*J. agric. Sci., Camb.* (1986), **106**, 271–278
*With 6 text-figures*
*Printed in Great Britain*



# Phosphorus requirements of ewes in pregnancy and lactation

By G. D. BRAITHWAITE
*National Institute for Research in Dairying, Shinfield, Reading, RG2 9AT*

(*Revised MS. received* 12 *August* 1985)

SUMMARY

The adequacy of the Agricultural Research Council (1980) recommendations for phosphorus for pregnancy and lactation has been investigated in ewes given a plentiful supply of dietary calcium.

The efficiency of absorption of P remained high and fairly constant throughout the whole experimental period and the rate of P absorption varied in direct relation to the P intake.

The endogenous faecal loss of P also varied with P intake and at all stages of pregnancy and lactation was higher than the value assumed by the Agricultural Research Council (1980) in their calculations of P requirements.

Bone mineral stores of Ca and P were lost in the normal way during late pregnancy and early lactation but were not replaced, as normal, in mid- to late lactation. At the end of the lactation, ewes were still in deficit of 125 g P and 100 g Ca.

It is argued that the Agricultural Research Council (1980) recommendations for P, particularly in mid- to late lactation, are too low and it is recommended that future calculations of requirements for pregnancy and lactation allow for the inevitable loss and subsequent replacement of skeletal mineral stores and also for the increased endogenous faecal losses of P that appear inevitable at the high P intakes needed to meet the increased demands.

## INTRODUCTION

A previous study (Braithwaite, 1983*a*, *b*) showed that ewes restricted in their dietary supply of calcium and phosphorus to recommendations of an Agricultural Research Council working party (1980), unlike ewes given a plentiful supply of Ca and P, were unable to replace in late lactation those inevitable losses of Ca and P that occurred in late pregnancy and early lactation. It was concluded from the study that the Agricultural Research Council (1980) Ca recommendations for pregnant and lactating ewes were inadequate, particularly in mid- to late lactation. Because of the underestimate of Ca requirements and the close association between Ca and P in bone and milk, it was not possible to draw any conclusions about the P recommendations.

The adequacy of these P recommendations have now been investigated in pregnant and lactating ewes given a plentiful dietary supply of Ca.

## MATERIALS AND METHODS

### *Animals, housing and diet*

Eight 4- to 5-year-old Suffolk ewes weighing 65–75 kg were used. After mating they were put in metabolism cages which allowed separate collection of urine and faeces. They were given a diet of hay and concentrates (Table 1) with added trace minerals, vitamins and $CaCO_3$, designed to supply the Agricultural Research Council (1980) recommended requirements for P, energy and protein but the Agricultural Research Council (1965) recommendations for Ca. Agricultural Research Council (1965) recommendations for Ca are considerably higher than Agricultural Research Council (1980) recommendations and are generally considered to be more than adequate.

The diet was adjusted at monthly intervals to meet the changing nutritional requirements of pregnancy and lactation and any uneaten food was

Table 1. *Composition of the diet (g/day per kg body weight) calculated to supply the phosphorus, energy and protein requirements recommended by the Agricultural Research Council (1980) for maintenance and for maintenance plus foetal or milk production (calculations based on a 70 kg ewe)*

| | | | Milk yield (l/day) | | | |
|---|---|---|---|---|---|---|
| Ingredient | Maintenance | Pregnancy (4–5 months) | 2 | 1·5 | 1 | 0·5 |
| Hay | 7 | 14 | 21 | 17·5 | 14 | 10·5 |
| Barley | 3 | 3 | 11·6 | 9·5 | 7·3 | 5·2 |
| Maize | 2 | 2 | 5·4 | 4·5 | 3·7 | 2·8 |
| Soya bean | 1·5 | 3 | 3 | 2·6 | 2·2 | 1·9 |

The diet was supplemented with trace minerals and vitamins and with $CaCO_3$ to supply the Ca requirements recommended by the Agricultural Research Council (1965).

weighed and analysed. Animals had free access to distilled water.

