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Fat and protein metabolism in growing steers fed either grass silage or dried grass

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Cattle fed grass silage diets have been reported to have high carcass fat:protein ratios. The effect of grass silage and dried grass diets, fed at different levels of intake to ensure a range of equivalent metabolisable energy intakes (MEI) from 1·1 × metabolisable energy requirement for maintenance to *ad libitum*, on fat and protein metabolism in twenty-four Hereford × Friesian steers was investigated. After about 84 d of dietary treatment rates of whole-body fat and protein metabolism were measured, as were rates of lipogenesis in omental, perirenal and subcutaneous adipose tissue. Carcass composition was determined. Animals fed silage had greater ($P<0·001$) carcass fat:protein ratios than animals fed dried grass at equivalent levels of MEI. Animals fed silage had lower ($P<0·001$) rates of protein gain. Rates of leucine entry and oxidation were lower ($P<0·001$) in animals fed silage, but there was no dietary difference in the rate of whole-body protein synthesis. There was no dietary difference in the rate of carcass fat gain, but rates of lipogenesis in perirenal adipose tissue were significantly ($P=0·007$) higher in animals fed silage. There was no dietary difference in the rate of palmitate and glycerol entry or palmitate oxidation. There were no interactions between MEI and diet, indicating that increments of energy were utilised with the same efficiency from both diets. It was concluded that the high carcass fat: protein ratios of young growing steers was due to limited rates of protein accretion and not to elevated rates of carcass fat accretion.

Ruminants: Grass silage: Dried grass: Fat metabolism: Protein metabolism: Carcass composition: Metabolisable energy

Grass silage forms the basis of many winter diets fed to beef cattle in the UK. The performance of animals fed silage is variable and often disappointing in terms of feed intake and the overall efficiency of energy (Thomas & Chamberlain, 1990) and amino acid (Beever *et al.* 1992; MacRae *et al.* 1995) utilisation. It has been reported that animals fed silage have a high carcass fat:protein ratio (Lonsdale, 1976; Moore & Steen, 1983; Baker *et al.* 1985, 1992; Steen & Moore, 1988, 1989; Steen, 1991). That protein deposition is limited in animals fed grass silage is now well established (Gill *et al.* 1987). Whether or not fat deposition is increased in these animals is unclear.

With continuing pressures to increase the utilisation of grass in ruminant production systems, a greater understanding of the partition of nutrients between fat and protein deposition in animals fed grass silage is required if useful models to predict animal performance are to be developed. The objective of the present study was to determine whether or not an increased fat deposition contributes to the high carcass fat:protein ratio of animals fed grass silage by simultaneously investigating fat and protein metabolism, using isotope dilution and incorporation techniques, and carcass composition of young, growing steers fed either grass silage or dried grass. As both the quantity and form of metabolisable energy (ME) are considered to be important determinants of animal performance (Beever *et al.* 1988; Thomas & Gill, 1988; Steen, 1992; Steen & Robson, 1995), the animals were fed silage or dried grass at different levels of intake to ensure a range of equivalent ME intakes (MEI), based on the animals' estimated ME requirement for maintenance (ME_m), with the objective of determining whether or not metabolism and carcass composition respond differentially to MEI from the two diets.

Materials and methods

Animals and experimental design

Thirty Hereford × Friesian steers were used, from which twenty-four were randomly selected and assigned a treatment in a 2 (diet: silage and dried grass) × 5 (MEI: 1·1, 1·2, 1·3, 1·4 and 1·5 × ME_m) factorial experiment with two additional levels of dried grass MEI (1·8 and 2·0 × ME_m), such that there were two steers per diet per MEI level. Animals were paired ensuring a minimal weight range between paired animals, and a similar weight range across dietary treatments. The six remaining steers were slaughtered at the start of

Abbreviations: IGF-I, insulin-like growth factor 1; KKCF, kidney knob and channel fats; LW, live weight; MADF, modified acid detergent fibre; ME, metabolisable energy; MEI, ME intake; ME_m, ME requirement for maintenance; Ra, rate of appearance.

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the experiment for carcass composition analysis (initial slaughter group).

The animals fed silage at $1{\cdot}5 \times ME_m$ and dried grass at $2{\cdot}0 \times ME_m$ were actually fed to appetite (fed 10 % in excess of the previous day's refusals), with these intake levels representing the average *ad libitum* intakes of the animal in each pair with the highest intake over the experimental period. Animals fed dried grass at $1{\cdot}5 \times ME_m$ were restricted to the voluntary MEI level of their silage-fed pair.

From weaning until the start of the experiment (approximately 5 weeks), the steers were group-penned on straw and fed Calf Starter (BOCM Pauls plc, Ipswich, Suffolk, UK) and hay. During this period the average live weight (LW) gain of the animals was 0·42 (SD 0·11) kg/d. At the start of the experiment the steers were approximately 13 weeks of age (mean LW: 94·2 (SD 7·2) kg). The steers were penned individually on rubber matting. The experimental period lasted approximately 13 weeks with all cattle spending the final 2 weeks in metabolism crates. During the whole duration of the experiment, except when animals were in the metabolism crates, steers were weighed twice weekly and their dietary intakes adjusted accordingly. They were fed equal-sized meals twice daily, and had free access to water and mineral licks (Standard Wright Blocks; Frank Wright Ltd, Ashbourne, Derbyshire, UK) at all times.

Once in metabolism crates steers were not weighed. Dietary feeding levels were adjusted according to previous rates of LW gain using linear regression. Final LW was determined in the same way. Diets were fed hourly using automatic feeders. The steers were allowed a 7 d adjustment period to the metabolism crates before the start of metabolism measurements, involving three intravenous infusions of isotope over a period of 4 d: [1-^{13}C]leucine, a combined [1-^{13}C]palmitate and [2-^{3}H]glycerol infusion, and a combined $NaH^{13}CO_3$ and [1-^{14}C]acetate infusion. On the eleventh day in metabolism crates the steers were slaughtered.

The experiment was carried out under licence of the Animals (Scientific Procedures) Act, 1986.

Diets

Big bale silage made from unchopped, unwilted primary growth perennial ryegrass (*Lolium perenne* cv. Cropper) was used. The silage was prepared on the same day from a single sward. The dried grass had been chopped, artificially dried and pelleted, and all came from the same batch. The modified acid detergent fibre (MADF) and DM contents of each bale of silage and the dried grass were determined prior to feeding. Silage bales of similar MADF and DM composition were selected for use in the experiment. The MADF values were used to estimate dietary ME values (Agriculture and Food Research Council, 1993). During the experiment samples (approximately 0·5 kg) of silage and dried grass were taken daily. These samples were pooled over weekly intervals, mixed thoroughly, sub-sampled (approximately 0·5 kg) and stored until analysed. Silage samples were refrigerated (approximately 4°C) until pooled and then frozen (approximately − 20°C), and dried grass samples were stored at room temperature until analysed.

The desired levels of MEI were achieved by changing the total amount of feed provided rather than by changing the composition of the diets. Feed intake levels were determined based on the steers' estimated ME_m, which were calculated using the equation:

$$ME_m\,(MJ/d) = \frac{(LW \times 0{\cdot}061) + 5{\cdot}67}{k_m},$$

where k_m is the efficiency with which ME is used for maintenance and was assumed to be 0·72 (Agriculture and Food Research Council, 1993). The intakes of DM, ME and total N used in this analysis are the average intakes recorded over the period prior to entering metabolism crates.

