Kinetic aspects of calcium metabolism in lactating sheep offered herbages with different Ca concentrations and the effect of protein supplementation

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1. Two experiments are described in which kinetic aspects of calcium metabolism were studied in housed lactating sheep consuming different fresh herbage species. The importance of protein supply was also investigated.

2. In Expt. 1, two groups (*n* 4) were offered, *ad lib.*, a freshly cut ryegrass (*Lolium perenne* L.)–white clover (*Trifolium repens* L.) pasture containing 5·48 g Ca/kg dry matter (DM). One group was supplemented daily with 100 g protected casein. A third group (*n* 4) was offered, *ad lib.*, freshly cut oats–Tama ryegass (*Lolium multiflorum* L.) herbage which had a lower Ca content of 3·07 g Ca/kg DM. Stable Ca and nitrogen balances were carried out during the first 7 weeks of lactation. At this stage 180 μCi ^{45}Ca were administered for Ca kinetic studies.

3. In Expt 2, eight sheep were offered, *ad lib.*, a fresh ryegrass–white clover pasture, and paired on the basis of their udder size. One member of each pair was supplemented daily with 100 g casein via the abomasum and the amount of milk removed was equalized between pairs. Ca and N balances (12 d) and Ca kinetic studies (280 μCi ^{45}Ca) were carried out during weeks 2 and 5 of lactation.

4. Rate of absorption of Ca increased, while rate of Ca secretion in milk and resorption from bone decreased as lactation progressed. Ca balances changed from negative to positive as lactation progressed in sheep offered ryegrass–white clover, but, while improving, were always negative in sheep offered oats–Tama ryegrass. Protein supplementation increased (18%) milk production of the ewes in Expt 1 and their retention of N in Expt 2.

5. The proportion of utilized Ca derived from the diet, as opposed to the skeleton, tended to increase as a result of protein supplementation.

6. Availability of Ca from ryegrass–white clover ranged from 0·19 to 0·32, even though only 50% of the net Ca requirement was derived from the diet. Availability of Ca from the oats–Tama ryegrass diet was similar, though in this case less than 20% of the net Ca requirement was derived from the diet. It was concluded that availability of Ca from forage diets may be lower than previously anticipated.

7. Faecal endogenous loss ranged from 16 to 40 mg Ca/kg body-weight per d, and was similar on both diets.

8. These and other findings are used to discuss more fully the subject of Ca nutrition in sheep, in particular, the implications of the strong homeostatic control of Ca absorption and the influence of protein status on the relative contribution of the diet and the skeleton in meeting the net Ca requirement of the ewe during lactation.

Estimates of the calcium requirement of grazing ruminants have been derived largely from information obtained from animals consuming conserved forages and concentrate feedstuffs rather than fresh pasture. This situation is unsatisfactory, particularly in view of the possible presence of rachitogenic factors in green forages (Ewer & Bartram, 1948; Grant & O'Hara, 1957) and the recent quantitative evidence for low availability of Ca in green feed oats when offered to red deer stags (Muir *et al.* 1987).

The interdependence of dietary Ca and protein for skeletal matrix growth and skeletal mineralization has been documented in rodents (Frandsen *et al.* 1954; El Maraghi *et al.* 1965) and in farm animals during pregnancy (Sykes & Field, 1972). More recently

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substantial negative Ca balances were observed in lactating ewes (Sykes & Geenty, 1986) despite unlimited access to high-quality pasture with a supposedly adequate mineral content. As the negative Ca balances were associated with large negative N balances it was postulated that this rather than a mineral deficiency *per se* was responsible for the negative balances.

The present study describes several experiments designed to investigate kinetic aspects of Ca metabolism in lactating ewes offered fresh herbages differing in Ca content. Further, an attempt was made to reverse the negative Ca balance associated with early lactation by protein supplementation.

EXPERIMENTAL
Expt 1
Animals, experimental design and management. Twelve Dorset sheep, 2 years of age, were brought indoors at about 130 d of gestation. At 2 d after parturition they were treated with an anthelmintic (5 mg/kg body-weight, Fenbendazole; Coopers Animal Health (NZ) Ltd) and placed in individual metabolism crates.

A schematic outline of the experimental design is shown in Fig. 1. All animals were initially offered, *ad lib.*, freshly cut ryegrass (*Lolium perenne* L.)–white clover (*Trifolium repens* L.) pasture. They were machine-milked twice daily after intramuscular injection of 10 i.u. oxytocin (Intravet Pty Ltd, Australia) to stimulate milk let down. A balance trial was conducted during 10–20 d after parturition. The sheep were then randomly divided into three groups of four animals. Two groups continued to be offered the ryegrass–white clover herbage, but one group was supplemented orally with a protected casein supplement. The third group was offered a freshly cut oat–Tama ryegrass (*Lolium multiflorum* L.) herbage with lower Ca but similar N content to that of the ryegrass–white clover. During six consecutive balance periods faeces were collected into polyethylene-lined bags attached to the body by harnesses. Urine was diverted into storage containers by bladder catheters. Intakes, feed residues and the amounts excreted in the faeces and urine along with milk production were measured. Suitable subsamples were taken for chemical analysis and stored at $-18°$. Urine and faeces samples were bulked within each balance period, except when [45]Ca was administered, then daily samples were kept.

