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Calcium and phosphorus balances of lactating ewes at pasture

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SUMMARY

Differences in maternal body weight (12–15 kg) were induced in two groups ($n = 43$–50) of ewes during pregnancy by controlled allowance of pasture. Following parturition equal numbers (9–12) were allocated to one of three herbage allowances in a 2×3 factorial design during 42 days of lactation. This design was employed on two occasions; in one (Expt 1) the sheep were machine-milked twice daily, in the second (Expt 2) they each suckled two lambs. Balances of Ca and P in the body were determined by comparative slaughter during early pregnancy, at parturition and after 42 days of lactation. Herbage Ca intake and milk Ca output were determined on a weekly basis.

Mean daily milk production during the 42 days of lactation ranged from 1·48 to 2·68 kg/day amongst treatment groups. The general trend, irrespective of level of milk production, was for negative Ca balances, ranging from 0·71 to 1·42 g Ca/day. It is argued that variation in apparent absorbability (0·17–0·46) and in rate of absorption (30–113 mg/kg W per day) of Ca among groups suggest that these factors are not in themselves responsible for the poor Ca balances observed.

It is suggested that demand for Ca from the diet during early lactation is reduced as a consequence of bone Ca resorption due to matrix osteoporosis resulting from a protein deficiency state. Negative Ca balances in sheep undernourished during pregnancy were similarly attributed to poor protein balances.

Although negative P balances were observed these were not attributed to low P intake.

INTRODUCTION

The data on which the Agricultural Research Council (1980) based their estimates of Ca requirement and the most extensive series of kinetic studies of Ca metabolism in lactating sheep (Braithwaite & Glascock, 1976; Braithwaite, 1983) were derived from experiments in which conserved, mixed roughage–concentrate diets were used. Whether these data can be adapted to lactating sheep at pasture does not appear to have been assessed. There is evidence from ewes grazing hill pastures during summer that skeletal remineralization following lactation may be slow (Sykes, Field & Gunn, 1974*b*) despite adequate Ca and P intake. Moreover, the early studies of Ewer & Bartrum (1957) described poor skeletal mineralization in sheep consuming herbages which Grant & O'Hara (1957) attributed to high vitamin A and low vitamin D content of the green fraction.

In a previous paper (Geenty & Sykes, 1986) comparative slaughter was employed to derive energy and protein balances of lactating sheep at pasture. The opportunity was therefore taken to determine Ca and P balances and these, together with estimated rates of Ca absorption, are presented here.

MATERIALS AND METHODS

The sheep and their management were described in detail by Geenty & Sykes (1986). Briefly, mixed age Dorset sheep were used in two experiments. In Expt 1 two groups ($n = 43$ and 45) were formed during early pregnancy; they were offered herbage (ryegrass–white clover) *ad libitum* (HP) or in restricted amounts (LP) in order to achieve differences in maternal body weight between groups, *post-partum*, of 12–15 kg. The lambs were then removed and 9–12 sheep allocated from within each group to one of three herbage allowances during lactation, 8 (HL), 5 (ML) and 2 (LL) kg dry matter (D.M.)/head per day. All sheep were machine-milked twice-daily for 42 days. The same design

Table 1. *Experimental design and numbers of ewes in treatment groups in Expts 1 and 2*

Herbage allowance		Treatment group	Number of ewes in each group		Time of slaughter
Pregnancy	Lactation		Expt 1	Expt 2	
—	—	Control	10	10	Week 6 of pregnancy
LP	—	LP	11	10	Post-partum
HP	—	HP	9	10	
LP	LL	LPLL	11 (m)*	10 (s)*	Week 6 of lactation
HP	LL	HPLL	12 (m)	9 (s)	
LP	LM	LPML	10 (m)	10 (s)	
		LPMML	—	11 (m)	
HP	LM	HPML	12 (m)	10 (s)	
		HPMML	—	10 (m)	
LP	LH	LPHL	11 (m)	9 (s)	
HP	LH	HPHL	12 (m)	10 (s)	
		Total	98	109	

L, low; M, medium; H, high. * m, machine-milked; s, twin-suckled.
Balanced for post-partum body weight and lamb litter size.

and procedure was followed in Expt 2 except that all ewes suckled two lambs. An additional group from each pregnancy allowance was maintained on the ML allowance during lactation, and machine-milked to maintain continuity with Expt 1. The objective was to create situations of diversity in body condition, nutrient intake and demand for nutrients for milk synthesis. The overall design and numbers of sheep involved are given in Table 1.