Preparation of infusates

[1-^{13}C]Leucine. The [1-^{13}C]leucine (99·3 atom% ^{13}C; MassTrace Inc., Woburn, MA, USA) infusate (3·3 μg [1-^{13}C]leucine/kg LW per ml infused at 2 ml/min) and priming dose (393 μg/kg LW) were prepared as described by Dawson *et al.* (1998).

[1-^{13}C]Palmitate and [2-^{3}H]glycerol. The [1-^{13}C]palmitate (99 atom% ^{13}C; MassTrace Inc.) infusate (2·5 μg [1-^{13}C]palmitate/kg LW per ml infused at 2 ml/min) was prepared as described by Dawson *et al.* (1998). To this was added [2-^{3}H]glycerol (355 GBq/mmol; ICN Biomedicals Ltd, Thame, Oxfordshire, UK) such that the final specific radioactivity of the infusate was 0·23 kBq/kg LW per ml.

$NaH^{13}CO_3$ and [1-^{14}C]acetate. The $NaH^{13}CO_3$ infusate (8·0 μg $NaH^{13}CO_3$/(kg LW)$^{0·75}$ per ml infused at 2 ml/min) was prepared in saline bags (0·9 % (w/v) NaCl; Baxter Healthcare Ltd, Thetford, Norfolk, UK). $NaH^{13}CO_3$ (99 atom% ^{13}C; MassTrace Inc.) was dissolved in a small volume of saline removed from the saline bag and the resulting solution filter-sterilised (0·2 μm Minisart syringe filter; Sartorius GmbH, Göttingen, Germany) as it was returned back to the saline bag. To this was added [1-^{14}C]acetate (2·2 GBq/mmol; ICN Pharmaceuticals Ltd, Thame, Oxfordshire, UK) such that the final infusate specific radioactivity of [1-^{14}C]acetate was 0·5 kBq/kg LW per ml.

Priming doses of $NaH^{13}CO_3$ were administered immediately before the start of the [1-^{13}C]leucine, the combined [1-^{13}C]palmitate/[2-^{3}H]glycerol (106·3 μg $NaH^{13}CO_3$/kg LW) and the combined $NaH^{13}CO_3$/[1-^{14}C]acetate (1·36 mg $NaH^{13}CO_3$/(kg LW)$^{0·75}$) infusions. Priming doses were prepared in 20 ml saline and then filter-sterilised (0·2 μm Minisart syringe filter).

Priming doses of $NaH^{13}CO_3$ and [1-^{13}C]leucine were administered to ensure that stable equilibrium ^{13}C-enrichments were rapidly achieved for expired CO_2 and plasma leucine, respectively, due to the slower fractional turnover rates of the plasma $NaHCO_3$ (Allsop *et al.* 1978) and leucine (Matthews *et al.* 1980) pools.

Precautions for the use of radioactive isotopes towards humans subjects, animals and the environment were those stipulated in the University of Nottingham's Code of Practice.

Experimental procedures

On the fourth day that steers were in metabolism crates, both of their jugular veins were fitted with indwelling cannulae and

kept patent by daily flushing with 3·8 % (w/v) tri–sodium citrate in sterile saline (0·9 % NaCl).

On the eighth day in metabolism crates the steers received a $NaH^{13}CO_3$- and [1-^{13}C]leucine–primed 6 h continuous intravenous infusion of [1-^{13}C]leucine to determine the rate of appearance (Ra) of plasma leucine and the rate of leucine oxidation.

On the ninth day in metabolism crates each steer received a $NaH^{13}CO_3$-primed 6 h continuous intravenous infusion of [1-^{13}C]palmitate and [2-^{3}H]glycerol, for determination of the Ra of plasma palmitate and the rate of palmitate oxidation, and determination of the Ra of plasma glycerol, respectively. A 1 d 'rest period' followed to allow clearance of residual ^{13}C label remaining from the previous infusions.

On the eleventh day in metabolism crates each steer received a combined, $NaH^{13}CO_3$-primed, 6 h continuous intravenous infusion of $NaH^{13}CO_3$ and [1-^{14}C]acetate, for determination of the Ra of whole-body CO_2 and rates of acetate incorporation into lipid, respectively. At the end of the 6 h infusion the steers were killed with a lethal dose of pentobarbitone (Euthesate®, 200 g pentabarbitone sodium/l; Williams Francis Veterinary, Crawley, West Sussex, UK) and exsanguinated. Adipose tissue samples from the subcutaneous (tailhead region), omental and perirenal depots were taken within 2 min of death. The carcass was then dressed, weighed and halved.

Samples of blood and breath were taken at 30 min intervals for a period of 2 h before the start of the infusions, for the determination of background levels of isotope. Following the start of the infusions breath samples were taken at 15 min intervals for the first 2 h, and then at 30 min intervals until the end of the infusions; blood samples were taken at 30 min intervals throughout the infusions. Blood samples taken on the day of the combined [1-^{13}C]palmitate/[2-^{3}H]glycerol infusion were collected into tubes containing K-EDTA (15 μg/ml blood). All other blood samples were collected into tubes containing heparin (25 units/ml blood). Plasma was isolated from the blood samples by centrifuging at 1000 ***g*** for 15 min, which was then stored in a freezer (approximately –20°C) until analysed.

Breath samples were collected into 2-litre breath bags, fitted with one-way valves, via a facemask that was placed over the animal's nostrils. Triplicate samples of breath were drawn from the bag into evacuated tubes and stored at room temperature until analysed.

Adipose tissue samples were prepared as described by Greathead *et al.* (2001). From both halves of the dressed carcass, the kidney knob and channel fats (KKCF) were dissected, pooled and weighed. The longissimus dorsi and semitendinosus muscles were dissected from the left half of the carcass and weighed. The entire right half of the carcass (excluding the kidney and KKCF) was weighed and then frozen (approximately –20°C) for subsequent compositional analysis.

Analytical methods

Dietary analysis. The chemical composition of the silage and dried grass were determined as described by Scollan *et al.* (2001).

Plasma palmitate ^{13}C-enrichment and concentration. These were determined by a modified version of the method described by Hachey *et al.* (1991). The modification involved substitution of the derivatising agent pentafluorobenzyl bromide with iodomethane such that fatty acid methyl esters rather than pentafluorobenzyl esters were formed, enabling analysis by electron impact-GC/MS.

Plasma NEFA concentration. These were determined colorimetrically using a commercially available assay kit (NEFA-C, Wako #994-75 409; Alpha Laboratories, Eastleigh, Hampshire, UK).

Plasma leucine and ketoisocaproic acid ^{13}C-enrichment and concentration. Using the method described by Calder & Smith (1988), amino acids and keto acids were first isolated from plasma, then transformed to t-butyldimethylsilyl and quinoxalinol-t-butyldimethylsilyl derivatives respectively, and finally analysed by electron impact-GC/MS.

Breath $^{13}CO_2$-enrichment. The ^{13}C-enrichment of expired air was determined with a Europa Scientific Tracermass IRMS (Europa Scientific, Crewe, Cheshire, UK). Breath samples were introduced to the isotope ratio mass spectrometer using Roboprep-G (Europa Scientific). Samples were measured against a standard reference gas of 5 % CO_2 in air of known ^{13}C-enrichment.

