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AN INVESTIGATION INTO POSSIBLE METHODS OF ASSESSING THE INTAKE OF CALCIUM AND PHOSPHORUS BY GRAZING SHEEP*

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ABSTRACT

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A study was made, over a period of 29 weeks, of the interrelationship between calcium (Ca) and phosphorus (P) levels while monitoring those of magnesium (Mg) in ovine bone, soft tissues, faeces, urine and rumen fluid as well as blood plasma, where the P was determined as inorganic P (Pi) in order to determine whether the results reflected the dietary intake of P and Ca. Three groups of 6-month-old South African Mutton Merino × Merino wethers were fed 3 different rations containing, respectively, approximately the same amounts of crude protein (7,28; 7,67; 7,53%) and Mg (0,12; 0,13; 0,11%), but increasing amounts of P (0,05; 0,27; 0,63%) and varying amounts of Ca (0,22; 0,62; 0,20%). A comparison of the results for these Low, Medium and High phosphate (P) groups of animals showed that plasma Ca did not reflect dietary intake and, although the plasma Pi levels of the Low P group were low, those of the Medium and High groups rarely differed. Thus, in the assessment of the dietary intake of Ca and P by grazing sheep, plasma Ca and Pi levels must be viewed with extreme caution.

On the other hand, the mean faecal Ca and P levels did reflect the dietary levels of these 2 elements. The Ca content of small faecal grab samples was closely correlated with the Ca content of 4-day pooled samples from the same animal ($P<0,01$, $r=0,927$). A similar finding was true for P ($P<0,01$, $r=0,963$). Thus, taking rectal faeces from a number of sheep in a flock for individual or pooled analyses for Ca and P appears to be the best method for assessing the dietary intake of these elements.

The Ca percentage in bone increased as dietary Ca increased but was influenced by the availability of P since the ratio of P to Ca in bone remained virtually constant. There was a significant correlation between the ash percentage and the midshaft width of the cortex of the femur ($P<0,01$, $r=0,879$). Significant negative correlations ($P<0,01$) were also found between whole dry bone fat and ash percentage in cervical vertebrae ($r=-0,963$), ribs ($r=-0,903$) and femurs ($r=-0,885$). Thus ash analyses on whole dry bone will probably suffice to indicate the mineral status of the animals. Body composition estimated from tritiated water space was of little value as total body ash percentage was the reverse of the ash percentage actually found in the bones. Bone turnover as measured by urinary hydroxyproline excretion was significantly lower ($P<0,01$) in the Low P group.

The concentration of Ca and/or P in soft tissues did not reflect dietary intake. Similarly the Ca concentration in the case of urine and ruminal fluid did not reflect dietary intake whereas P concentrations did. Although the dietary intake of Ca and P was reflected in the mean daily urinary excretion, this measurement is unpractical in grazing animals.

Although dietary Mg was almost equal in the 3 groups, faecal Mg was the lowest and plasma Mg the highest in the Low P group. Bone Mg was significantly lower ($P<0,01$) in the Low P group than in the other 2 groups in respect of all 3 types of bone analysed.

Résumé

INVESTIGATION SUR LA POSSIBILITE DE METHODES VISANT A ÉVALUER L'ABSORPTION DU CALCIUM ET DU PHOSPHORE PAR DES MOUTONS EN PÂTURE

On a étudié la relation entre les niveaux de calcium (Ca) et de phosphore (P) chez le mouton, tandis qu'on suivait les niveaux du magnésium (Mg) dans l'os, les tissus mous, les fèces, l'urine et les sécrétions du rumen aussi bien que dans le plasma sanguin, où le P était déterminé comme P inorganique (Pi) afin de vérifier si les résultats reflétaient l'absorption alimentaire de P et C. L'étude a duré 29 semaines. Trois groupes de béliers chatrés "South African Mutton Merino × Merino" ont été nourris au moyen de 3 rations différentes contenant, respectivement, à peu près les mêmes quantités de protéine brute (7,28; 7,67; 7,53 %) et de Mg (0,12; 0,13; 0,11 %), mais des quantités croissantes de P (0,05; 0,27; 0,63 %) et des quantités variables de Ca (0,22; 0,62; 0,20 %). Une comparaison des résultats obtenus pour ces groupes d'animaux à phosphate (P) bas, moyen et élevé a montré que le Ca du plasma ne reflétait pas l'absorption alimentaire et que, malgré un faible niveau du Pi plasmatique dans le groupe à P bas, ces mêmes niveaux montraient rarement une différence entre les groupes moyen et élevé. En conséquence il faut être extrêmement prudent dans l'utilisation des niveaux de Ca et Pi plasmatiques pour évaluer l'absorption alimentaire de Ca et P par des moutons en pâture.