Milk production was assessed directly by machine-milking or, in ewes rearing lambs, by a combination of the lamb suckling and oxytocin methods (Geenty & Sykes, 1986). Milk samples obtained weekly by machine-milking were analysed for Ca and P after deproteinization by the methods of Sykes & Dingwall (1975).

Groups of sheep (n = 9–12) were slaughtered in early pregnancy, at parturition and after 42 days of lactation (Geenty & Sykes, 1986). The frozen empty bodies were reduced to small fragments in an industrial bone pulverizer and then passed through a 6 mm plate in an Autio cutter-grinder (Autio Co., Astoria, U.S.A.). Duplicate homogenate samples of this material were freeze dried and passed through a 2 mm sieve in an ultracentrifugal mill (Retch GmbH & Co., Germany). Duplicate 5 g samples were then ashed at 500 °C, taken up in 50% v/v HCl and stored for analysis.

Herbage D.M. intake was determined by chromic oxide dilution in faeces and *in vitro* estimates of herbage digestibility on oesophageal boluses (Geenty & Sykes, 1986) and was used for calculation of mean Ca intake during lactation. Herbage Ca and P were determined on samples cut at 3-day intervals from the sward prior to sheep moving to a new grazing. These samples were washed, dried, ground through a 1 mm sieve, ashed at 500 °C and taken up in HCl (50%, v/v).

Calcium and P were determined by atomic absorption spectroscopy and Technicon method SE4-004FH4, adapted from Kraml (1966), respectively.

The data were analysed statistically by the methods described by Geenty & Sykes (1986).

RESULTS

Mean herbage Ca (g/kg D.M.) was 6·1 ± 0·81 and 6·1 ± 0·89 and P (g/kg D.M.) was 5·8 ± 0·46 and 3·4 ± 0·16 in Expts 1 and 2, respectively. The weekly pattern of herbage intake was given by Geenty & Sykes (1986). Mean daily herbage D.M., Ca and P intake during the 6 weeks of lactation, calculated from these data, are given in Table 2.

There were no differences between pregnancy or lactational treatment groups in milk Ca or P concentrations in Expt 1 or 2. Mean values (g/kg) were 1·9 ± 0·16 and 1·7 ± 0·22 for Ca and 1·3 ± 0·09 and 1·4 ± 0·12 for P in Expts 1 and 2, respectively.

The pattern of weekly milk production for the groups was given by Geenty & Sykes (1986). Overall mean daily secretion rates and milk Ca and P output during the 42 days of lactation are given in Table 3. There was generally little effect of pregnancy nutrition on milk secretion rate. Herbage allowances produced a range of mean milk Ca

Table 2. *Mean daily intake of dry matter (kg/day) and of Ca and P (g/day) of the groups during 42 days of lactation*

Group ...	HPHL	LPHL	HPML	LPML	HPMML*	LPMML*	HPLL	LPLL
				Expt 1*				
Dry matter	2·03	2·32	2·09	1·93	—	—	1·68	1·76
Ca	12·4	14·2	12·8	11·8	—	—	10·3	10·8
P	12·0	13·7	12·4	11·4	—	—	10·0	10·4
				Expt 2				
Dry matter	2·48	2·48	2·15	2·20	1·92	1·93	1·66	1·72
Ca	15·2	15·2	13·2	13·4	11·8	11·8	10·2	10·5
P	8·5	8·5	7·4	7·5	6·6	6·6	5·7	5·9

* These ewes were machine-milked; the remainder suckled two lambs.

Table 3. *Mean daily milk production (kg/day) and milk Ca and P (g/day) output for sheep in Expts 1 and 2 during 42 days of lactation*

	Expt 1*			Expt 2		
	Milk			Milk		
Group	Production	Ca	P	Production	Ca	P
HPHL	1·97	3·7	2·6	2·65	4·5	3·7
LPHL	2·04	3·9	2·6	2·68	4·6	3·7
HPML	1·82	3·4	2·4	2·59	4·4	3·6
LPML	1·88	3·6	2·4	2·49	4·2	3·5
HPLL	1·48	2·8	1·9	2·10	3·6	2·9
LPLL	1·53	2·9	2·0	1·88	2·7	2·6
HPMML*	—	—	—	1·58	2·7	2·2
LPMML*	—	—	—	1·75	3·0	2·5
S.D.	0·386	0·71	0·49	0·47† 0·24‡	0·86† 0·61‡	0·62† 0·46‡

† Suckled. ‡ Machine-milked.
* These ewes were machine-milked; the remainder suckled two lambs.

secretion rates from 2·8 to 3·9 g/day in Expt 1 and from 2·7 to 4·6 g/day in Expt 2.