Specific radioactivity of plasma glycerol. Plasma samples were deproteinised with perchloric acid (6 %, v/v) according to the method of Somogyi (1945). The concentration of glycerol in the deproteinised plasma was measured spectrophotometrically using a commercial glycerol assay kit (catalogue no. 148270; Boehringer Mannheim GmbH, Mannheim, Germany). The specific radioactivity of deproteinised plasma glycerol was determined using a modified version of the method described by Symonds *et al.* (1989), which involved using Dowex 1 (OH^- form, 200–400 mesh) ion-exchange resin to trap glucose in place of Dowex 1 (borate form, 200–400 mesh).

Specific radioactivity of plasma acetate, and total lipid extraction and specific radioactivity determination from adipose tissue

These were determined using methods described by Greathead *et al.* (2001).

Plasma insulin concentration. Plasma insulin concentrations were measured using a commercially available RIA kit (INS-RIA-100; Lifescreen Ltd, Watford, Hertfordshire, UK). *Chemical composition analysis of carcass and muscle samples*. This was determined using methods described by Gibb & Baker (1992).

Calculations

Initial carcass composition and live weight gains. Gains in carcass weight and its chemical components were calculated as the difference between the measured component at slaughter and the initial value estimated from regression analysis (Table 1) on the values derived from the initial slaughter group. The mean LW of the initial slaughter group at slaughter was 94·7 (SD 10·10) kg. The average LW gains of the steers were determined by linear regression using the twice-weekly measured LW of the steers.

Rates of appearance, oxidation, whole-body protein synthesis and acetate incorporation into lipid. In all cases,

Table 1. Constants (α) and coefficients (β) from the regression of carcass weight (kg) and the chemical components (kg) of the carcass *v.* live weight (kg) of the initial slaughter group of steers

(Mean values with their standard errors)

	α		β			
	Mean	SE	Mean	SE	r^2	RSD (n 6)
Carcass weight	4·4	7·06	0·46	0·074	0·88	1·68
Crude protein	−0·2	2·21	0·10	0·023	0·77	0·53
Fat	−3·1	1·09	0·07	0·011	0·87	0·26
Ash	0·7	0·479	0·02	0·005	0·81	0·11
Water	6·6	4·90	0·28	0·052	0·85	1·16

RSD, residual standard deviation.

For details of animals and procedures, see p. 28.

stable equilibrium ^{13}C- and ^{14}C-enrichments were achieved within 2 h of the start of the continuous infusions.

The rates of appearance of palmitate and total-body CO_2 were calculated by dividing the infusion rate of the tracers [1-^{13}C]palmitate and $NaH^{13}CO_3$ respectively, by the tracer:tracee ratios of palmitate and $NaHCO_2$ (measured as breath CO_2) respectively, at isotopic equilibrium. Tracer:tracee ratios were determined as described by Dawson *et al.* (1999).

The rate of plasma leucine appearance was calculated using the equation described by Wolfe *et al.* (1980). The plasma ^{13}C–enrichment of ketoisocaproic acid rather than leucine was used in the equation on the assumption that it more accurately reflects the true intracellular precursor pool enrichment (Matthews *et al.* 1982).

Rates of plasma glycerol and acetate appearance were calculated by dividing the infusion rate of the labelled substrate, [1-^{14}C]glycerol and [1-^{14}C]acetate respectively, by the specific radioactivity of the substrate at isotopic equilibrium (Pethick & Dunshea, 1993).

The oxidation rates of palmitate and leucine were calculated using the equation:

$$\text{Oxidation rate}(\mu\text{mol/min per kg}^{0\cdot75}) = \frac{\text{APE}_{CO_2} \times \text{Ra}_{CO_2}}{\text{APE}_S},$$

where APE_{CO2} is the isotopic enrichment of expired CO_2 at isotopic equilibrium measured during the continuous infusion of [1-^{13}C]palmitate or [1-^{13}C]leucine, respectively; Ra_{CO2} is the rate of appearance of total-body CO_2; and APE_S is the plasma isotopic enrichment of [1-^{13}C]palmitate or [^{13}C]ketoisocaproic acid, respectively, at isotopic equilibrium depending on which substrate oxidation rate is being calculated.

Rates of whole-body protein synthesis were calculated using the equation (Krishnamurti & Janssens, 1988):

$$\text{Protein synthesis (g/(kg LW)}^{0\cdot75}\text{ per d)} = \frac{(\text{Ra}_{\text{leucine}} - \text{leucine oxidation})}{0\cdot081} \times 0\cdot189,$$

where 0·081 is the average leucine concentration (g/g protein) in cattle carcass protein (Food and Agricultural Organization, 1970), and 0·189 converts from μmol/min to g/d.

The rate of acetate incorporation into adipose tissue lipid (R_{lipid}) was calculated from the increase in accumulation of ^{14}C-label in the total lipid over time as described by Greathead *et al.* (2001).

All rates of metabolism were expressed relative to the animals' metabolic LW ($kg^{0\cdot75}$).

Statistical analysis

Data were analysed by multiple linear regression using Genstat for Windows (Release 6·1; Lawes Agricultural Trust, Rothamstead, Hertfordshire, UK). Data were initially fitted to the model:

$$Y = \alpha + \beta_1 MEI + \beta_2 Diet + \beta_3 MEI \cdot Diet + \beta_4 MEI^2 + \beta_5 MEI^2 \cdot Diet,$$

where Y was the response variable, α was a constant, $\beta_1, \ldots, \beta_5$ were regression coefficients, MEI and MEI^2 were the explanatory variables and $Diet$ was the indicator variable. As the quadratic terms MEI^2 and $MEI^2 \cdot Diet$, and the linear interaction term $MEI \cdot Diet$, did not improve the fit of the model they were excluded from the final model. However, the P values for the linear interaction term $MEI \cdot Diet$, when included in the model, are presented for the purpose of illustrating the lack of a differential dietary response to MEI. Residuals were examined for homogeneity of variance and normality. As actual MEI differed from the planned MEI, the fitted model was used to predict responses at the planned levels of MEI, and comparisons between the two diets at the planned MEI were made by t test using the standard errors for the predicted response values and the residual degrees of freedom from the regression.

Results

Chemical composition of the silage and dried grass

The silage composition data presented (Table 2) are the average composition for all bales used. The silage had a DM content of 282·9 g/kg fresh weight and a total N content of 33·5 g/kg DM, of which 10·8 % was NH_3-N. The iso- and n-butyric acid contents were low at 0·27 and 0·29 g/kg DM, respectively. Lactic acid (50·6 g/kg DM) content was low whilst acetic acid (26·7 g/kg DM) content was considered to be relatively high. On the basis of these values the silage was considered to be of poor quality. This was reflected in the *ad libitum* ($1\cdot5 \times ME_m$) DM intake (21·4 g/kg LW per d, Table 3) and daily LW gain (0·4 (SD 0·02) kg/d, Table 4) of the animals fed silage, which were considered to be average and poor, respectively.