All ewes were allowed to lamb normally. Lambs from ewes used in experiments in late pregnancy were killed immediately after birth and the placenta and fluids collected. Other lambs were removed from their mothers 2 days after birth and all ewes were machine milked twice daily (Treacher, 1970).

### *Experimental procedure*

Ca and P kinetic studies were carried out at 28–35 and 130–137 days gestation, at 14–21, 42–49, 63–70 and 94–99 days lactation and at 7–14 and 28–35 days post-lactation. To allow at least 5 weeks between kinetic studies in each of the eight ewes, ewes were divided into two groups of four, groups A and B which were used alternately. Thus each of the ewes was used four times; in early or late pregnancy, in early or mid-lactation, in mid- or late lactation and during the post-lactation period. It must be emphasized, however, that dietary treatment for both groups of ewes was the same throughout the experiment.

A known amount of $^{45}$Ca as calcium chloride and $^{32}$P as orthophosphate (Amersham International, Amersham, Bucks, 2·5 and 6 $\mu$Ci/kg body weight respectively) in aqueous solution, was injected into a jugular vein and samples of blood, urine, faeces and milk were collected over a period of 7 days as previously described (Braithwaite, Glascock & Riazuddin, 1969). At the same time Ca and P balance measurements were made.

### *Methods*

The Ca content of food, urine, faeces, foetuses and milk was measured by methods already described (Braithwaite *et al.* 1969, 1970). The total P content of these materials was determined by the procedure of Fiske & Subbarow (1925) modified (Technicon Instruments Corporation, 1967) for use with an AutoAnalyzer. Serum Ca and inorganic P were measured by the same procedures after first precipitating the protein with trichloroacetic acid (Manston, 1966).

Radioactivity was measured in a Packard liquid-scintillation spectrophotometer (model 2450B) by a dual-label technique with external standardization. Samples of serum (1 ml of TCA supernatant fraction), urine (1 ml acidified with three drops 2 M hydrochloric acid), milk (0·5 ml plus 0·5 ml 2 M-HCl) and ashed faeces and foetuses in HCl (1 ml) were counted in 10 ml Insta-gel scintillator solution (Packard Instruments Co. Inc).

Kinetic analysis of the Ca and P results was done by the method of Aubert & Milhaud (1960) modified for use with sheep: Ca (Braithwaite *et al.* 1969; Braithwaite & Riazuddin, 1971; Braithwaite & Glascock, 1976), P (Braithwaite, 1983*b*).

## RESULTS

Mean values of the various processes of P metabolism at different stages of pregnancy and lactation are shown in Table 2 and those of Ca metabolism in Table 3. The standard errors in the Tables are derived from the average animals × stages S.S. for means from the same animals at different stages, and on a pool of this and the between-animals S.S. for means from different animals at different stages. Figures 1, 4 and 5 are composite curves plotted from these mean values. Although these curves are obtained from different animals, they do nevertheless show the variations in the more important processes over the whole reproductive cycle.

### *P metabolism*

Demands for P associated with foetal development and milk production increased rapidly in late pregnancy to a peak in early lactation and then decreased (Fig. 1). Whereas the efficiency of absorption of dietary P remained high and fairly constant at about 63% throughout the whole experimental period, the rate of absorption ($P_a$)

Table 2. *Phosphorus metabolism in ewes restricted to the Agricultural Research Council* (1980) *recommendations for dietary P throughout pregnancy and lactation in two groups* (*A and B*) *of four sheep*