D'autre part, les niveaux moyens de Ca et P dans les fèces ont effectivement reflété les niveaux diététiques de ces 2 éléments. Le contenu en Ca de petits échantillons pris au hasard s'est montré en corrélation étroite ($P<0,01$, $r=0,927$) avec le contenu en Ca d'une accumulation d'échantillons portant sur 4 jours et venant du même animal. Des résultats semblables ont été obtenus avec P ($P<0,01$, $r=0,963$). Il apparaît donc que la prise de fèces dans le rectum chez un certain nombre de moutons d'un troupeau pour y analyser Ca et P individuellement ou sur un lot d'échantillons serait la meilleure méthode d'évaluation de l'absorption alimentaire de ces éléments.

Le pourcentage de Ca dans l'os a augmenté avec le taux de Ca dans l'alimentation mais a été influencé par les disponibilités en P puisque le rapport P/Ca dans l'os est resté pratiquement constant. On a trouvé une corrélation significative entre le pourcentage de cendres et le diamètre à mi-tige du cortex du fémur ($P<0,01$, $r=0,879$). Des corrélations négatives significatives ont aussi été trouvées entre la teneur en graisse de l'os entier sec et le pourcentage de cendres, pour les vertèbres cervicales ($r=-0,963$), les côtes ($r=-0,903$) et les fémurs ($r=-0,885$). En conséquence, l'analyse des cendres de l'os entier sec suffira probablement à indiquer l'état de minéralisation des bêtes. L'estimation de la composition dans le corps à partir du volume d'eau tritiée n'a pas été de grande valeur car le pourcentage global de cendres dans le corps était l'inverse du pourcentage effectivement trouvé dans les os. Le métabolisme osseux tel que mesuré par l'excrétion urinaire d'hydroxyproline à été significativement moindre ($P<0,01$) dans le groupe à P bas.

La concentration en Ca et/ou en P dans les tissus mous n'a pas reflété l'absorption alimentaire. De même les concentrations en Ca dans l'urine et les sécrétions du rumen n'ont pas reflété l'absorption alimentaire, mais les concentrations en P l'ont fait. Bien que l'absorption alimentaire de Ca et P se soit reflétée dans l'excrétion urinaire moyenne journalière, cette dernière mesure est peu pratique s'il faut l'effectuer sur des animaux en pâture.

Quoique le Mg alimentaire ait été pratiquement le même dans les trois groupes, le Mg fécal a été le plus bas et le Mg plasmatique le plus élevé dans le groupe à P bas. Le Mg osseux à été significativement plus faible ($P<0,01$) dans le groupe à P bas que dans les 2 autres groupes, et ceci par rapport aux 3 types d'os qui ont été analysés.

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Introduction

Calcium and phosphorus, being important constituents in the diet of sheep, have attracted much attention to their requirements, dietary levels and interrelationship. In a review of the literature, McDonald (1968) could find no authentic record of a primary calcium deficiency in grazing cattle or sheep, whereas a primary phosphorus deficiency was widespread in cattle. Nowhere had this phosphorus deficiency been clearly demonstrated in sheep despite the fact that much of the world's grazing is low in phosphorus. In South Africa, Du Toit, Malan & Rossouw (1930) noted that sheep could remain apparently normal and healthy in areas where cattle would suffer from extreme phosphorus deficiency. This could be attributable in part, at least, to the highly selective grazing habits of sheep for the most nutritious plants (McDonald, 1968), a situation which also applies to South African sheep (Engels, 1972).