The dried grass had a lower total N content (28·4 g total N/kg DM), but the insoluble N content was much greater than that of the silage (811 *v.* 349 g/kg total N). The ME content, calculated from MADF content, was slightly higher for the dried grass (10·8 MJ/kg DM) than for the silage (10·4 MJ/kg DM). The dried grass, compared with the sil age, had a considerably higher content of water-soluble carbohydrate (149·6 *v.* 11·5 g/kg DM). The fact that starch was present in the silage (7·6 g/kg DM) was somewhat surprising, as temperate grasses, unlike tropical grasses, are believed to not accumulate starches (McDonald *et al.* 1991). This result

Table 2. Chemical composition (g/kg DM) of the silage and dried grass diets

(Mean values and standard deviations for twenty-six samples)

	Silage		
	Mean	SD	Dried grass
DM (g/kg fresh diet)	282·9†	36·0	916·8
Organic matter	914	8·0	914·9
Total N	33·5	3·3	28·4
Ammonia-N (g/kg total N)	107·8	14·4	
Soluble N (g/kg total N)	651·4	54·8	187·7
Insoluble N (g/kg total N)	348·6	54·8	810·9
Ash	86·0	8·0	85·1
Neutral detergent fibre	529·4	25·6	536·7
Acid detergent fibre	326·1	19·9	270·9
Modified acid detergent fibre	330·4	18·7	271
Acid detergent fibre-N	0·9	0·3	2·7
Starch	7·6	3·1	25·1
Water-soluble carbohydrate	11·5	4·0	149·6
Lactic acid	50·6	10·77	
Acetic acid	26·7	13·24	
Propionic acid	1·06	0·70	
iso-Butyric acid	0·27	0·19	
n-Butyric acid	0·29	0·54	
iso-Valeric acid	0·04	0·07	
n-Valeric acid	0·02	0·07	
Gross energy (MJ/kg DM)	18·9	0·3	18·7
Metabolisable energy (MJ/kg DM)*	10·4	0·25	10·8
pH	4·38	0·10	
Buffering capacity (meq/kg DM)	770·9	179·6	

*Estimated from modified acid detergent fibre.
†Toluene DM, corrected for ethanol content.
For details of diets and procedures, see p. 28.

is likely to be indicative of contamination with water-soluble carbohydrates.

Dietary intakes

The experiment was designed to provide silage or dried grass to animals over a range of, and where possible, equivalent MEI, from $1{\cdot}1 \times ME_m$ to *ad libitum*. The average recorded MEI of the animals in the different treatment groups closely matched the planned MEI levels, with the exception of the *ad libitum* MEI levels, where intakes were more variable (Table 3).

As the concentration of ME in the silage was approximately 0·96 that of the dried grass (Table 2), animals were fed proportionately less dried grass DM than animals fed silage at equivalent levels of MEI. Consequently, animals fed silage had higher ($P<0{\cdot}001$) recorded total N intakes than animals fed dried grass at equivalent levels of MEI. However, animals fed dried grass had approximately 85 % higher intakes of insoluble N than animals fed silage at equivalent levels of MEI. Water-soluble carbohydrate intakes for the silage diets were only approximately 8 % of those for the dried grass diets at equivalent MEI. Therefore, over the MEI range of 1·1–$1{\cdot}5 \times ME_m$, intakes of insoluble N and water-soluble carbohydrate differed markedly.

At *ad libitum* levels of intake there was no difference ($P>0{\cdot}05$) in the mean recorded DM intake between the two diets.

Table 3. Recorded intakes* of steers fed grass silage or dried grass at planned levels of metabolisable energy intake (MEI)

(Mean values and standard deviations of two determinations)

		Planned MEI level ($\times ME_m$)													
		1·1		1·2		1·3		1·4		1·5		1·8		2·0	
Parameter	Diet	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM intake (g/kg LW per d)	Silage	17·7	0·43	18·5	1·42	19·9	0·90	22·0	0·94	21·4	1·39				
	Dried grass	16·4	0·4	17·7	0·35	18·9	0·43	20·5	1·04	21·1	0·15	25·1	0·84	24·3	2·72
MEI ($\times ME_m$)	Silage	1·13	0·007	1·24	0·059	1·29	0·010	1·41	0·007	1·42	0·166				
	Dried grass	1·11	0·029	1·23	0·009	1·31	0·027	1·44	0·006	1·50	0·116	1·76	0·103	1·75	0·236
Total N intake (g/kg LW per d)	Silage	0·59	0·014	0·62	0·047	0·67	0·030	0·74	0·031	0·72	0·047				
	Dried grass	0·47	0·012	0·50	0·010	0·53	0·012	0·58	0·029	0·60	0·004	0·71	0·024	0·69	0·077

ME_m, metabolisable energy requirement for maintenance; LW, live weight.
*Intakes are the average recorded during the period prior to entering metabolism crates.
For details of diets and procedures, see p. 28.

Table 4. Constants (α) and coefficients (β) from the regression of performance data of steers fed either silage or dried grass *v.* metabolisable energy intake (MEI)

(Mean values with their standard errors)

	α				β				*P* value		
	Dried grass		Silage								
	Mean	SE	Mean	SE	Mean	SE	r^2	RSD (21 df)	MEI	Diet	MEI·Diet†*
Weights at slaughter											
LW (kg)†	57·7	14·0	54·0	12·8	50·9	9·6	59·9	9·43	<0·001	0·385	0·993
Carcass weight (kg)	33·9	7·9	29·7	7·2	23·7	5·4	55·8	5·33	<0·001	0·086	0·319
LD (g)	1424	494	1058	449	982	337	46·7	332·2	<0·001	0·021	0·397
ST (g)	719	182	529	166	250	124	48·8	122·7	0·003	0·002	0·183
KKCF (g)	−905	242	−759	221	1124	165	65·8	163·1	<0·001	0·054	0·733
Daily gains (g/d)											
LW	−320	96	−371	87	531	66	79·0	64·6	<0·001	0·083	0·653
Carcass weight	−168	61	−229	55	281	41	77·9	40·8	<0·001	0·002	0·154
Carcass CP	−20·2	13·1	−37·9	11·9	51·2	8·9	77·4	8·79	<0·001	<0·001	0·090
Carcass fat	−60·1	13·6	−55·7	12·4	58·2	9·3	62·4	9·18	<0·001	0·280	0·701
Carcass water	−84·3	42·9	−132·9	39·0	161·3	29·2	73·8	28·86	<0·001	<0·001	0·214
Carcass ash	−2·0	3·4	−0·5	3·1	8·2	2·3	32·2	2·25	0·004	0·149	0·645
Carcass energy (MJ/d)	−2·83	0·75	−3·06	0·68	3·47	0·51	71·3	0·501	<0·001	0·294	0·341
Carcass composition at slaughter (g/kg carcass)											
CP	209·5	8·1	202·1	7·4	−6·9	5·6	24·8	5·47	0·818	0·006	0·413
Fat	16·0	14·2	30·1	13·0	42·8	9·7	48·2	9·58	0·002	0·003	0·661
Fat:protein	0·06	0·07	0·15	0·06	0·23	0·05	56·4	0·047	0·002	<0·001	0·460
Water	712·4	19·2	700·6	17·5	−30·4	13·1	18·8	12·92	0·100	0·049	0·909
Ash	56·3	4·2	62·6	3·8	−7·1	2·9	65·5	2·81	<0·001	<0·001	0·528
Energy (MJ/kg carcass)	5·50	0·63	5·88	0·57	1·52	0·43	33·3	0·421	0·006	0·052	0·885

RSD, residual standard deviation; LW, live weight; LD, longissimus dorsi; ST, semitendinosus; KKCF, kidney knob and channel fats; CP, crude protein.
*The MEI·Diet term was not included in the model that generated the rest of the data in this table.
†Final LW was predicted from the rate of LW gain prior to entering metabolism crates using linear regression.
For details of diets and procedures, see p. 28.