| | Pregnancy | | Lactation | | | | Post-lactation | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Day no. ... | 28–35 | 130–137 | 14–21 | 42–49 | 63–70 | 94–99 | 7–14 | 28–35 | | S.E.† |
| Group ... | A | B | A | B | A | B | A | B | S.E.* | (18 D.F.) |
| Rate (mg/day per kg body weight) of: | | | | | | | | | | |
| Ingestion of P | 37·0 | 53·3 | 82·6 | 68·2 | 76·9 | 48·8 | 29·0 | 36·1 | 10·66 | 9·70 |
| Loss of P in faeces | 35·2 | 48·1 | 66·2 | 56·7 | 59·4 | 42·2 | 30·2 | 38·0 | 5·23 | 5·64 |
| Excretion of P in urine | 0·2 | 0·4 | 0·4 | 0·3 | 0·3 | 0·2 | 0·3 | 0·3 | 0·13 | 0·13 |
| Loss of P to foetus or milk | — | 16·8 | 50·9 | 25·3 | 23·0 | 11·6 | — | — | — | — |
| Retention of P | +1·6 | −12·1 | −34·9 | −13·8 | −5·7 | −5·2 | −1·5 | −1·9 | 5·69 | 6·08 |
| Endogenous loss of P in faeces | 21·2 | 28·7 | 37·2 | 30·3 | 35·9 | 25·7 | 19·3 | 21·8 | 4·34 | 4·75 |
| Absorption of dietary P | 23·0 | 33·9 | 53·6 | 41·8 | 53·4 | 32·3 | 18·1 | 19·9 | 9·97 | 9·14 |
| P incorporation into non-exchangeable pool of bone and soft tissues | 12·8 | 11·5 | 13·2 | 13·9 | 12·2 | 17·3 | 18·4 | 18·8 | 3·58 | 3·41 |
| P loss from non-exchangeable pool of bone and soft tissues | 11·2 | 23·6 | 48·1 | 27·7 | 17·9 | 22·5 | 19·9 | 20·7 | 5·43 | 5·53 |
| P absorbed (% P ingested) | 62·2 | 63·4 | 64·9 | 61·3 | 69·4 | 66·2 | 62·4 | 55·1 | 6·53 | 6·31 |
| Serum P (mmol/l) | 1·8 | 1·4 | 1·2 | 1·3 | 1·4 | 1·5 | 1·5 | 1·2 | 0·18 | 0·18 |
| Milk yield (ml/day) | — | — | 1906 | 1125 | 800 | 539 | — | — | — | — |

* Average S.E. of a mean when comparing it with a mean for a different group of animals. Derived from a between-animals S.S. with 6 D.F. and an animals × stages S.S. with 18 D.F.

† Average S.E. of a mean when comparing it with another mean for the same group of animals. Derived from an animal × stages S.S. with 18 D.F.

Table 3. *Calcium metabolism in ewes restricted to the Agricultural Research Council* (1980) *recommendations for dietary P throughout pregnancy and lactation in two groups (A and B) of four sheep*

| | Pregnancy | | Lactation | | | | Post-lactation | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Day no. ... | 28–35 | 130–137 | 14–21 | 42–49 | 63–70 | 94–99 | 7–14 | 28–35 | | |
| Group ... | A | B | A | B | A | B | A | B | S.E.* | S.E.† |
| Rate (mg/day per kg body weight) of: | | | | | | | | | | |
| Ingestion of Ca | 86·1 | 113·8 | 139·5 | 123·1 | 138·4 | 113·1 | 78·4 | 85·3 | 20·93 | 16·69 |
| Loss of Ca in faeces | 86·9 | 114·9 | 129·0 | 105·1 | 110·2 | 88·5 | 65·7 | 78·4 | 17·68 | 15·13 |
| Excretion of Ca in urine | 0·2 | 1·1 | 0·7 | 0·8 | 1·0 | 1·0 | 0·6 | 2·4 | 0·45 | 0·51 |
| Loss of Ca to foetus or milk | — | 16·8 | 54·3 | 28·2 | 25·4 | 14·1 | — | — | — | — |
| Retention of Ca | −0·9 | −19·0 | −44·5 | −11·0 | +1·8 | +9·8 | +12·2 | +4·5 | 6·70 | 6·82 |
| Endogenous loss of Ca in faeces | 9·4 | 11·9 | 19·0 | 15·9 | 13·3 | 12·4 | 6·5 | 13·8 | 3·14 | 3·11 |
| Absorption of dietary Ca | 8·7 | 10·8 | 29·5 | 33·9 | 41·5 | 37·0 | 19·2 | 20·7 | 9·74 | 9·33 |
| Accretion of Ca into bone | 9·9 | 7·1 | 7·9 | 8·5 | 8·5 | 9·7 | 10·8 | 14·2 | 3·32 | 3·45 |
| Resorption of Ca from bone | 10·8 | 26·1 | 52·4 | 19·5 | 6·7 | −0·1 | −1·3 | 9·7 | 6·99 | 6·86 |
| Ca absorbed (% Ca ingested) | 10·1 | 9·4 | 21·1 | 27·5 | 30·0 | 32·7 | 24·5 | 24·3 | 6·36 | 6·82 |
| Serum Ca (mmol/l) | 2·5 | 2·4 | 2·3 | 2·2 | 1·9 | 1·9 | 1·9 | 2·0 | 0·11 | 0·10 |
| Food intake (g/day per kg body weight) | 13·5 | 18·4 | 33·0 | 27·3 | 30·1 | 23·4 | 13·6 | 13·9 | 4·21 | 3·78 |

