two stages of lactation. Therefore the larger proportion of endogenously derived C in the milk solids in early than in late lactation indicates a more than twofold greater use of fat from the adipose tissue and amino acids from tissue proteins in early lactation. A greater turnover of body tissue was also indicated by the greater proportion of endogenous C in the urine in early than in late lactation (Table 7). Stage of lactation and the nutritional state of the animal can lead to alterations in the rates of protein (Swick & Benevenga, 1977) and fat turnover (Oldham, 1984) and the cows in early lactation were in greater negative energy balance than those in late lactation. The amounts of metabolites 'released' to the plasma pool can be estimated if assumptions are made concerning the amounts absorbed directly from the diet.

Thus, if the amount of amino acid absorbed from the diet was 560 g (estimated from ME and undegradable dietary protein intakes; Agricultural Research Council, 1980) then the

casein produced by the HBI cows early in lactation was synthesized from a pool of 848 g amino acids (560 ÷ (1 − 0·34)) while the precursor pool in late lactation was 691 g. The corresponding pool sizes for the LBI cows were 746 and 651 g respectively. It is of interest that these estimated plasma amino acid pool sizes were consistently higher in HBI than LBI cows, which is also in accord with the observation that a much greater proportion of the lactose-C was synthesized from body C sources (e.g. glucogenic amino acids) by the HBI animals in early lactation.

The amino acid pool sizes, estimated previously, are remarkably small compared with published estimates of total body-protein turnover (Oldham, 1984) which would be expected to be about 1500 g in our 450–500-kg cows. A possible explanation for this discrepancy is that not all the amino acids mobilized in body tissues during protein turnover become part of the pool available to the mammary gland. Furthermore, it is tempting to speculate that the size of the plasma amino acid pool may be a factor which influences the differences in production between HBI and LBI cows.

Since 54% of the C in milk fat of HBI cows (and 43% in LBI) in early lactation was from body reserves (Table 4), and $C_{18} + 0{\cdot}5\ C_{16}$ fatty acids (which may arise from plasma lipids; Moore & Christie, 1981) were about 63% of the total, it follows that about 86% (68% for LBI) of these LCFA originated in adipose tissue. Thus if it is assumed that 300 g LCFA are absorbed from the diet then the total pool available to the mammary gland of the HBI cows would be 2143 g (300 ÷ (1 − 0·86)) and 938 g for the LBI cow. The corresponding LCFA pool sizes in late lactation were calculated as 422 g and 448 g for HBI and LBI cows respectively. These calculations indicate extremely variable rates of adipose tissue turnover during lactation. While the endogenous metabolites made a much greater contribution to the precursor pools of HBI than LBI cows in early lactation, this apparent genetic effect (based on few animals) could equally well have been due to the relative amounts of tissue available for mobilization, as the HBI cows were in slightly better body condition than the LBI cows before calving.

Accuracy of estimates

It was assumed in making the estimates of the body tissue contributions to milk synthesis that the $\delta^{13}C$ values for the individual milk solids may be expected to be similar to that for the C_4 diet, or in other words that no discrimination between C-isotopes occurred. This assumption was not tested as it would have necessitated an extended feeding period with C_4 plant material to allow complete replacement of body-tissue C (see Tieszen *et al.* 1983). However, the variation in $\delta^{13}C$ values between individual milk solids during C_3 feeding may provide a guide to the possible extent of the error which could be associated with the estimates, arising from discrimination between ^{13}C and ^{12}C in the various metabolic pathways during C_4 feeding.

On the C_3 diet, milk fat was consistently depleted in ^{13}C (on average, 1·8 ‰ units) whereas casein was enriched to a similar extent. In contrast $\delta^{13}C$ values for lactose remained fairly similar to those for the diet, indicating very little discrimination and hence little error in the estimates. Adjustment of the $\delta^{13}C$ values for the C_4 diet by −1·8 ‰ units in the case of fat synthesis has the effect of decreasing the estimates of the proportion of C in milk fat derived from body fat by 5–6% in early lactation and by about 9% in late lactation. The corresponding values for casein-C derived from body protein would be increased by 8–10%. These are likely to be maximum errors as careful examination of published values indicates that the $\delta^{13}C$ values for individual tissues of ungulates on different diets differed less from the whole-body mean for animals on a C_4 diet than when fed on a C_3 diet (DeNiro & Epstein, 1978; van der Merwe, 1982).

The estimates of contribution of body tissues to milk synthesis are, however, all likely to be underestimates because rapid recycling of newly deposited body tissues during C_4 feeding would not have been measured using the techniques described.