Animal performance

Daily LW gains and final LW increased ($P<0·001$) with increasing MEI (Table 4). Although not significant ($P=0·083$), animals fed grass silage had lower daily LW gains than animals fed dried grass, although this was not reflected by a dietary difference in final LW. This was not due to a dietary difference in the initial LW of the steers at the start of the experiment (96·0 (SD 7·95) *v.* 93·0 (SD 6·56) kg for animals fed silage and dried grass, respectively).

Carcass and dissected tissue weights all increased ($P<0·01$) with increasing MEI (Table 4). Animals fed dried grass had significantly ($P=0·002$) higher carcass weight gains than animals fed silage and, although not significant ($P=0·086$), had heavier carcasses at slaughter. Animals fed dried grass had heavier longissimus dorsi (mean: 2·70 *v.* 2·33 kg; $P=0·021$) and semitendinosis (mean: 1·04 *v.* 0·85 g; $P=0·002$) muscles than animals fed silage at equivalent levels of MEI. Although not significant ($P=0·054$), the KKCF of animals fed grass silage was heavier than that of animals fed dried grass at equivalent levels of MEI (mean: 702 *v.* 556 g; Fig. 1). However, at *ad libitum* levels of MEI, animals fed dried grass had significantly ($P<0·001$) more KKCF than animals fed silage (1342 *v.* 927 g).

Daily gains in carcass protein, fat, water and ash all increased significantly ($P<0·01$) with increasing MEI (Table 4). The response of carcass fat gain to MEI was reflected in carcass fat content, which also increased ($P=0·002$) with increasing MEI. The increasing carcass fat content with increasing MEI presumably occurred at the expense of carcass ash content, which decreased ($P<0·001$) with increasing MEI, and carcass protein and water content, which were not affected by MEI. Daily rates of carcass protein and water gain were significantly ($P<0·001$) lower in animals fed silage, resulting in carcasses that contained significantly less crude protein (mean: 193 *v.* 201 g/kg carcass; $P=0·006$) and water (mean: 661 *v.* 673 g/kg carcass; $P=0·049$) than the carcasses of animals fed dried grass at equivalent levels of MEI. Despite the fact that there were no dietary differences in the daily rates of carcass fat, ash and energy gain, the carcasses of animals fed silage contained significantly more fat (mean: 86 *v.* 72 g/kg carcass; $P=0·003$) and ash (mean: 53 *v.* 47 g/kg carcass; $P<0·001$) and, although not significant ($P=0·052$), they contained more energy (mean: 7·77 *v.* 7·44 MJ/kg carcass) than the carcasses of animals fed dried grass at equivalent levels of MEI. The dietary differences in carcass fat and protein composition resulted in animals fed grass silage having significantly ($P<0·001$) greater carcass fat:protein ratio than animals fed dried grass at equivalent levels of MEI (mean: 0·45 *v.* 0·36; Fig. 1). There were no significant differences ($P>0·05$) in carcass fat and protein contents, and thus in the carcass fat:protein ratio, between animals fed *ad libitum* on silage and dried grass.

Metabolism

Fat metabolism. There was no effect ($P>0·05$) of diet on any of the rates of whole-body fat metabolism measured, i.e.

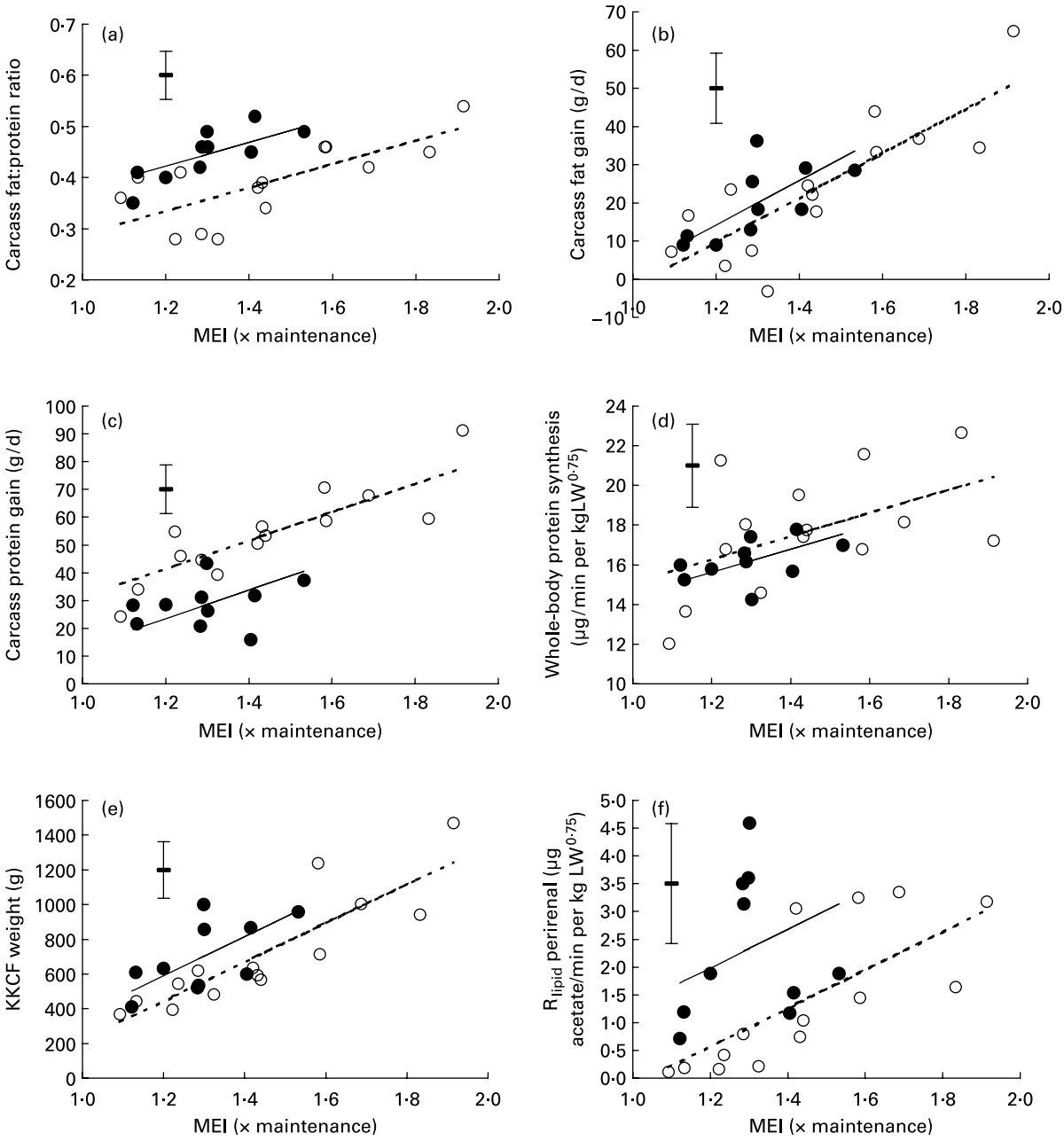

Fig. 1. The relationships between metabolisable energy intake (MEI; × maintenance) and carcass fat:protein ratio (a), carcass fat (b), protein gain (c), whole-body protein synthesis (d), kidney knob and channel fats weight (KKCF; e) and the rate of acetate incorporation into the total lipid of perirenal adipose tissue (f) in cattle fed grass silage (●) or dried grass (○). Also shown are the lines through the silage (—) and dried grass (----) data from the fitted model $Y = \alpha + \beta_1 MEI + \beta_2 Diet$, the constants and coefficients of which can be found in Tables 4, 5 and 6. Values are means with the residual standard deviation shown by vertical bars (21 df).

palmitate entry, palmitate oxidation and glycerol entry, or on plasma concentrations of NEFA and glycerol (Table 5). There was a significant ($P=0{\cdot}033$) effect of MEI on the rate of palmitate oxidation, which decreased with increasing MEI. Although not significant ($P=0{\cdot}055$), plasma NEFA concentrations decreased with increasing MEI.