* Average S.E. of a mean when comparing it with a mean for a different group of animals. Derived from a between animals S.S. with 6 D.F. and an animals × stages S.S. with 18 D.F.

† Average S.E. of a mean when comparing it with another mean for the same group of animals. Derived from an animal × stages S.S. with 18 D.F.

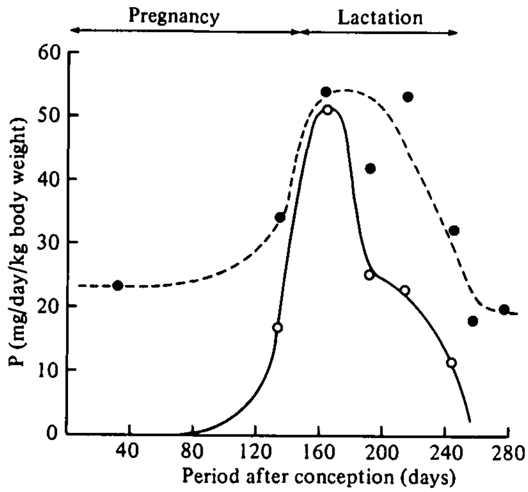


Fig. 1. Variations in P demands for foetal and milk production (○) and in P absorption (●) in ewes during pregnancy and lactation.

varied in direct relation to the P intake ($P_i$) (Table 2, Fig. 2).

$$P_a = -5{\cdot}2+0{\cdot}74\ (\pm 0{\cdot}032)\ P_i.$$

Thus, absorption tended to be high at peak lactation but low in early pregnancy and the dry period (Fig. 1).

Endogenous losses of P in the urine remained fairly constant and low but endogenous losses in the faeces ($P_f$) varied in direct relation to the intake ($P_i$) and absorption ($P_a$) of P (Figs 3 and 4).

$$P_f = 11{\cdot}5+0{\cdot}30\ (\pm 0{\cdot}035)P_i,$$
$$P_f = 14{\cdot}1+0{\cdot}39\ (\pm 0{\cdot}047)\ P_a,$$

P was mobilized from the bone and soft tissues in late pregnancy and early lactation, when the rate

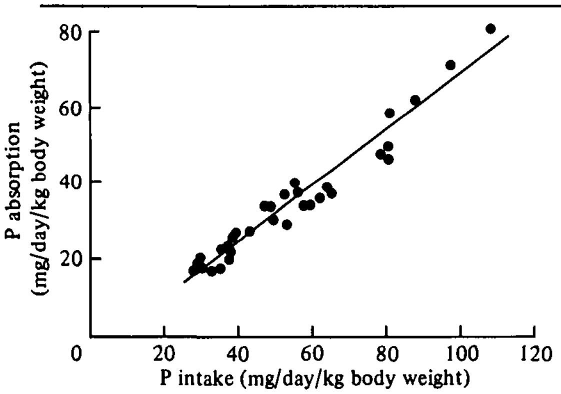


Fig. 2. Relationship between P intake ($P_i$) and dietary P absorption ($P_a$) in pregnant and lactating ewes. $P_a = -5{\cdot}2+0{\cdot}74\ (\pm 0{\cdot}032)\ P_i$.