The dramatic, widely publicized improvements brought about by phosphorus supplementation in cattle have resulted in this practice becoming common also in the sheep industry. However, Steenkamp (1967) found that phosphorus supplementation had no beneficial effect on the body mass of sheep, while wool production actually decreased slightly. On the other hand, Kotze (1950), Van der Vyver & Van Niekerk (1965) and Cloete (1971) have recorded improved body mass gain and a decreased mortality rate during protracted droughts in sheep that received phosphorus supplementation. Thus it appears that, although sheep are better able than cattle to exist on pastures which are low in phosphorus, supplementation may be necessary under certain limited circumstances. In view of this, phosphorus supplementation can be economically justified only when the phosphorus status of the grazing sheep warrants it.

Although the required intake of phosphorus for sheep has been established under controlled conditions [National Research Council (NRC), 1968], it is virtually impossible to determine the actual intake of the grazing animal because of its habit of selection (McDonald, 1968). Engels (1972) used oesophageal-fistulated sheep to demonstrate that the herbage which they selected from natural pasture contained, on the average, 125,7% more crude protein and 37,6% less crude fibre than samples collected by hand from the same pasture. Thus, in general, hand-collected samples do not truly reflect the animal's intake. The use of oesophageal-fistulated sheep is unpractical for large scale diagnostic procedures, while the material harvested is diluted with saliva which is high in phosphorus (McDougall, 1948). Thus there is an urgent need for reliable diagnostic methods to assess the phosphorus status of the grazing animal.

Existing diagnostic tests are based on blood analyses. The level of inorganic phosphate in the blood has been used as a measure of the phosphorus status of animals in the classical series of articles "Studies in Mineral Metabolism" by Theiler, Green & Du Toit (1928 *et seq.*). The Compton Metabolic Profile Test is used on the blood of lactating cows, but it is limited to being a test of metabolic status within a herd, within a season (Payne, Dew, Manston, & Faulks, 1970; Payne, Rowlands, Manston & Dew, 1973; Payne, Rowlands, Manston, Dew & Parker, 1974). Blood analyses, particularly of phosphorus alone, are difficult to interpret and give little or no information about the mineral reserves in the body (Benzie, Boyne, Dalgarno, Duckworth, Hill & Walker, 1955). As the metabolism of calcium and phosphorus is closely interrelated, and, as most of the phosphorus supplements are in the form of calcium salts, it is imperative that calcium be included in any investigation into phosphorus metabolism. Nevertheless, Sykes & Field (1974) found that plasma levels of calcium and phosphorus are inadequate as indicators of the nutritional status of the sheep since they give no information about reserves of these elements.

Thus it was decided in the present experiment to study the interrelationship between the levels of calcium and phosphorus while monitoring those of magnesium in sheep, since magnesium metabolism is closely related to that of calcium and phosphorus (Jacobson, Hemken, Button & Hatton, 1972). Three diets containing approximately the same amounts of crude protein and magnesium but differing in calcium and phosphorus content were used. Analyses for calcium, phosphorus and magnesium were performed not only on blood plasma but also on bone, various soft tissues, faeces, rumen fluid and urine to determine whether the results reflected the dietary intake of calcium and phosphorus. In addition, bones were analysed for ash and fat content, urine for hydroxyproline excretion to indicate bone turnover, and total body composition was estimated *in vivo* from tritiated water space. Evidence was obtained that rectal faeces from a number of sheep in a flock for individual or pooled analyses provides the best method of assessing the dietary intake of calcium and phosphorus. Ash analyses on whole dry bone probably suffice to indicate the mineral status of the animals.

Materials and Methods

Animals

Eighteen healthy 6-month-old South African Mutton Merino × Merino wethers were selected. They were blocked by body mass into 3 equal groups. During the 29 weeks of the experiment all 3 groups were kept, at intervals, either in concrete floored camps with a roof and uncovered runway, or on slatted floors in a shed with an open side allowing plenty of reflected sunlight to enter.