[45]*Ca kinetic studies.* At the beginning of balance 5, 7 weeks after parturition, three sheep in each group were given, as a single dose via an indwelling catheter placed in the right jugular vein, 180 μCi [45]Ca as $CaCl_2.2H_2O$ (Amersham International plc, Amersham) in 20 ml sterile saline (9 g sodium chloride/l). Blood samples were then taken from an indwelling catheter in the left jugular vein at accurately recorded time intervals of about 1, 2·5, 5, 10, 20, 40, 60, 80 min after the administration of [45]Ca. Further samples were then taken at 2, 4, 6, 9, 12, 18, 24, 27, 30 h and then daily at 09.00 hours for the next 14 d. The plasma collected was stored at $-20°$.

Expt 2
Animals, experimental design and management. Nine Poll Dorset ewes, 2–3 years of age, were brought indoors at 100 d of gestation and two-way balloon catheters (Folatex Eschmann) fitted into the abomasum using the techniques of T. N. Barry (personal communication). The sheep were then returned to pasture until 10 d before parturition when they were placed in individual pens, indoors, for lambing. Parturition was synchronized by induction at about 125 d of gestation using 20 mg oestradiol benzoate (Intervet Pty Ltd, Australia). On the day after parturition the ewes were treated with an anthelmintic and placed in individual metabolism crates.

Two groups, each containing one member of four pairs of sheep selected on the basis of
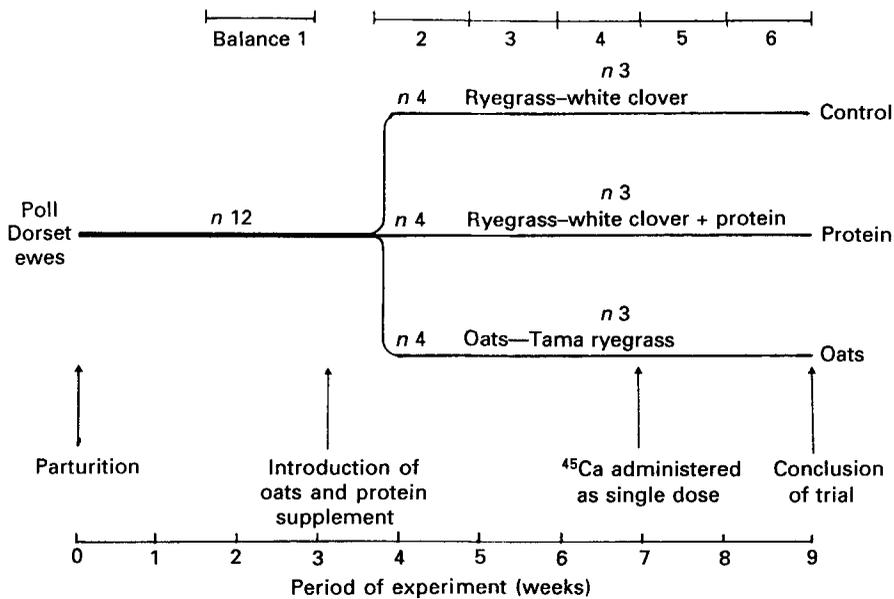
Balance 1 2 3 4 5 6

n 3
n 4 Ryegrass–white clover ———————— Control

Poll
Dorset ————— n 12 ——— n 4
ewes

n 3
Ryegrass–white clover + protein ——— Protein

n 3
n 4 Oats—Tama ryegrass ———————— Oats

Parturition Introduction of oats and protein supplement ^{45}Ca administered as single dose Conclusion of trial

0 1 2 3 4 5 6 7 8 9
Period of experiment (weeks)

Fig. 1. Design of Expt 1.

body condition and udder volume, were offered ryegrass–white clover herbage. One member of each pair received continuous intra-abomasal infusion of casein (protein group). Its pair (control group) received a similar volume of infusate containing the same concentrations of phosphorus and sodium.

Milk production within pairs was equalized. Control sheep were milked first, the volume of milk recorded and the same volume subsequently taken from its pair.

Kinetic and stable Ca balance studies. Two 9 d balance studies were carried out, commencing during weeks 2 and 5 of lactation when feed intake, feed refusals and the amounts of faeces and urine excreted as well as milk output were measured. Suitable subsamples of feed, faeces, urine and milk were taken for chemical analysis and stored at $-18°$. At the start of each balance period 280 μCi ^{45}CaCl$_3$.2H$_2$O were administered to each sheep. Blood was collected at 1, 5, 10, 20, 40, 60, 80, 100 and 120 min after injection. Further samples were obtained at 2 h intervals until 12 h after injection, then at 8 h intervals for 7 d, and then 12 h intervals until the end of the balance period.

Diets and protein supplements

In Expt 1 a mixed herbage of 'Grasslands Ruanui' perennial ryegrass and 'Grasslands Huia' white clover, or a mixed herbage of oats and 'Grasslands Tama' westerwolds tetraploid ryegrass was offered to the sheep. Both herbages were cut daily at a height of 100–200 mm and offered twice daily (09.00 and 17.00 hours) to provide *ad lib.* intakes. The protein supplement, given at the rate of 100 g/d, was formaldehyde-protected casein. This was prepared by mixing 100 ml formaldehyde (formaldehyde–water, 1:10, v/v) with 1 kg casein (Stobbs *et al.* 1977). The product was then air-dried at 50° for 2 h and administered orally as a paste.