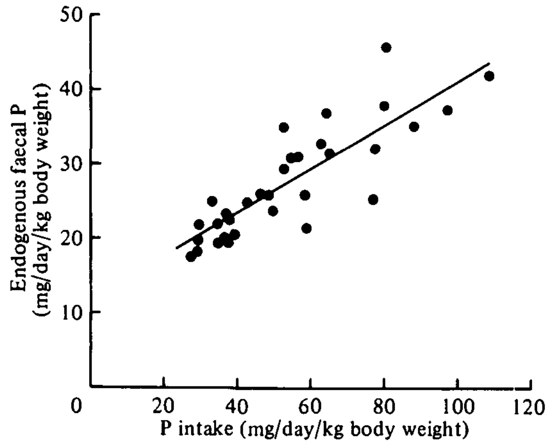


Fig. 3. Relationship between P intake ($P_i$) and endogenous faecal P ($P_f$) in pregnant and lactating ewes. $P_f = 11{\cdot}5+0{\cdot}30\ (\pm 0{\cdot}035)\ P_i$.

of resorption of P was increased to a high level compared with the rate of accretion. Mobilization was then decreased in mid- to late lactation, when the rate of bone and soft tissue resorption was decreased to approximately that of accretion. Resorption and accretion then remained at about the same level until the end of the experimental period, 1 month after the end of lactation.

Retention of P was strongly negative in late pregnancy and early lactation (Fig. 5) and although the rate of loss of P was reduced to almost zero in mid- to late lactation, it then remained at this level

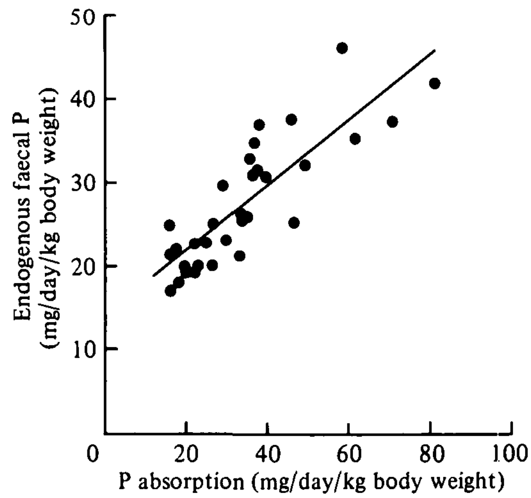


Fig. 4. Relationship between absorption of dietary P ($P_a$) and endogenous faecal P ($P_f$) in pregnant and lactating ewes.

$$P_f = 14{\cdot}1+0{\cdot}39\ (\pm 0{\cdot}047)\ P_a.$$

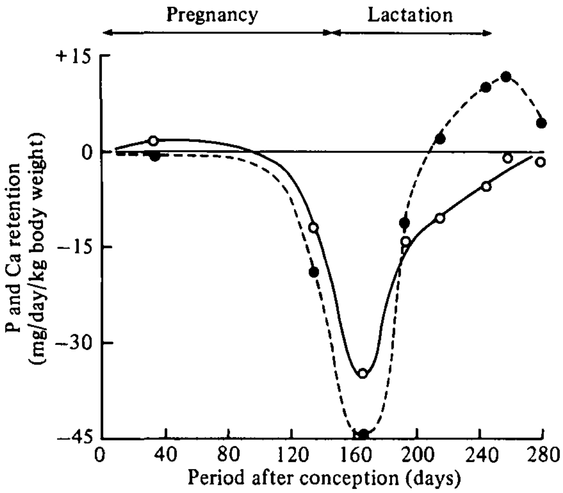


Fig. 5. Variations in P (○) and Ca (●) retention in ewes during pregnancy and lactation.