Before the commencement of the experiment, the animals were treated with a broad spectrum anthelmintic (Tramisol[a]) and a coccidiostat (Bonaid[b]). At the end of the 4th week they were treated again with Tramisol. At the end of the 8th week they were treated with an anthelmintic which is also effective against nasal worm (Dylox[c]), Bonaid and injectable vitamin A (Inject A[d]). At the end of the 12th week they were given a final treatment with the anthelmintic, Bonlam[e], and the coccidiostat, Amprol RH[e]. When the animals were slaughtered during the 29th week they were found to be free from internal parasites and there was no indication of coccidial infestation.

Specimens could not always be taken from all 6 animals in each group. However, in any single analysis never fewer than 5 of the animals in a group were represented.

Feed

Three different rations containing approximately the same amounts of crude protein and magnesium but differing in calcium and phosphorus content were formulated (Table 1).

[a] Imperial Chemical Industries, U.K.
[b] Norwich Pharmaceuticals, U.K.
[c] Bayer Agro-Chem, Johannesburg
[d] A.S. Ruffel, Isando, Transvaal
[e] M.S.D., Halfwayhouse, Transvaal

TABLE 1 The constituents and amounts (kg) used to compound the rations fed to the 3 groups of sheep

Ration 1 for Low phosphate group		Ration 2 for Medium phosphate group		Ration 3 for High phosphate group	
Veldgrass[a]	100	Oat hay[b]	100	Oat hay	100
Molasses meal[c]	8	Molasses meal	8	Molasses meal	8
Urea	1,5	Urea	1,5	Urea	1,5
		Deproteinized bone meal	1,3	NaH_2PO_4	2

[a] From Armoedsvlakte, N. Cape. Known to contain very low levels of P (Bisschop, 1964)
[b] From Stellenbosch, S.W. Cape
[c] Voermol Products, Cape

The constituents were thoroughly mixed while passing through a hammermill with a 12,5 mm sieve, then through a hammermill with a 6 mm sieve into a mixing drum from which they were fed into a pelleting machine and cubed. The animals were gradually adapted to the new diets over a period of 4 weeks. Daily intake per group was limited to the total amount consumed by the group eating the least the previous day. By this means the total intake of all 3 groups over the whole experimental period was about equal.

Specimens and data collected before slaughter

Food samples: Grab samples of each of the rations were taken periodically and stored. The resultant composite sample was then milled fine, mixed, and a 500 g subsample kept for analysis.

Body mass: Except during the 5th week, the animals were mass measured weekly for the first 23 weeks. They were mass measured again during the 28th week after having been shorn and deprived of food and water for 24 h. Their final mass was used in all calculations involving body mass.

Blood samples: Samples of jugular blood for plasma calcium, inorganic phosphate, magnesium and total protein were first taken during the 5th week after the animals had been put onto the experimental rations. Further specimens were taken during the 10th, 14th, 18th and 24th weeks.

During the 28th week, the animals were shorn and blood samples were taken before and after the injection of tritium for the determination of body composition from tritiated water space.

Faecal and urine samples: During the 24th week the sheep were fitted with harnesses with faecal bags and urine funnels. The urine was drawn away from the funnel through a plastic pipe into a bottle kept cool under the slatted floor of the shed.

Three days were allowed for the animals to become used to carrying the harness. Thereafter faeces and urine were collected daily for 4 days. Each day the total faecal production was measured, mixed well and 10% aliquot taken and frozen. The 4 aliquots were later mixed together before analyses were performed. On the 4th day a small subsample was taken from the top of the bag of faeces of each sheep and kept frozen in a separate plastic bag as a grab sample.

The daily urine production was measured, mixed, and a 10% aliquot taken and frozen. The 4 aliquots were later mixed together before analyses were performed. A 10 ml subsample of urine was also taken from the final 24 h urine specimen of each sheep and frozen for hydroxyproline analysis.

Specimens and data collected after slaughter

During the 29th week the sheep were slaughtered and the following samples were collected:

Muscles: The tip of the tongue from the junction of the frenum linguae forward, the wall of the left ventricle and the gluteus medius were used for analysis. Each specimen was sliced thinly and laid flat in a plastic bag which was sealed and then frozen.