In Expt 2 a 'Grasslands Ariki' ryegrass (*Lolium* × *hydridum* Hausskn) and 'Grasslands Huia' white clover were offered to provide a dry matter (DM) intake of 1·6 kg/d. The protein supplement was sodium caseinate (non-protected) and was administered intra-abomasally by gravity feed from an overhead reservoir by a modification of the method of
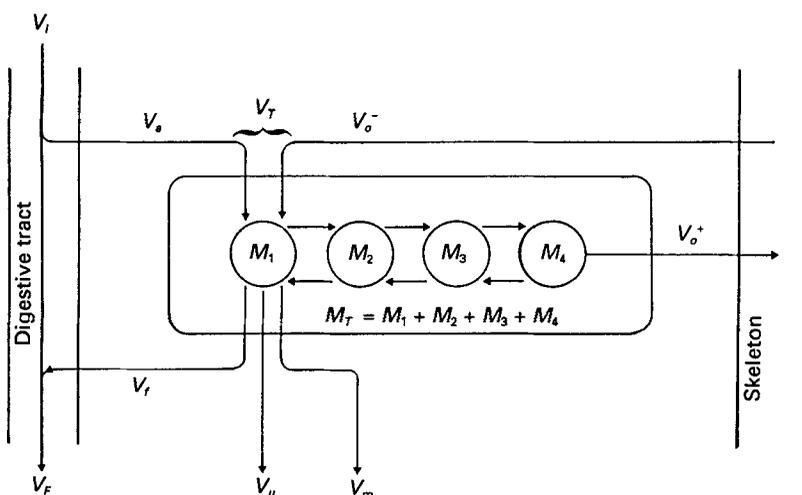
Fig. 2. The model of calcium metabolism. M_1, M_2, M_3 and M_4 are compartments of the exchangeable Ca pool M_T. M_1 is the site of tracer administration and is sampled via the blood. Inflows of Ca entering M_1 are from the gut (V_a) and bone (V_o^-) while outflows from M_1 are losses to milk (V_m), urine (V_u) and endogenous faecal losses (V_f). M_4 is the site of Ca loss (V_o^+) in the non-exchangeable bone, V_i is Ca intake, V_T is Ca transport and V_F is total Ca in faeces.

Grobbelaar et al. (1981). The objective was to achieve an infusion rate of 100 g/d in 1 litre water uniformly distributed during 24 h. A solution with P and Na contents similar to the casein infusate was given to the pair-mate.

Analytical procedures

Dried and ground samples of diet and faeces from Expt 1 were wet ashed in a concentrated nitric acid–perchloric acid (700–720 ml/l) (5:1, v/v) mixture at 150–200° and diluted in 0·1 M-hydrochloric acid for Ca determination. In Expt 2 samples of diet and faeces were dry ashed at 600° and made up to volume in 2 M-HCl. Plasma Ca was determined by atomic absorption spectroscopy in the trichloroacetic acid (TCA) supernatant fraction after precipitation in TCA (50 g/l). N in the feedstuffs, faeces, urine and milk was determined by a modification of the Kjeldahl method. P concentration in the diet was measured by the method of Kraml (1966).

The radioactivity of ^{45}Ca in the HNO_3–$HClO_4$ or HCl digests of the faeces, in urine and in the TCA supernatant fraction of milk and plasma, following addition of TCA (100 g/l), was counted using a Phillips liquid scintillation counter (model PW4700). The composition of the scintillation fluid added to the samples was that described by Braithwaite et al. (1969). Radioactivity counts were corrected for quenching using an external standard and a specific quench series for each type of sample. All radioactivity counts were corrected to zero time, that is the time the isotope was administered to the sheep, to account for natural decay.

The Ca model

The Ca model used in the present study was similar to that described by Aubert et al. (1963) and Ramberg et al. (1970) and is represented diagrammatically in Fig. 2.

The use, assumptions and limitations of the model have been discussed previously (Berman et al. 1962; Boston·et al. 1981). Basically, the simulation, analysis and modelling program of the National Institute of Health (SAAM27), was used to derive a four-compartment

series model of Ca metabolism by fitting (Chabay *et al.* 1982) the plasma decay curve of ^{45}Ca and submitting directly derived variables of Ca metabolism, i.e. urinary (V_u) excretion, milk Ca (V_m) secretion, Ca intake (V_I), total Ca excretion in faeces (V_F) and an estimate of faecal endogenous loss (V_f) derived independently from the total radioactivity received in faeces divided by the mean plasma specific activity for the same time-period. The calculations of the rate of Ca transport (V_T) and the mass of the Ca exchangeable pool (M_T) involves the assumption of a steady-state during the experiment; i.e. Ca inflow equals Ca outflow from the exchangeable pool.

The Ca outflow is represented by:

$$V_T = V_u + V_f + V_m + V_o^+,$$

and the Ca inflow by:

$$V_T = V_a + V_o^-,$$

where V_a is the absorption of Ca from the digestive tract and is calculated as the difference between Ca intake (V_I) and faecal Ca output (V_F) after correcting for the faecal Ca endogenous loss (V_f), i.e.

$$V_a = V_I - (V_F - V_f).$$

The efficiency of Ca absorption is given by V_a/V_I; V_o^+ is the accretion of Ca into bone and is determined as

$$V_o^+ = V_T - (V_u + V_f + V_m);$$

while V_o^- is the removal of Ca from stable bone and is determined as $V_o^- = V_T - V_a$.