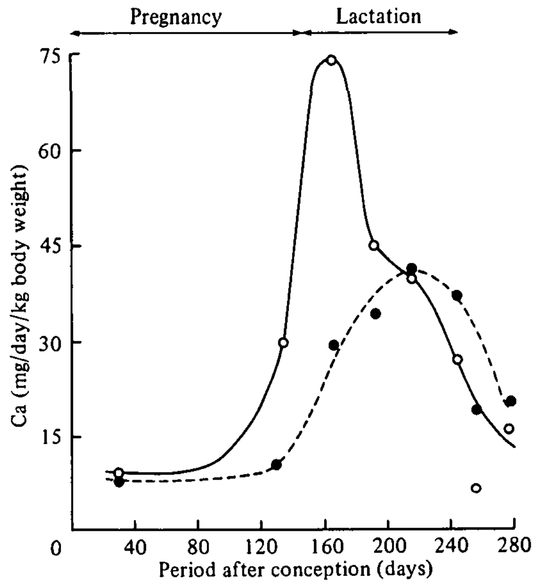


Fig. 6. Variations in Ca demands (○) and in absorption of dietary Ca (●) in ewes during pregnancy and lactation.

until the end of the experimental period and retention never became positive. Integration of the balance curve (Fig. 5) showed that ewes lost an average of 125 g P throughout the pregnancy and lactation.

*Ca metabolism*

Demands for Ca (the sum of losses in the urine, endogenous faecal losses and losses to the foetus in pregnancy or the milk in lactation)increased rapidly in late pregnancy, reached a peak in early lactation and then decreased throughout the remainder of lactation (Fig. 6). Although these changes occurred mainly as a result of the changing Ca demands for foetal development or milk production (Table 3), the endogenous faecal loss of Ca also increased in early lactation, probably as a result of the increased food intake at this time (see Braithwaite, 1982).

Despite the plentiful supply of dietary Ca, the rate of absorption, which was low in early pregnancy, did not increase quickly enough during late pregnancy and early lactation to meet the high Ca demands (Fig. 6). In mid- to late lactation, however, Ca was absorbed in excess of demands largely as a result of a small increase in absorptive efficiency.

The deficit between Ca losses and absorbed dietary Ca in late pregnancy and early lactation was made good by mobilization of skeletal Ca, achieved by an increased rate of bone resorption relative to bone accretion. Some, but not all, of the lost reserves were then replaced in late lactation when the rate of bone resorption was decreased relative to bone accretion.

These various changes in Ca metabolism were reflected in the Ca balance of the ewes (Fig. 5). Retention was negative in late pregnancy and early lactation when skeletal Ca reserves were mobilized to meet the high demands. Retention then became positive in mid- to late lactation when dietary Ca was absorbed in excess of immediate demands. Skeletal reserves, however, were only partially replaced and integration of the balance curve shows that ewes lost an average 135 g Ca during pregnancy and early lactation, but at 1 month after the end of lactation had replaced only 35 g (26%).

## DISCUSSION

Previous studies in this laboratory have shown that even those ewes given a plentiful supply of dietary Ca and P throughout pregnancy and lactation are unable to meet the high demands of late pregnancy and early lactation without mobilizing a considerable amount of their skeletal Ca and P reserves. This failure to supply the increased mineral demands from the diet appears to be due to the inability of ewes to increase Ca absorption quickly enough (Braithwaite, 1983*a*, *b*; Braithwaite *et al.* 1969, 1970). No harm is done, however, as reserves are normally replaced in late lactation and the following dry period when Ca is absorbed in excess of demands.

The present results show that ewes, given an intake which was plentiful in Ca but restricted to Agricultural Research Council (1980) recommendations for P, were unable to replace in late lactation those skeletal reserves of Ca and P lost in late pregnancy and early lactation.