Rates of acetate incorporation into total lipid of all three adipose tissue depots measured increased ($P<0{\cdot}05$) with increasing MEI (Table 6). The rate of acetate incorporation into the total lipid of perirenal adipose tissue was significantly ($P=0{\cdot}007$) greater in animals fed silage than in animals fed dried grass at equivalent levels of MEI (mean: 2·33 *v.* 0·91 μg acetate/min per g lipid; Fig. 1). However, there was no difference ($P>0{\cdot}05$) between animals fed *ad libitum* on silage and dried grass, and no effect of diet on the rates of acetate incorporation into the total lipid of subcutaneous ($P=0{\cdot}484$) and omental ($P=0{\cdot}398$) adipose tissue.

Protein metabolism. Animals fed dried grass had significantly ($P<0{\cdot}001$) higher rates of leucine entry (mean: 10·23 *v.* 8·37 μmol/min per kg $LW^{0{\cdot}75}$) and oxidation (mean: 3·00 *v.* 1·41 μmol/min per kg $LW^{0{\cdot}75}$) and significantly ($P<0{\cdot}001$) higher plasma leucine concentrations

Table 5. Constants (α) and coefficients (β) from the regression of parameters of whole-body metabolism measured in steers fed either silage or dried grass *v.* metabolisable energy intake (MEI)

(Mean values with their standard errors)

	α				β						
	Dried grass		Silage						*P* value		
	Mean	SE	Mean	SE	Mean	SE	r^2	RSD (21 df)	*MEI*	*Diet*	*MEI·Diet**
$RaCO_2$ (μmol/min per kg $LW^{0·75}$)†	605	238	631	217	542	163	29·7	160·5	0·003	0·718	0·980
RaPalmitate (μmol/min per kg $LW^{0·75}$)	1·68	0·52	1·75	0·47	−0·44	0·35	2·1	0·349	0·149	0·62	0·324
Palmitate oxidation (μmol/min per kg $LW^{0·75}$)	0·98	0·34	1·04	0·31	−0·45	0·23	13·3	0·227	0·033	0·555	0·430
RaGlycerol (μmol/min per kg $LW^{0·75}$)	8·73	3·49	9·50	3·12	4·84	2·38	8·8	2·273	0·072	0·479	0·227
RaAcetate (μmol/min per kg $LW^{0·75}$)	21·6	55·9	38·6	50·9	151·0	38·1	37·5	37·62	0·001	0·316	0·116
RaLeucine (μmol/min per kg $LW^{0·75}$)	4·91	1·47	3·04	1·34	4·09	1·00	68·9	0·987	<0·001	<0·001	0·488
Leucine oxidation (μmol/min per kg $LW^{0·75}$)	0·96	0·73	−0·63	0·66	1·57	0·50	79·4	0·489	<0·001	<0·001	0·558
Protein synthesis (g/kg $LW^{0·75}$ per d)	9·20	3·12	8·57	2·84	5·88	2·13	27·0	2·101	0·005	0·499	0·660
Plasma NEFA concentration (μm)	208·3	56·7	210·0	51·6	−72·8	38·7	8·5	38·17	0·055	0·921	0·176
Plasma glycerol concentration (μm)	14·14	4·08	14·49	3·72	4·10	2·78	0·9	2·747	0·159	0·773	0·315
Plasma leucine concentration (μm)	181·0	22·9	85·5	20·8	6·3	15·6	90·8	15·41	<0·001	<0·001	0·459
Plasma acetate concentration (μm)	627·0	145·0	444·0	132·0	268·1	99·2	60·6	97·83	<0·001	<0·001	0·675
Plasma insulin concentration (U/ml)	1·05	3·24	−2·81	2·98	4·73	2·21	54·5	2·138	0·002	0·001	1·000

RSD, residual standard deviation; Ra, entry rate.
*The MEI·Diet term was not included in the model that generated the rest of the data in this table.
†The calculation used to determine $RaCO_2$ did not correct for CO_2 sequestered in the body.
For details of diets and procedures, see p. 28.

Table 6. Constants (α) and coefficients (β) from the regression of rates of acetate incorporation into the total lipid (R_{lipid}, μg acetate/min per g lipid) of different adipose tissue depots of steers fed silage or dried grass *v.* metabolisable energy intake (MEI)

(Mean values with their standard errors)

	α				β						
	Dried grass		Silage						*P* value		
Depot	Mean	se	Mean	se	Mean	se	r^2	RSD (21 df)	MEI	Diet	MEI·Diet*
Subcutaneous	−10·03	2·08	−9·61	1·86	8·89	1·40	66·0	1·280	<0·001	0·484	0·467
Omental	−7·00	1·47	−6·62	1·34	6·01	1·00	60·6	0·990	<0·001	0·398	0·434
Perirenal	−3·58	1·61	−2·16	1·46	3·46	1·10	34·7	1·081	0·033	0·007	0·392

RSD, residual standard deviation.
*The *MEI·Diet* term was not included in the model that generated the rest of the data in this table.
For details of diets and procedures, see p. 28.

(mean: 189·15 *v.* 93·66 μM) than animals fed silage at equivalent levels of MEI (Table 5). There was no effect ($P=0·499$) of diet on the rate of whole-body protein synthesis (Fig. 1). All parameters of protein metabolism measured increased ($P<0·01$) with increasing MEI.

Plasma acetate kinetics. Plasma acetate entry rate and plasma acetate concentration (Table 5) increased ($P<0·001$) with increasing MEI. There was no effect ($P=0·316$) of diet on plasma acetate entry rate. However, animals fed silage had significantly ($P<0·001$) lower plasma acetate concentrations than animals fed dried grass at equivalent levels of MEI (mean: 792·5 *v.* 975·9 μM). Consequently the calculated plasma acetate clearance rates for animals fed silage were significantly ($P=0·004$) greater than those calculated for animals fed dried grass at equivalent levels of MEI (mean: 239·5 *v.* 176·9 ml/min per kg $LW^{0·75}$). There was no difference in acetate clearance rate between animals fed *ad libitum* on silage and dried grass.

Plasma insulin concentration. Animals fed dried grass had significantly ($P=0·001$) higher plasma insulin concentrations than animals fed silage (mean: 7·19 *v.* 3·33 U/ml) at equivalent levels of MEI (Table 5). Plasma insulin concentrations increased ($P=0·002$) with increasing MEI.

There were no significant interactions between MEI and diet for any of the parameters measured, i.e. there were no difference between the two diets in the responses to increasing MEI.