The Ca balance (Δ) is determined from the Ca intake and Ca losses ($\Delta = V_I - [V_F + V_u + V_m]$). As about 99 % of the Ca is associated with the skeleton then the Ca balance is also equal to the difference between the Ca deposition and that removed from stable bone ($\Delta = V_o^+ - V_o^-$).

Statistics

Means are given in tables and the text with their standard errors. The differences between treatments in the ^{45}Ca kinetic values were analysed by Student's *t* test. The differences between treatments repeated in time and time-effects (periods 2–6) were analysed after adjustment, where significant, by covariance using the variable established in period 1 as covariate. The degrees of freedom were appropriately reduced to reflect the correlation between observations on individual sheep repeated in time (Rowell & Walters, 1976).

RESULTS

Expt 1

The mean Ca, P and N contents and DM digestibility (DMD) of the Ruanui perennial ryegrass–white clover pasture are given in Table 1. Ca and crude protein (N × 6·25) concentrations were higher in the ryegrass–white clover diet than in the oats–Tama ryegrass ($P < 0.001$ in both cases). There was a trend, apparent in Fig. 5 (see p. 51) for Ca concentration in the ryegrass–white clover to increase and in oats–Tama ryegrass to decline during the last three balance periods. The oats–Tama ryegrass and ryegrass–white clover had similar DMD, though there was a trend for decline in DMD in all diets with time which was greater with the oat–Tama ryegrass diet than the ryegrass–white clover. Mean DMD values declined from 0·76 to 0·65 and from 0·74 to 0·68 between the first and last two periods of the trial respectively.

Table 1. *Expts 1 and 2. Herbage composition and body-weight changes of sheep offered fresh herbages during early lactation*

(Sheep were offered fresh ryegrass (*Lolium perenne* L.)–white clover (*Trifolium repens* L.) supplemented (RG + P) or unsupplemented (RG) with casein, or were offered oats–Tama ryegrass (*Lolium multiflorum* L.). Values are means with their standard errors)

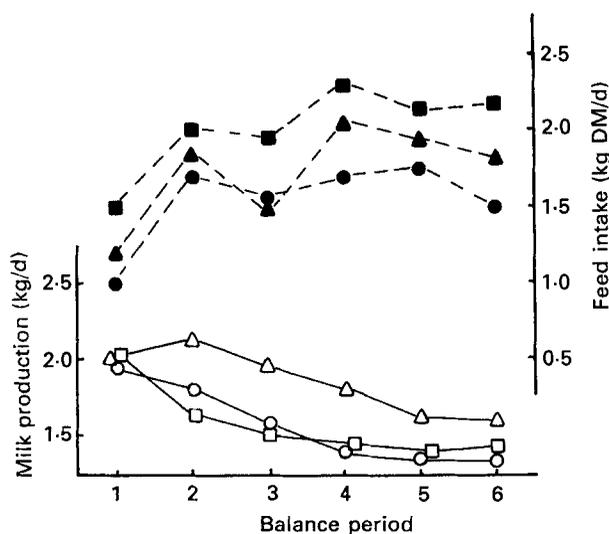|	Expt 1					Expt 2		
	RG	RG + P	Oats	SE		RG	RG + P	SE
Body-wt (kg)								
Initial	47·5	48·3	42·0	1·90		57·4	54·1	1·95
Final	47·3	48·5	52·1	2·00		56·6	58·1	2·00
Herbage composition (g/kg DM)								
	Mean	SE	Mean	SE			Mean	SE
Calcium	5·48	0·19	3·79	0·22			6·50	0·22
Phosphorus	2·95	0·17	3·03	0·19			3·12	0·16
Crude protein (nitrogen × 6·25)	222	6·0	163	5·6		Period: 1	196·4	
						2	156·2	
DMD	0·70	0·041	0·71	0·045		Period: 1	0·80	
						2	0·76	