In their calculations of P recommendations, the Agricultural Research Council (1980) made the assumption that dietary P is absorbed at a constant efficiency of 60% and that the endogenous faecal loss of P is maintained at the level (10–14 mg/day/kg body weight) expected if animals are given a P-free diet. The higher endogenous faecal losses of P, reported by various workers, were then taken to represent elimination of P absorbed in excess of demands.

In the present study, the efficiency of absorption of dietary P (62·8%) was almost the same as the value used by the Agricultural Research Council but the endogenous faecal loss of P, whilst variable, was at all stages of pregnancy and lactation, much higher than the Agricultural Research Council value.

Since skeletal reserves of Ca and P had to be mobilized in late pregnancy and early lactation to meet the increased demands for Ca, it is possible that the high endogenous faecal loss of P at this time might have been due to elimination of unwanted skeletal or dietary P. This could hardly be true in mid- to late lactation, however, because Ca absorption at this time had increased and P was also needed to allow replacement of skeletal reserve. In spite of this need for extra P in mid- to late lactation, the endogenous faecal loss of P was maintained at a high level (25–35 mg/day/kg body weight) and was not reduced to the 10–14 mg/day/kg body weight as assumed by the Agricultural Research Council (1980) in their calculation of P requirements.

It is well known that endogenous faecal P is increased in direct relation to the P intake (Preston & Pfander, 1964; Young, Lofgreen & Luick, 1966; Agricultural Research Council, 1980; Grace, 1980; Braithwaite, 1984), and recently Braithwaite (1984) suggested that the increase may be inevitable. This suggestion is supported by the present study and, indeed, has been confirmed in a further study from this laboratory (Braithwaite, 1985), in which actively growing lambs were given a diet either grossly deficient, moderately deficient or nearly adequate in P. Despite the high P requirement of the lambs, the endogenous faecal loss of P was increased in direct relation to the increased P intake and the increase occurred at the expense of P retention.

Normally sheep retain or lose Ca and P at a fairly constant ratio of about 1·5:1. Indeed, such a ratio was found during late pregnancy and early lactation when skeletal mineral was mobilized to meet increased demands. This ratio was not found during mid- to late lactation, however, when despite a positive Ca retention, P retention remained negative.

The fact that Ca:P retention ratios much higher than normal have also been observed in P deficient growing animals (Boxebeld *et al.* 1983; Braithwaite, 1985), suggests that during periods of P shortage, mineralization and remodelling of the skeleton can continue to a limited extent, but with a bone salt rich in Ca but poor in P.

*P requirements for pregnancy and lactation*

The present study suggests that the Agricultural Research Council (1980) recommendations of P requirements are inadequate, particularly in mid- to late lactation. There are two main reasons for underestimation of requirements. First, their recommendations are based on immediate day-to-day needs for maintenance and foetal or milk production and no allowance is made in their calculations for the inevitable loss and subsequent replacement of skeletal reserves associated with pregnancy and lactation. Since their calculations assume that absorption of dietary P is always maximal and endogenous P loss always minimal, there is no safety factor and little chance of animals fed strictly according to recommendations being able to find any additional P.

Secondly, the actual endogenous faecal loss of P may be much greater than assumed by the Agricultural Research Council in their calculations.

As a result of the present study, it is recommended that, rather than feed pregnant and lactating ewes according to their immediate day to day needs for P, it would be better to give less mineral than is indicated from calculated requirements during late pregnancy, when because of a limitation on Ca absorption, some mobilization of skeletal reserves is inevitable, and to give more mineral in mid- to late lactation when animals need to replace skeletal mineral reserves.

It is further recommended that all future estimates of P requirements for pregnant and lactating ewes take into account the possibility that the inevitable endogenous faecal loss of P is not constant but varies in direct relation to the P intake needed to achieve a particular requirement.

The author thanks Miss K. E. Masters and Mr J. K. Tweed for skilled technical assistance, Mr A. Mowlem for supervising the care of the experimental animals, and Dr S. V. Morant for the statistical analysis.