Discussion

The present experiment had two objectives. The first was to determine whether rates of fat accretion in cattle fed grass

silage are higher than in cattle fed dried grass and thus whether this, together with impaired rates of protein accretion, contributes to the high carcass fat:protein ratio of cattle fed grass silage. The second objective was to determine whether or not responses to increasing MEI differ between grass silage and dried grass diets. To fulfil these objectives measures of fat and protein metabolism, using isotope dilution and incorporation techniques, were combined with measures of carcass composition in young growing cattle fed either grass silage or dried grass over a range of equivalent MEI levels.

Before discussing the results for the present experiment there are a number of points relating to the experimental design and methodology worth considering. First, the range of MEI fed was achieved by changing the amount of feed offered rather than by changing the composition of the diet. While this ensured that the proportion of nutrients in the diet remained unchanged, it did mean that MEI was not the only dietary component changed, and thus intake effects were not limited to ME. Second, the experiment relied on the assumption that metabolism, measured in animals confined to metabolism crates and fed hourly, reflects that responsible for carcass composition, which would have been largely defined by the growth period outside metabolism crates, during which time they were fed twice daily. It is acknowledged that the direct relevance of metabolism measurements made on hourly fed animals in practical production systems could be challenged (Thorp *et al.* 2000).

Carcass composition

The experiment confirmed that the carcasses of cattle fed grass silage do indeed have a high carcass fat:protein ratio. This was shown to be true when carcass fat:protein ratios of cattle fed grass silage were compared with those fed dried grass at equivalent levels of MEI. Interestingly, there was no difference in carcass fat:protein ratio between animals fed *ad libitum* on the two diets. This was attributable to the combined effects of the increase in fat:protein ratio with increasing MEI (Table 4) and the greater MEI of animals fed *ad libitum* on dried grass compared with grass silage (Table 3). This result may provide a plausible explanation as to why in some comparative carcass composition experiments, involving cattle fed grass silage- and dried grass-based diets, no differences in carcass fat content have been reported, as these experiments did not use diets formulated to be isoenergetic (Steen & Moore, 1988, 1989; Steen, 1991). Studies that have reported differences in carcass fat content between cattle fed silage-based diets and dried forage-based diets have all fed isoenergetic diets (Lonsdale, 1976; Moore & Steen, 1983; Steen, 1991).

Protein metabolism

Clearly, a high carcass fat:protein ratio can be the result of a limited rate of protein deposition, an increased rate of fat deposition, or a combination of both. That protein deposition is limited in cattle fed grass silage is well established (Gill *et al.* 1987), and the performance data (Table 4) from the present experiment clearly support this, with cattle fed grass silage having lower daily carcass protein gains than cattle fed dried grass. However, there was no difference in rates of whole-body protein synthesis between the diets. If it is assumed that skeletal muscle accounts for a fixed proportion of whole-body protein synthesis, e.g. 20 % (Lobley, 1993), irrespective of diet and level of MEI, and that carcass protein is predominantly associated with skeletal muscle, then the ratio of carcass protein gain:synthesis in cattle fed grass silage was lower than that for cattle fed dried grass (0·24 (SD 0·06) *v.* 0·39 (SD 0·09), respectively; $P<0·001$), i.e. rates of carcass protein turnover were greater in cattle fed grass silage. However, this is unlikely as turnover rates are normally positively associated protein accretion rates (Lobley *et al.* 1987), and thus one would expect turnover rates to be greatest for the dried grass diet. In any case, where animals are fed different diets and at different levels of MEI, the assumption that skeletal muscle accounts for a fixed proportion of whole-body protein synthesis would be inappropriate. It has been suggested that a relationship between dietary fibre content and gut protein turnover exists (Seal & Parker, 2000). While there was little difference in neutral detergent fibre concentration between the two diets, the grass silage contained approximately 6 % more acid detergent fibre. It is therefore possible that gut protein turnover in cattle fed grass silage accounted for a larger proportion of the whole-body protein synthesis than in cattle fed the dried grass.

Based on the current understanding of the problem, i.e. the limited protein deposition in cattle fed grass silage, it was hypothesised that cattle fed grass silage would have had lower rates of whole-body protein synthesis. This is because amino acid intake and rates of protein synthesis have been shown to be positively related (Reeds & Davis, 1992) – in cattle fed grass silage flows of duodenal amino acids have been shown to be limiting (Baker *et al.* 1985; Gill *et al.* 1987; Thomas & Gill, 1988; Veira *et al.* 1994). Based on the lack of a dietary difference in the rate of whole-body protein synthesis, it might therefore be concluded that in the present experiment duodenal flows of amino acids were similar for the two diets. This is unlikely though, as the greater leucine entry and oxidation rates, and greater plasma leucine concentrations of animals fed dried grass (Table 5), are indicative of greater leucine absorption on this diet, as these parameters have been shown to be a function of intake (Meguid *et al.* 1986; Hammond *et al.* 1987; Lapierre *et al.* 2002; Savary-Auzeloux *et al.* 2003). This is supported by the effect of MEI, and thus protein intake as the relative composition of the diets remained unchanged, on these parameters associated with whole-body protein metabolism.

Based on the evidence of previous reports and the evidence of the present experiment, the limited rates of carcass protein gain, the lower plasma leucine concentration and the lower leucine entry and oxidation rates of cattle fed grass silage, it therefore seems likely that the result for rates of whole-body protein synthesis is misleading through failure to reveal a dietary difference. Based on the residual standard deviation (2·101) and degrees-of-freedom (21) for rates of protein synthesis (Table 5), it can be calculated that the least significant difference ($P=0·05$) between two means required would have been 1·78 g/kg $LW^{0·75}$ per d, i.e. a 10·5 % difference. Where differences as small as 0·1 % in the rate of protein synthesis can effect rates of tissue protein gains of 20–100 % (Lobley, 1993), the sensitivity of the isotope dilution technique as used to measure rates of whole-body protein synthesis rates is clearly inadequate.

Fat metabolism

Evidence to support the hypothesis that rates of fat accretion in cattle fed grass are elevated and thus contribute to the high carcass fat:protein ratios was inconclusive. There was no difference in the rate of carcass fat gain between animals fed grass silage and dried grass. The lack of a dietary difference in the rate of lipogenesis in adipose tissue from the subcutaneous depot, a depot associated with the carcass, corroborates this result. Thus, the higher concentration of carcass fat in cattle fed grass silage would appear to be due to the effect of the reduced rates of protein and water accretion on the proportions of the carcass constituents measured. The fact that young, growing animals were used in this experiment, combined with their overall relatively poor rates of LW gain (Table 4), could explain the lack of a dietary effect on rates of carcass fat gain. However, the reason for using young, growing animals was that it was in similarly aged animals that Lonsdale (1976) reported differences in carcass composition. Also, if rates of fat deposition were elevated in young animals then this could have important implications on animal mature size (Owens *et al.* 1993).

However, the experiment does provide evidence for elevated rates of fat accretion in non-carcass fat depots. There was a trend ($P=0{\cdot}054$) for cattle fed grass silage to have more KKCF than cattle fed dried grass, a result given credence by the higher rates of lipogenesis measured in adipose tissue from the perirenal depot. As for protein accretion, fat accretion is dependent upon the relative rates of lipogenesis and lipolysis and therefore an increase in the rate of lipogenesis does not necessarily infer an increase in the rate of fat accretion. However, the fact that there was this trend for a dietary difference in the KKCF weight, and that there was no difference in the rate of glycerol appearance, an index of lipolysis, suggest that the difference is indicative of an increased rate of fat accretion in this depot. Lonsdale (1976) similarly reported that dietary differences (silage *v.* silage plus dried grass diets) in fat deposition appeared to be more marked in non-carcass than carcass components in young cattle (approximately 110 kg LW at slaughter).