Synthetic pathways

Different $\delta^{13}C$ values for individual milk solids during C_3 feeding offers the opportunity to trace synthetic pathways and examine precursor product relations, provided that the site(s) of discrimination between ^{13}C and ^{12}C can be identified.

The faeces of the cows were always depleted in ^{13}C (Table 3) as were most of the 'indigestible-fibre' fractions obtained following in vitro fermentation of the feeds (Table 2). The $\delta^{13}C$ values of the faeces were on average 1·7 ‰ more negative than those of the feed, similar to that found by Jones *et al.* (1979, 1981). Thus by inference the digestible nutrients were enriched a little with ^{13}C. However, measurement of the $\delta^{13}C$ values for rumen CO_2 and methane are needed to get an understanding of the gross discrimination which occurs in the rumen.

The differences between the $\delta^{13}C$ values of milk solids during the fed and fasted states (Table 3) probably lie mainly in the contribution during the fed state of precursors from the rapidly fermented barley meal which was relatively enriched with ^{13}C, but mobilization of tissue reserves differing in $\delta^{13}C$ may also have contributed.

The $\delta^{13}C$ values for body fat (−30·2 ‰) and milk fat during feeding (−29·5 to −30·8 ‰) or fasting (−30·5 to −30·9 ‰), early (−30·0 to −30·9 ‰) or late (−29·5 to −30·6 ‰) in lactation were sufficiently similar to suggest that the depletion of ^{13}C relative to the C_3 diet was relatively constant irrespective of whether the fat was synthesized in the adipose tissue or in the mammary gland under various physiological states. Fat-free muscle on the other hand (−25·4) was a little more enriched than casein (−25·8 to −27·9) which suggests that some sources of precursors may be different in these two pathways.

The finding that protein extracted from the leaves of C_3 plants was 3–5 ‰ more positive than that of whole leaf (Vogel, cited by van der Merwe, 1982) suggests the main site of differential incorporation of ^{13}C between protein and non-protein fractions may occur in the plant. However, in ruminants much of the absorbed amino acids are derived from digested micro-organisms which use C skeletons obtained from dietary carbohydrates together with ammonia from degraded plant proteins to form protein. So the finding that muscle protein and casein are relatively enriched in ^{13}C suggests fractionation in rumen micro-organisms or ruminant tissues in addition to plant tissues.

It may be concluded that concurrent measurement of $\delta^{13}C$ values in a wider range of tissues and metabolic intermediates may offer considerable potential for further quantification of synthetic pathways.

Endogenous losses in excreta

The values for the proportions of urinary and faecal C derived from body tissues provides evidence on the quantitative importance of endogenous losses in lactating cows. The likely significance of these losses in contributing to the energy requirements of cows can be predicted by making the assumption that the heats of combustion of individual constituents in the excreta are approximately proportional to their C concentrations. Thus our results (Table 6) suggest that approximately 9–14% of the energy in faeces, equivalent to 4–6% of digestible energy (DE) intake, is of metabolic origin in early lactation and that this proportion may decrease a little with stage of lactation. There is apparently no previous quantitative information available relating to the heat of combustion of the faecal metabolic products (Agricultural Research Council, 1980) with which our estimate could be compared.

The endogenous contribution to urinary C averaged about 23% in early lactation when the cows were mobilizing body tissue, and 15% in late lactation. Thus if urinary losses account for up to 9% of DE requirements, then endogenous losses in urine may range up to 2% DE.

The findings on the endogenous faecal contribution may also provide an estimate of metabolic faecal N in lactating cows, a measurement which is difficult to determine in ruminants using conventional methods (Agricultural Research Council, 1980). In this case it is necessary to assume that the endogenous C is present in the faeces mainly as protein residues. The protein equivalent of 6–14% of the C in faeces becomes 4–10% (or 6·4–16·0 g N/kg faecal DM (FDM)), if allowance is made for the higher C content of proteins compared with carbohydrates. This estimate is comparable with the mean value of 10·9 g N/kg FDM calculated by Swanson (1982) on the basis of fifty-seven experiments with dry cattle on low-N diets, but much higher than the values estimated by Ørskov & MacLeod (1982) using abomasal N-free infusates. Confirmation of our results using the novel technique described, which has the important attribute of using milking cows fed and managed under relatively normal feeding conditions, seems warranted.

The authors gratefully acknowledge the skilled technical assistance of Mrs J. A. Rumbal (Massey University) with sample collection, preparation and analysis and Mr M. E. F. Oelsner and Ms K. Stanley (DSIR) with the carbon-isotope analyses. Thanks are also due to Dr M. P. Rolston (Grasslands Division, DSIR) for providing the paspalum hay.

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