DM, dry matter; DMD, dry matter digestibility

Fig. 3. Expt 1. Mean daily dry matter (DM) intake (■, ▲,●) and milk production (□, △,○) during six consecutive 1-week balance periods in lactating sheep offered Ruanui ryegrass (*Lolium perenne* L.)–white clover (*Trifolium repens* L.) either unsupplemented (○, ●) or supplemented with 100 g protected casein orally (△, ▲), or oats–Tama ryegrass (*Lolium multiflorum* L.) (□, ■) (*n* 4).
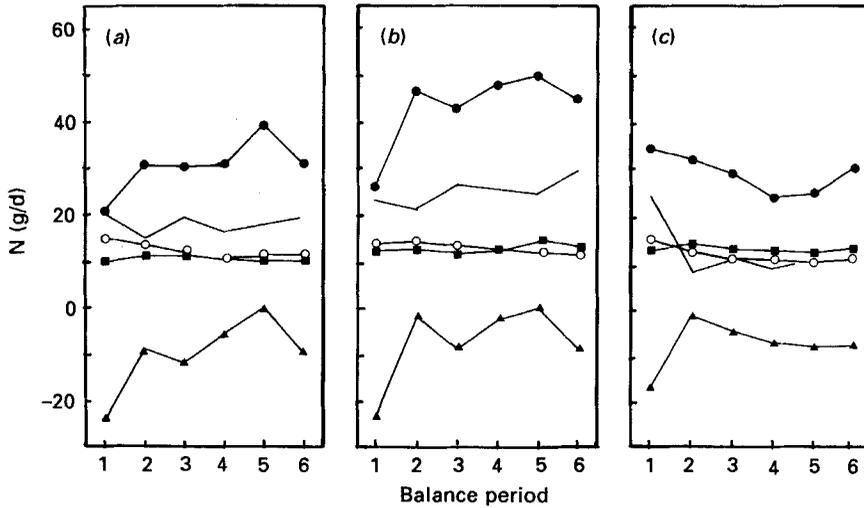
Fig. 4. Expt 1. Mean daily nitrogen intake (●), N output in faeces (■), urine (—) and milk (○) and N balance (▲) during six balance periods in lactating sheep offered either Ruanui ryegrass (*Lolium perenne* L.)–white clover (*Trifolium repens* L.) (*a*) without and (*b*) with a casein supplement, or (*c*) oats–Tama ryegrass (*Lolium multiflorum* L.) (*n* 4).
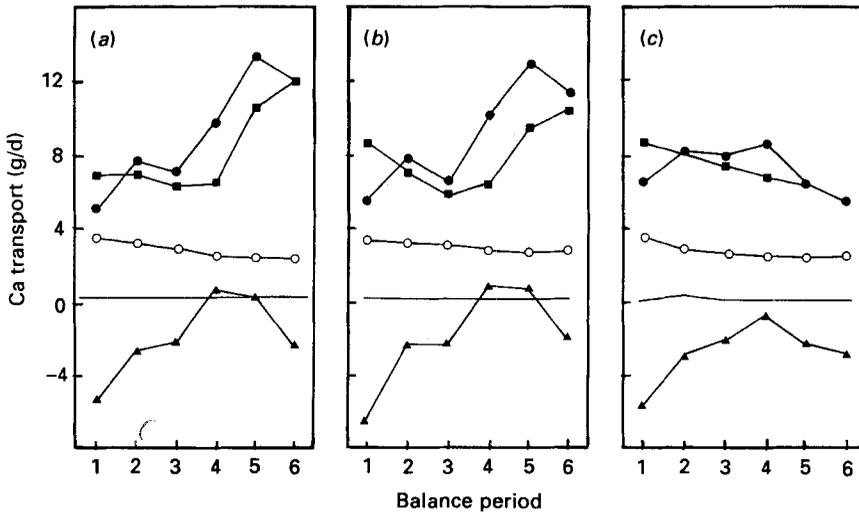

Fig. 5. Expt 1. Mean daily calcium intake (●), Ca transport in faeces (■), urine (—), milk (○) and Ca balance (▲) during six balance periods in lactating sheep offered Ruanui ryegrass (*Lolium perenne* L.)–white clover (*Trifolium repens* L.) (*a*) without and (*b*) with a casein supplement, or (*c*) oats–Tama ryegrass (*Trifolium multiflorum* L.) (*n* 4).

The mean DM intake and milk production of the groups is given in Fig. 3. Feed intake, adjusted for a significant ($P < 0.001$) covariate effect of intake during period 1, was higher ($P < 0.05$) in sheep offered oats–Tama ryegrass than in those offered perennial ryegrass–white clover, but there was no effect of protein supplementation. There was a significant time effect ($P < 0.01$) reflecting the fact that from parturition until period 4 of the trial intake gradually increased in all groups.

Milk production, after adjustment for individual milk production during period 1, was

Table 2. *Expt 1, 5th balance period. Rates of calcium transport (mg/body-weight per d) and exchangeable Ca (g/kg body-weight) in sheep offered either Ruanui ryegrass (*Lolium perenne L.*)–white clover (*Trifolium repens L.*) without and with a protected casein supplement (100 g/d by mouth), or oats–Tama ryegrass (*Lolium multiflorum L.*)*

(Mean values with their standard errors for three sheep)

	Symbol	Ruanui ryegrass– white clover		Ruanui ryegrass– white clover +casein		Oats–Tama ryegrass	
		Mean	SE	Mean	SE	Mean	SE
Intake	V_I	266·9	11·41	252·2	20·38	115·3	8·65
Faeces	V_F	238·9	19·09	204·0	28·87	115·5	5·17
Absorption	V_a	51·7	15·33	75·5	4·38	20·5	7·54
Absorption coefficient	V_a/V_I	0·19	0·06	0·30	0·06	0·17	0·02
Faecal endogenous loss	V_f	23·7	0·87	28·1	2·95	20·7	1·25
Milk	V_m	50·4	3·81	56·3	3·40	46·7	2·38
Urine	V_u	2·86	1·88	2·38	0·21	2·15	0·72
Bone accretion	V_o^+	41·7	3·76	48·5	5·26	50·4	2·89
Bone reabsorption	V_o^-	66·9	14·1	60·0	8·60	99·5	10·1
Balance	Δ	−25·2	14·1	−11·5	4·18	−49·1	7·62
Irreversible loss	V_T	118·6	17·22	135·3	5·17	110·9	10·12
Exchangeable Ca	M_T	0·29	0·107	0·30	0·068	0·32	0·038

significantly affected by treatment, due to greater milk production ($P < 0.01$) in the protein supplemented group than in the other two groups. There was, however, no significant effect of treatment on body-weight (Table 1) nor any significant change with time.