It is possible that the result is a function of depot activity, depot activity being related to the stage of animal maturity. Carcass fat depots have been shown to be late-developing relative to non-carcass depots (Cianzio *et al.* 1985; Scollan *et al.* 2003). Therefore, it is tempting to speculate that had the animals been allowed to advance in maturity before slaughter, dietary differences in rates of carcass fat accretion may have been observed as they were for the perirenal depot.

Lonsdale (1976) proposed that elevated rates of fat accretion in cattle fed grass silage are the result of a limiting supply of amino acids for protein synthesis on this diet, with the consequent increase in energy available above maintenance stored as fat. This has been supported by experiments where reductions in carcass fat content have been achieved in cattle fed grass silage by increasing the duodenal supply of amino acids, through supplementation (Baker *et al.* 1985, 1992) and formaldehyde treatment (Thompson *et al.* 1981).

The principal energy substrate in ruminants is acetate. Animals fed grass silage had lower plasma concentrations of acetate than animals fed dried grass. Differences in circulating acetate concentrations normally arise through differences in rumen (Pethick *et al.* 1981) and endogenous acetate production (Cronjé *et al.* 1991). There was no dietary difference in acetate entry rate, yet animals fed grass silage had lower plasma acetate concentrations. This implies that animals fed grass silage had greater clearance rates (acetate disappearance rate, which under 'steady-state' conditions is assumed to equal acetate entry rate divided by plasma acetate concentration) of acetate than animals fed dried grass (239 (SD 61) *v.* 185 (SD 28) ml/min per kg $LW^{0{\cdot}75}$, respectively; $P<0{\cdot}01$). However, there is no obvious explanation for this.

Insulin

Hormones are important mediators of the effect of diet on growth and development. In the present experiment plasma insulin concentrations were measured. The increase in plasma insulin concentration with increasing MEI is consistent with the understanding that insulin status in ruminants is regulated by energy and amino acid supply (Lobley, 1992). The fact that animals fed dried grass had higher concentrations of insulin than animals fed grass silage at equivalent levels of MEI could indicate that there was a dietary difference in the duodenal supply of amino acids in the present study, as discussed earlier, although insulin release is believed to be more affected by propionate than amino acid absorption (Mineo *et al.* 1994; Gonda *et al.* 1997).

There was a significant correlation between plasma insulin concentration and both carcass protein gain (r 0·722, $P<0{\cdot}001$) and carcass fat gain (r 0·475, $P<0{\cdot}05$), which is consistent with insulin's role as a modulator of feed induced anabolic activity. Breier & Gluckman (1991) suggested that the importance of insulin as a regulator of growth may be increased when nutrients for growth are limiting. In the present experiment there was no evidence of an effect of MEI on the significance of the correlation between insulin and carcass protein and fat gain. The strength of the correlation with carcass protein gain might suggest a greater role for insulin in directing protein metabolism than for fat metabolism. This suggestion is supported by the higher plasma insulin concentrations in animals fed dried grass, which had higher rates of protein gain than animals fed grass silage. Thorp *et al.* (2000) reported higher plasma insulin concentrations in cattle fed grass silage supplemented with barley, diets for which the group had previously reported reduced carcass fat: protein ratios, than cattle fed unsupplemented silage. Indeed, the role of insulin in directing fat metabolism in ruminants is uncertain; for example, Smith *et al.* (1992) were unable to demonstrate a correlation between insulin and fatty acid synthesis *in vitro*, while Mills *et al.* (1989) were able to.

The importance of the growth hormone/insulin-like growth factor I (IGF-I) axis as a major factor controlling postnatal growth and its interaction with nutritional status is well documented. Plasma growth hormone and IGF-I concentrations were not measured in the present experiment. However, circulating insulin concentrations have been shown to broadly correlate with circulating IGF-I concentrations (Dawson *et al.* 1998), and thus it is logical to speculate that animals fed dried grass might have had higher plasma IGF-I concentrations. IGF-I production is believed to be associated with the hepatic response to growth hormone, an association modulated by nutritional status (Brameld *et al.* 1996) and insulin

(McGuire *et al.* 1995). As IGF-I is believed to mediate the protein anabolic effects of growth hormone (Douglas *et al.* 1991), it is likely that the greater rates of carcass protein gain in animals fed dried grass compared with grass silage were in part mediated by this hormone.

Metabolisable energy intake

The objective of feeding diets over a range of MEI was to determine whether there was an interaction between form of ME, grass silage *v*. dried grass, and quantity of ME, $1{\cdot}1 \times ME_m$ to *ad libitum*, both of which are considered important determinants of animal performance (Beever *et al.* 1988; Thomas & Gill, 1988; Steen, 1992; Steen & Robson, 1995). As the overall efficiency of energy (Thomas & Chamberlain, 1990) and amino acid (Beever *et al.* 1992; MacRae *et al.* 1995) utilisation of animals fed grass silage is considered to be poor, it was hypothesised that animals fed the different diets would respond differentially to changes in MEI. However, the lack of an interaction between diet and MEI for any of the parameters measured refutes this hypothesis. The fact that the ratio of dietary components remained constant irrespective of MEI may explain this result, as the inefficiencies in nutrient utilisation have been related to imbalances in nutrient supply (Beever *et al.* 1992).

The fact that linear regression best described the response to MEI for all the parameters measured indicates that there was no threshold requirement for MEI below which responsiveness was minimal. This is contrary to what has been reported by Smith *et al.* (1992), who did demonstrate the presence of a threshold requirement for MEI for *in vitro* adipose tissue anabolic activities, but not for *in vivo* measures of N metabolism. However, their range of MEI did include below maintenance requirement levels of MEI, whereas the present experiment did not.

With regard to the rates of acetate incorporation into the total lipid of subcutaneous, omental and perirenal adipose tissue *per se*, there did appear to be a differential response to MEI (Table 6). At low MEI acetate incorporation into lipid was greatest in perirenal adipose tissue, whereas at high intakes the greatest rates of acetate incorporation into lipid were measured in subcutaneous adipose tissue. Rates of acetate incorporation into the omental adipose tissue were low to intermediate at all levels of intake. This would imply that when nutrient supply is limiting non-carcass depots are favoured over carcass depots; in other words, that depot-specific metabolic activity is not only determined by the animal's stage of maturity, as mentioned earlier, but also by levels of nutrient supply. However, Broad & Ulyatt (1980) reported no differences in the relative rates of *in vivo* lipogenic activity between depots in sheep fed at maintenance, $1{\cdot}3 \times$ maintenance and *ad libitum*.

Summary

From the results of the present study it was concluded that the high carcass fat:protein ratios of young growing steers is the result of limited rates of protein accretion. While there was evidence that rates of non-carcass fat accretion were elevated in cattle fed grass silage, there was no evidence to support the hypothesis that, in cattle of the age used in the present experiment, elevated rates of carcass fat accretion contribute to the high carcass fat:protein ratio. The lack of any interaction between diet and MEI suggests that the efficiency of ME utilisation is dependent upon form, form including the source of the ME and ratio of ME relative to the other dietary components, rather than quantity.

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