Whole body Ca and P contents of sheep slaughtered during early pregnancy, at parturition and at the end of lactation are given in Table 4 and concentrations of Ca and P in the empty body in Table 5. Changes in whole body Ca and P contents occurred as a result of pregnancy treatment in both experiments. In Expt 1 there was net gain of Ca in HP and loss of P in LP groups. In Expt 2 similar losses of body P occurred but differences in body Ca content at parturition reflected gain and loss of Ca, respectively, in HP and LP groups.

There was no consistent pattern in the change in body Ca content during lactation; all groups except two, LPHL and LPLL in Expt 1, lost considerable quantities of Ca. The trend of change in P was generally similar amongst groups, though groups LPHL and LPMML in Expt 2 did appear to maintain P balance while in negative Ca balance.

The changes in concentrations of Ca and P in the body reflected primarily the losses of non-skeletal tissue, since concentrations remained constant or increased in all groups during pregnancy irrespective of whether the animals were in negative or positive Ca balance. Concentrations generally remained constant during lactation, though they tended to increase further in sheep on low (LL) allowances.

DISCUSSION

One must be cautious about definitive interpretation of mineral balance results based on comparative slaughter. While they overcome the difficulties of measuring small daily balances of a nutrient in situations, like the present, where a large dietary throughput occurs, errors due to variation in mineral composition of animals can be large. This problem is illustrated in Table 6 in which the balances of Ca during the whole lactation period have been calculated. One group, LPHL in Expt 1, exhibited an apparent positive Ca balance

Table 4. *Body Ca and P content (g) of sheep in early pregnancy, at parturition and after 42 days of lactation*

	Expt 1*		Expt 2	
	Ca	P	Ca	P
Early pregnancy	527	331	551	349
S.D.	63·2	28·3	83·0	37·8
Parturition				
HP	599	337	578	351
LP	516	292	522	296
S.D.	73·1	30·7	60·5	32·4
After 42 days of lactation				
HPHL	533	316	543	343
LPHL	535	312	489	303
HPML	534	314	520	313
LPML	483	282	463	285
HPLL	570	315	531	321
LPLL	514	285	469	266
HPMML*	—	—	513	316
LPMML*	—	—	475	296
S.D.	73·3	34·7	80·4	43·6

* These groups were machine-milked; the remainder suckled two lambs.

Table 5. *Concentration of Ca and P in the bodies of sheep (g/kg empty body weight) in early pregnancy, at parturition and after 42 days of lactation*

	Expt 1*		Expt 2	
	Ca	P	Ca	P
Early pregnancy	9·8	6·1	9·8	6·2
S.D.	1·30	0·48	1·30	0·66
Parturition				
HP	11·0	6·2	10·2	6·2
LP	12·5	7·1	12·1	6·9
S.D.	1·70	0·72	1·53	0·91
After 42 days of lactation				
HPHL	11·2	6·4	11·1	7·1
LPHL	12·0	7·0	12·7	7·9
HPML	10·9	6·4	11·5	6·9
LPML	11·8	6·9	12·4	7·6
HPML	12·3	6·8	12·1	7·3
LPLL	12·8	7·1	13·7	7·8
HPMML*	—	—	9·7	6·0
LPMML*	—	—	11·3	7·0
S.D.	1·52	0·72	1·66	0·87

* These groups were machine-milked, the remainder suckled two lambs.