Mean intakes, outputs and balances of N for the sheep during the six balance periods are given in Fig. 4. Significant treatment differences ($P < 0.01$ in all cases) for N intake, milk and urinary N outputs were attributable to the protein supplement. A significant interaction ($P < 0.01$) between treatment and balance period for N balance reflected an improvement with time in N balance of both groups offered the ryegrass–white clover while the N balances of sheep offered oats–Tama ryegrass declined.

The Ca balances during the six periods are shown in Fig. 5. A significant interaction ($P < 0.01$) between dietary treatments and balance period for Ca intake reflected the increasing Ca intake of the sheep offered ryegrass–white clover compared with that of those offered the oats–Tama ryegrass which decreased, mainly as the result of change in dietary Ca concentration discussed earlier. There were significant effects of treatment on milk Ca and Ca balance ($P < 0.01$ in both cases). The former was due to greater Ca output in the protein-supplemented than in the other two groups and the latter due to sheep offered oats–Tama ryegrass having consistently lower balances than the other groups.

The total exchangeable Ca pool and the amounts of Ca transported to and from the exchangeable pool in sheep from the three groups during balance period 5 are given in Table 2. There was large between-sheep, within-treatment variation attributable largely to one animal in the unsupplemented ryegrass–white clover group. There was a trend for greater irreversible loss (V_T) of Ca in the protein-supplemented group compared with the two non-supplemented groups. The low Ca intake (V_I) of sheep offered the oats–Tama ryegrass diet was associated with a lower rate of Ca absorption (V_a), a higher rate of resorption of Ca from the skeleton (V_o^-) and a more-severe negative Ca balance (Δ) than the other two groups, differences which were significant ($P < 0.05$ in all cases) when compared

Table 3. *Expt 2. Dry matter (DM) intake and milk production (kg/d), and nitrogen intake, N output in faeces, urine and milk and N balance (g/d) for sheep offered Ariki ryegrass* (Lolium perenne *L.)–white clover* (Trifolium repens *L.) herbage without or with a casein supplement*

(Mean values with their standard errors for four sheep)

Week of lactation	Balance period		DM intake	Milk production	N				
					Intake	Faeces	Urine	Milk	Balance
2	1	Ryegrass–white clover	1·60	2·24	50·3	12·7	17·5	16·7	3·46
		Ryegrass–white clover + casein (70 g/d)	1·44	2·06	61·7	11·5	30·0	17·0	3·17
5	2	Ryegrass–white clover	1·64	1·56	36·0	12·0	10·3	11·7	2·01
		Ryegrass–white clover + casein (100 g/d)	1·77	1·48	53·1	13·3	20·0	12·6	7·23
		Pooled SE	0·05	0·05	1·6	0·3	0·8	0·3	1·42

with the protein-supplemented group. Correspondingly, faecal endogenous loss (V_f) was lowest in the oats–Tama ryegrass group, and there was evidence that it increased as a result of protein supplementation, though only the difference between the oats–Tama ryegrass and the supplemented group was significant ($P < 0.05$). Protein supplementation tended to increase the rate of Ca absorption (V_a) and, as a consequence, the proportion of dietary Ca absorbed (V_a/V_i). There was, however, no effect of treatment on the size of the exchangeable Ca pool (M_T).

Expt 2

The mean Ca, P and crude protein contents and DMD of the herbage are given in Table 1. The DMD of the herbage was generally high, but it did tend to be lower during period 2 than in period 1. Values for DM intake, milk production and N balance variables are given in Table 3. The adjustment of the casein infusion rates presented some difficulties and a mean rate of only 70·5 g/d was achieved during period 1 rather than the 100 g/d anticipated (see Table 3). These difficulties were overcome during period 2.

There were no significant differences between groups in DM intake, DMD or milk production during balance periods 1 and 2. DM intake tended to be lower during period 1 than in period 2, while milk production was significantly lower ($P < 0.01$) during the latter period.

Casein supplementation increased milk protein concentration by about 10%, which resulted in a higher milk protein output in the protein supplemented group despite a slightly lower milk output, the result of an inability to balance precisely milk output of the paired animals. N balances of unsupplemented and supplemented groups were positive during both balance periods. Protein supplementation increased N balance only during period 2 ($P < 0.05$).

Values for the rates of Ca transport in the two groups during weeks 2–3 and 5–6 of lactation are given in Table 4. Protein supplementation had no significant effect on Ca transport variables, though there was a consistent trend for a 22% greater exchangeable pool of Ca (M_T) in supplemented than in non-supplemented sheep. Milk Ca secretion

Table 4. *Expt 2. Rates of calcium transport (mg/kg body-weight per d) and the exchangeable Ca pool (g/kg body-weight) in sheep offered Ariki ryegrass* (Lolium perenne *L.)–white clover* (Trifolium repens *L.) without and with abomasal infusion of a casein supplement*