## REFERENCES


AGRICULTURAL RESEARCH COUNCIL (1965). *The Nutrient Requirements of Farm Livestock. No. 2, Ruminants.* London: H.M.S.O.

AGRICULTURAL RESEARCH COUNCIL (1980). *The Nutrient Requirements of Ruminant Livestock.* Slough: Commonwealth Agricultural Bureaux.

AUBERT, J.-P. & MILHAUD, G. (1960). Methode de measure des principles voies du metabolisme calcique chez l'homme. *Biochimica et Biophysica acta* **39**, 122–139.

BOXEBELD, A., GUEGUEN, L., HANNEQUART, G. & DURAND, M. (1983). Utilization of phosphorus and calcium and minimal maintenance requirement for phosphorus in growing sheep fed a low-phosphorus diet. *Reproduction Nutrition Développement* **23**, 1043–1053.

BRAITHWAITE, G. D. (1982). Endogenous faecal loss of calcium by ruminants. *Journal of Agricultural Science, Cambridge* **99**, 355–358.

BRAITHWAITE, G. D. (1983*a*). Calcium and phosphorus requirements of the ewe during pregnancy and lactation. 1. Calcium. *British Journal of Nutrition* **50**, 711–722.

BRAITHWAITE, G. D. (1983*b*). Calcium and phosphorus requirements of the ewe during pregnancy and lactation. 2. Phosphorus. *British Journal of Nutrition* **50**, 723–736.

BRAITHWAITE, G. D. (1984). Some observations on phosphorus homoeostasis and requirements of sheep. *Journal of Agricultural Science, Cambridge* **102**, 295–306.

BRAITHWAITE, G. D. (1985). Endogenous faecal loss of phosphorus in growing lambs and the calculation of phosphorus requirements. *Journal of Agricultural Science, Cambridge* **105**, 67–72.

BRAITHWAITE, G. D. & GLASCOCK, R. F. (1976). Metabolism of calcium in the sheep. *Biennial Reviews, National Institute for Research in Dairying*, pp. 43–59.

BRAITHWAITE, G. D., GLASCOCK, R. F. & RIAZUDDIN, Sh. (1969). Calcium metabolism in lactating ewes. *British Journal of Nutrition* **23**, 827–834.

BRAITHWAITE, G. D., GLASCOCK, R. F. & RIAZUDDIN, Sh. (1970). Calcium metabolism in pregnant ewes. *British Journal of Nutrition* **24**, 661–670.

BRAITHWAITE, G. D. & RIAZUDDIN, Sh. (1971). The effect of age and level of dietary Ca intake on calcium metabolism in sheep. *British Journal of Nutrition* **26**, 215–225.

FISKE, C. H. & SUBBAROW, Y. (1925). The colorimetric determination of phosphorus. *Journal of Biological Chemistry* **66**, 375–400.

GRACE, N. D. (1980). Effect of increasing phosphorus intake on the P faecal endogenous loss in the sheep. *Proceedings of the New Zealand Society for Animal Production* **40**, 211–225.

MANSTON, R. (1966). The effect of large doses of vitamin A on calcium and phosphorus metabolism in the cow. *British Veterinary Journal* **122**, 443–449.

PRESTON, R. L. & PFANDER, W. H. (1964). Phosphorus metabolism in lambs fed varying phosphorus intakes. *Journal of Nutrition* **83**, 369–378.

TECHNICON INSTRUMENTS CORPORATION (1967). Inorganic phosphate. *Technicon Method Sheet*, N-4B. Tarry Town, New York: Technicon Instruments Corporation.

TREACHER, T. T. (1970). Apparatus and milking techniques used in lactation studies with sheep. *Journal of Dairy Research* **37**, 289–295.

YOUNG, V. R., LOFGREEN, G. P. & LUICK, J. (1966). The effects of phosphorus depletion and of calcium and phosphorus intake, on the endogenous excretion of these elements by sheep. *British Journal of Nutrition* **20**, 795–805.