Table 6. *Calcium and protein balances of sheep and estimates of rate of absorption and of apparent absorbability of Ca during 42 days of lactation*

	Calcium						
Group	Intake (g/42 (days)	Milk (g/42 days)	Balance (g/42 days)	Endogenous loss† (g/42 days)	Rate of absorption (mg/kgW/day)	Apparent absorbability	Protein balance (g/day)
				Exp 1*			
HPHL	521	147	−46	58	47	0·24	−15
LPHL	596	168	+19	65	113	0·45	+13
HPML	538	147	−65	60	63	0·30	−13
LPML	496	147	−33	55	75	0·34	+2
HPLL	433	122	−29	48	55	0·32	−21
LPLL	454	126	−2	50	80	0·38	0
				Expt 2			
HPHL	638	185	−35	71	77	0·34	−17
LPHL	638	193	−33	69	103	0·36	−3
HPML	554	189	+2	61	94	0·46	−22
LPML	563	181	−59	62	84	0·33	−9
HPLL	428	143	−47	52	56	0·34	−30
LPLL	441	134	−53	32	56	0·26	−24
HPMML*	496	93	−65	54	30	0·17	−10
LPMML*	496	134	−45	45	60	0·27	+3

* These groups were machine-milked; the remainder suckled two lambs.

† From Braithwaite (1982), where endogenous loss (mg Ca/kg W per day) = 1·357 + 0·6477 × D.M. intake (g/kg W per day).

of 19 g or 0·45 g/day during this period and two others, LPLL in Expt 1 and HPML in Expt 2, showed zero net Ca balance. One cannot be confident that these represent real effects of treatments, particularly since the HPML groups behaved so differently in the two experiments. The general pattern during lactation was, however, one of negative balances of between 30 and 60 g or 0·71 to 1·42 g/day of Ca (Table 4).

For P a similar picture of negative balances of 20–40 g or 0·4–1·0 g P/day emerged. Group LPMML in this case also appeared anomalous, apparently maintaining P balance despite substantial negative Ca balance. It seems unlikely, however, that P deficiency was responsible for the generally poor mineral balances. Even in Expt 2, in which herbage P concentration was low and intake therefore restricted to 5·7–8·5 g P/day, a maximum absorbability of only 0·51 would readily have met the range of demand (2·8–4·3 g P/day) for milk and endogenous P calculated for all groups. Phosphorus absorbability is generally considered to be much higher than this and the Agricultural Research Council (1980) adopted a cautious value of 0·6.

The present sheep consumed, on average, between 10 and 15 g Ca/day. The apparent absorbability of dietary Ca was therefore low, ranging from 0·17 to 0·46 amongst groups, and very much lower than the value of 0·68 adopted by Agricultural Research Council (1980). This latter value, however, represents the estimated maximum absorbability of Ca. Since Ca absorption is heavily regulated through the parathyroid gland, parathyroid hormone (PTH) and the metabolites of vitamin D (Care, Barlet & Abdel-Hafeez, 1980) and absorption of Ca in excess of requirement does not appear to occur in ruminants (Braithwaite, 1978*b*) it follows that maximum absorbability will be expressed only when Ca intake is close to or below requirement. This was well demonstrated by Braithwaite (1983); when dietary Ca intake was decreased from 270 to 105 mg/day per kg body weight (W) during early lactation, by decreasing dietary Ca concentration, Ca availability increased from 0·18 to 0·68. We must assume, therefore, either that availability of Ca from these herbages was inherently low, that the demand for Ca from the diet was low, or that the capacity of the animal to absorb Ca was impaired.

High negative Ca balances were observed in most groups, largely independent of Ca demand. Indeed, groups HPMML and LPMML in Expt 2, in which milk production was deliberately restricted by machine-milking, had relatively high negative Ca balances. It seems unlikely that, in the latter case in particular, this occurred as a result of low inherent absorbability of dietary Ca because higher absorbabilities were calculated for other groups. Similarly, limitation of the capacity of the digestive tract to absorb Ca seems unlikely because the calculated rates of absorption, 30 and 50 mg Ca/kg W per day, were much lower than those recorded in other groups (Table 6). The maximum calculated rate of absorption, > 100 mg Ca/kg W per day, observed in LPHL groups was, in fact, very high, being greater than the maximum of 60–80 mg/kg W per day observed in lactating sheep consuming conserved feeds by Braithwaite (1978*a*, 1983). In part this reflects their depleted body condition and therefore weight, but even if calculated relative to mating weight the rate of absorption (> 90 mg Ca/kg W per day) is still high. The value is, however, close to that of 115 mg/kg W per day calculated to have been absorbed by sheep consuming semi-purified diets during lactation after skeletal Ca had been depleted by undernutrition during pregnancy (Sykes & Dingwall, 1975). It therefore seems possible that other factors may be involved in the poor Ca balances.