(Mean values with their standard errors for four sheep)

| | | 2nd week of lactation | | | | 5th week of lactation | | | |
| | | Ryegrass–white clover | | Ryegrass–white clover +casein (70 g/d) | | Ryegrass–white clover | | Ryegrass–white clover +casein (100 g/d) | |
	Symbol	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Intake	V_I	179·9	5·61	167·8	20·49	187·0	4·79	195·9	5·78
Faeces	V_F	154·7	9·15	143·8	16·14	149·5	5·42	149·0	5·60
Absorption	V_a	43·9	4·07	46·1	8·66	59·2	1·32	69·9	7·27
Absorption coefficient	V_a/V_I	0·24	0·03	0·27	0·03	0·32	0·01	0·36	0·03
Faecal endogenous loss	V_f	18·7	1·68	22·0	1·53	21·8	0·81	23·1	1·34
Milk	V_m	77·3	2·37	80·2	11·00	54·4	2·17	57·6	3·36
Urine	V_u	0·12	0·02	0·19	0·04	0·14	0·02	0·49	0·09
Bone accretion	V_o^+	26·4	2·92	27·0	2·61	34·0	2·51	33·8	4·77
Bone reabsorption	V_o^-	78·6	6·07	83·5	5·37	52·0	4·14	45·0	1·84
Balance	Δ	−52·1	4·44	−56·5	5·27	−17·0	2·52	−11·2	3·03
Irreversible loss	V_t	122·5	5·97	129·4	12·63	111·2	9·03	115·0	3·23
Exchangeable Ca	M_T	0·23	0·016	0·28	0·044	0·22	0·037	0·30	0·065

(V_m) was lower ($P < 0·05$) and rate of absorption of Ca (V_a) higher ($P < 0·05$) during period 2 than period 1. These changes were associated with a decrease in rate of reabsorption of Ca from bone (V_o^-) ($P < 0·01$) and an improvement in Ca balance (Δ) ($P < 0·01$). There were trends for increase in the coefficient of absorption of Ca, in milk Ca output, in faecal endogenous loss of Ca and in urinary Ca excretion, but these were not significant.

DISCUSSION

Ca metabolism in animals is tightly regulated through the reaction of the parathyroid–thyroid axis to fluctuation in plasma ionic Ca concentration (Halloran *et al.* 1979; Care *et al.* 1980; Robinson *et al.* 1982). Homeostasis in ruminants is maintained predominantly through regulation of the rate of absorption of Ca from the alimentary tract since urinary Ca losses in these species are very low (Agricultural Research Council, 1980) and the response to an intravenous infusion of Ca is a reduction in the rate of Ca absorption (Braithwaite, 1978 *b*).

The availability of Ca is therefore a function of the intake and requirement of a particular animal rather than a unique characteristic of a particular feed (Field, 1983). Braithwaite (1974) observed, in sheep, an increase in Ca availability from 0·12 to 0·18 simply by reducing Ca concentration. The maximum or 'potential' availability can only be measured in animals in which intake ≤ requirement. The values for the availability of Ca in the ryegrass–white clover in the present experiments, i.e. 0·18–0·30, probably, therefore, represent 'realized' availability values. The diet is, however, only one source of supply of Ca to the plasma ionic Ca pool. Parathyroid hormone secretion in response to low plasma ionic Ca stimulates not only Ca absorption but also, concurrently, bone Ca resorption (Care *et al.* 1980; Stern, 1980). Because of this dual source of Ca it is, perhaps, sounder
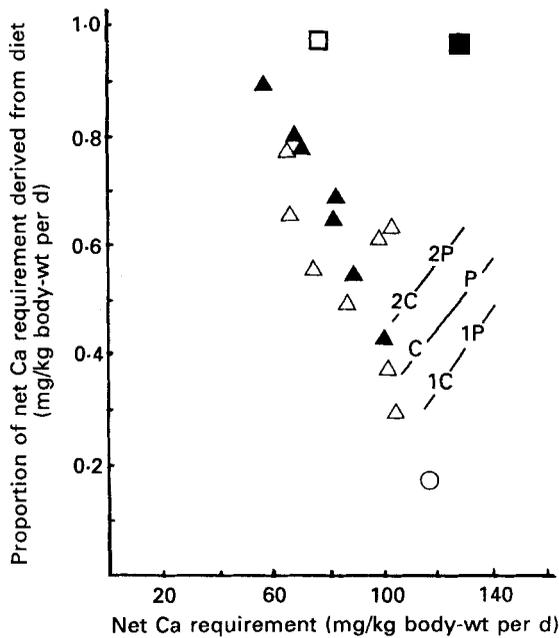
Fig. 6. The relation between daily net calcium requirement ($V_f + V_m + V_o^+ + V_u$) and the proportion of the Ca utilized which is derived from the diet ($V_a/V_a + V_o^-$) in lactating sheep. ($\triangle$, $\blacktriangle$), Values derived from the results of Braithwaite *et al.* (1969) and Braithwaite (1983) respectively, C and P represent the control and supplemented sheep respectively in Expt 1, while 1C–1P and 2C–2P represent the control and supplemented sheep during balance periods 1 and 2 respectively in Expt 2. ($\bigcirc$), Value for sheep offered green feed oats in Expt 1. ($\square$, $\blacksquare$), Values for sheep previously deprived of Ca or protein energy from the experiments of Braithwaite (1978*a*) and Sykes & Dingwall (1975) respectively. Note that $V_a + V_o^- = V_m + V_f - V_o^+ + V_u$.

from a physiological and nutritional standpoint to consider what proportion of Ca requirement will be furnished by the diet and the skeleton at different levels of Ca demand (requirement), than the traditional approach (Agricultural Research Council, 1980) which seeks to define realized availability in order to translate net Ca requirement into a dietary requirement.