Poor skeletal mineralization occurs as a result of protein deficiency in rodents (Frandsen *et al.* 1954; El-Maraghi, Platt & Stewart, 1965) and in pregnant sheep (Sykes & Field, 1972). All sheep in the present experiment, with the exception of group LPHL in Expt 1 and LPML in Expt 2, were in negative energy and protein balance during lactation (Table 6) and Geenty & Sykes (1986) argued the precarious protein status of all groups of sheep. In his kinetic studies Braithwaite (1983) observed that increase in the rate of absorption of Ca from the intestine appeared to lag behind increase in skeletal Ca resorption in response to demand for Ca during lactation. Why this occurs is not clear if both respond to circulating levels of PTH and 1:25 dihydroxy-cholecalciferol, as a consequence of reduced plasma ionic Ca concentration (Care *et al.* 1980). Furthermore, the timing of increase in rate of absorption appeared to coincide with an increase in the rate of accretion of Ca in the skeleton (Braithwaite, 1983). It seems just as plausible that, in part at least, resorption of Ca from the skeleton during early lactation occurs as a result of net loss of bone matrix consequent upon protein and energy deficiency. This would furnish Ca for milk synthesis and diminish the requirement for intestinal Ca absorption. Delay in accretion of Ca into bone until milk production declines can be interpreted on the basis of delay until the animal comes closer to positive protein balance and net deposition of bone matrix occurs.

In the one study in the literature in which positive Ca and P balances were achieved during early lactation, they were associated with positive protein balance (Sykes & Dingwall, 1975). Moreover, in a study of seasonal variation in mineralization of the skeleton of hill sheep (Sykes, Field & Gunn, 1974*a*, *b*), regain of skeletal Ca at the end of lactation was observed in 2½-year-old sheep but not in older age groups. It is perhaps not coincidental that only the former age group restored soft tissue and semitendinosus muscle protein contents to the levels operating prior to skeletal demineralization. Preliminary findings (J. S. Chrisp, A. R. Sykes and N. D. Grace, unpublished) have suggested that Ca balance during mid-lactation in sheep consuming herbages is improved when protein status is enhanced by feeding protected casein.

The studies of Braithwaite (1983) can, in fact, be interpreted in this way. On the basis of a delay in the remineralization of the skeleton, following lactation, in sheep offered the Agricultural Research Council (1980) allowance of Ca compared with a second group offered generous Ca allowances, Braithwaite concluded that Agricultural Research Council (1980) allowances were inadequate. It was perhaps significant, however, that the feed offered was restricted by almost 50% during mid-lactation in the former group, but not in the group offered high Ca intakes. On the basis of the present data and arguments advanced above it seems just as likely that the failure of bone accretion during late lactation in the sheep offered Ca at the rate of the Agricultural Research Council (1980) estimate of requirement was the result of the reduced feed allowance and therefore a lower rate of bone matrix deposition.

There was further evidence in the present experiment to support the findings of Sykes & Field (1972) for an association between protein status during pregnancy and Ca balance. Maternal balances of protein (kg) and Ca (g) during pregnancy were, respectively, +0·44 and +72 in HP and −1·06 and −11 in LP sheep in Expt 1 and −0·12 and +27 in HP and −1·72 and −29 in LP in Expt 2. Moreover, the positive Ca balance in group LPHL during lactation in Expt 1 was associated with a large positive protein balance though, as pointed out earlier, sampling errors cannot be excluded.

There was no evidence, however, that the differences in Ca balances induced in HP and LP groups during pregnancy (Table 4) influenced ability to maintain Ca balance or calculated rate of Ca absorption during lactation. This appears to contrast with the finding of a positive effect of a low Ca intake, and presumed skeletal demineralization, during pregnancy on rate of Ca absorption during lactation (Braithwaite, 1978*a*). If the demineralization of the skeleton during pregnancy in the present sheep occurred as a result of matrix-osteoporosis (Sykes, Nisbet & Field, 1973) consequent upon poor protein status, remineralization and rate of Ca absorption would be dependent on net rate of bone matrix deposition rather than change in inherent ability for enhanced rates of Ca absorption.

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