In order to consider this approach and to compare our findings on the contribution of dietary Ca to total Ca utilized with values in the literature for lactating sheep, the relation between the proportion of net Ca requirement supplied by the diet ($V_a/V_a + V_o^-$) and total net Ca requirement ($V_f + V_m + V_o^+ + V_u$) for the present results and those of Braithwaite *et al.* (1969) and of Braithwaite (1983), has been derived and is presented in Fig. 6. Empirically these suggest that as Ca demand declines with stage of lactation, at constant Ca intake, the proportion of Ca derived from the diet increases and, conversely, that a larger proportion is contributed by the skeleton during periods of high Ca demand. Further, they suggest good agreement between findings from sheep given mixed hay and concentrate feeds (Braithwaite *et al.* 1969; Braithwaite, 1983), and the present work with fresh herbage. It would appear, therefore, that Ca requirement has a major impact on the source of supply (diet *v.* skeleton) and that, superficially at least, availability of Ca in fresh herbages may be similar to that in hay–concentrate diets.

It is likely, however, that factors which, independently of the Ca homeostatic mechanism, affect net transfer of Ca between the skeleton and the plasma and extracellular pool of Ca will complicate the relation. An example is the loss of bone matrix as a result

of protein deficiency (Frandsen *et al.* 1954; El-Maraghi *et al.* 1965; Sykes *et al.* 1973). Indeed, in grazing studies with sheep during the same stage of lactation as in the present study, Sykes & Geenty (1986) observed that, on average, a 1·8 g/kg body-weight per d reduction in N balance was associated with a 10 mg/kg body-weight per d reduction in the calculated rate of Ca absorption though, as would be expected, the absolute rate of absorption of Ca at any daily N balance was dependent on milk Ca output. Protein supplementation in the present work was designed to reverse bone protein loss, increase bone Ca accretion (V_o^+) and thereby increase rate of Ca absorption (V_a) and, as a consequence, realized Ca availability (V_a/V_i). Sheep in Expt 1 did, as expected, suffer negative N balances during early lactation. Protein supplementation simply increased milk production by, on average, 18%. Interestingly the increased Ca demand was met by increased absorption from the alimentary tract rather than from the skeleton. Pair-milking in Expt 2 was designed to prevent expression of improved protein supply in milk synthesis and, therefore, provide maximum opportunity for body protein to be spared. The latter was, however, achieved to only a limited extent and there was no associated improvement in body Ca balance. However, protein supplementation did tend to increase the proportion of Ca derived from the diet (Fig. 6), possibly reflecting greater avidity of the skeleton for Ca as a result of increased matrix deposition. It is interesting that very high values for the proportion of Ca derived from the diet during lactation have only been observed in sheep with positive Ca balances and, moreover, following skeletal mineral deprivation during pregnancy. In one situation the sheep had previously suffered severe protein depletion (Sykes & Dingwall, 1975) and in the other had previously been subjected to prolonged Ca deficiency (Braithwaite, 1978*a*). These values are superimposed on Fig. 6.

Together, the findings suggest that any model of Ca metabolism must recognize not only Ca demand but also how the tight physiological regulation of Ca metabolism and the current avidity of bone matrix for Ca determine the proportion of Ca utilized which will be derived from the diet. A simple framework is provided in Fig. 6 within which such a model for lactating animals could be developed. Clearly further quantitative findings are required.

The low realized availability of Ca in the ryegrass–white clover observed in both experiments (0·18–0·30), despite the fact that the diet provided only 0·50 of net Ca requirement, could be interpreted as confirmation of the suggestion of the Insitut National de la Recherch Agronomique (1978) that the potential availability of Ca in herbages is less than 0·45. The increase in coefficient of absorption of Ca from 0·19 to 0·30 as a result of protein supplementation of the ryegrass–white clover diet in Expt 1 and similar trends in Expt 2 suggest, however, that potential availability was not fully exploited.

Availability of Ca from the oats–Tama ryegrass diet was very low at 0·17 and considerably lower than that from the ryegrass–white clover, despite the fact that only 0·18 of net Ca requirement was met from the diet. While extrapolation of the findings of Braithwaite *et al.* (1969) and Braithwaite (1983) for hay–concentrate-based diets (Fig. 6) suggests similar values for $V_a/V_a + V_o^-$ at similar levels of Ca utilization, comparisons of realized availability of Ca from oats–Tama ryegrass and ryegrass–white clover in red deer with a high Ca requirement during antler growth, have allowed the same conclusion of low availability in the former herbage (Muir *et al.* 1987). Clearly more definitive work is required. The Agricultural Research Council (1980) made no provision for variation in potential availability of Ca between diets or for particularly low Ca availabilities in certain fresh forages. In the light of the present findings, adoption of a single value of 0·68 would seem to be incautious.

The rate of endogenous loss of Ca, which varied from 16 to 40 mg/kg body-weight per d between animals, was considerably greater than the value of 16 mg/kg body-weight

per d derived by the Agricultural Research Council (1980), suggesting that the latter may underestimate net endogenous losses of high-producing animals, at least in the short term. This subject is developed further by Chrisp *et al.* (1989).

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