Organs: Brain, liver and kidney specimens were taken for analysis. To prevent contaminating the brain specimen with bone during its removal, the head was opened by a sagittal section well off-centre. The exposed area was flushed with distilled water and the brain removed. The undamaged cerebral hemisphere was then taken as a specimen as were the ventral lobe of the liver from the umbilical fissure and 1 whole kidney. Each specimen was sliced thinly and laid flat in a plastic bag which was then sealed and frozen.

Bones: Cervical vertebrae, ribs and femurs were analysed. The 2nd, 3rd and 4th cervical vertebrae were removed as a unit. The 7th rib was sectioned at the costochondral junction and dislocated at the vertebral extremity. The femur was dislocated from the pelvis and tibia and the patella was removed. Most of the flesh was removed from the bones before they were sealed in plastic bags and frozen.

Rumen fluid: The total rumen content was removed and strained through several layers of fine cheese cloth. The rumen fluid was then well mixed and a sample taken and frozen.

Processing of samples and analytical methods

Food: Triplicate specimens were taken from each of the 3 rations. These were dried to a constant mass at 103°C.

For mineral analyses, about 2 g of sample was accurately measured and then ashed in a muffle oven at 580°C. The ash was dissolved in 20% HCl over a boiling water bath. The solution was cooled and transferred to a 100 ml volumetric flask, washed with distilled water and made up to the mark with distilled water. This stock solution was filtered through Whatman No. 40 filter paper and then suitably diluted for analyses. Calcium and magnesium were determined by atomic absorption spectrophotometry using a nitrous oxide flame. To suppress ionization, potassium was added to the samples and standards as described in the Varian–Techtron Manual (1972). Phosphorus was determined by the phosphovanado-molybdate photometric method of Hanson (1950).

These methods were also employed for samples of faeces, urine, muscles, organs, bones, and rumen fluid.

For crude protein determinations the nitrogen content of the rations was determined on separate subsamples by the Kjeldahl method of the A.O.A.C. (1960) and then multiplied by 6,25 for calculating the crude protein equivalent.

Blood: Free-flowing jugular blood samples were taken with a 10 ml syringe containing 100 IU dry heparin. Plasma was removed after centrifugation for inorganic phosphate determinations (Delsal & Manhouri, 1958) which commenced within 30 min of bleeding. The remaining plasma was kept frozen and

later analysed for calcium and magnesium by atomic absorption as described above, and for total protein (Weichselbaum, 1946).

Faeces: The faecal specimens were dried at 103°C for 5 days, milled fine and mixed well. Subsamples were again dried at 103°C until constant mass and then ashed and analysed for calcium, phosphorus and magnesium.

Urine: For mineral analyses, 25 ml of the mixed urine aliquots was placed in a porcelain dish, dried over a boiling water bath and ashed at 580°C. The ash was dissolved in HCl, transferred to a 25 ml volumetric flask and made up to the mark with distilled water. This solution was analysed for calcium, phosphorus and magnesium.

The urine subsamples taken from the urine produced during the 4th day were used to determine the hydroxyproline excretion in mg/24 h/body mass^{0,75} (Hypronosticon, Organon).

Body composition: The method of Searle (1970) was used to determine body composition *in vivo* from tritiated water space and body mass. Body composition was calculated by means of the following formulae:

(a) Total body water (kg) = —0,01 + 0,92X
(b) Total body fat (kg) = 0,01 — 1,05X + 0,90Y
(c) Total body protein (kg) = 0,007 + 0,139X + 0,05Y
(d) Total body ash (kg) = 0,04 + 0,04X —0,004Y + 0,03Z
(e) Total body energy (Mcal) = —0,30 —8,87X + 8,54Y

Where X = tritiated water space (kg)
Y = body mass (kg) of shorn animals after 24 h without food or water
Z = age of animals in months (12)

From these data the components could be expressed on a percentage basis.

Muscles and organs: The individual muscle and organ specimens were dried at 103°C for 3 days. To ensure brittleness and to prevent the fat from clogging the mill, the samples were deep frozen in dry ice and then passed through the mill together with a large quantity of dry ice. These fine-milled samples were then dried to a constant mass at 103°C and then a subsample was ashed and analysed for calcium, phosphorus and magnesium. Another subsample was used to determine fat content by ether extraction in a Goldfish apparatus. The results of the fat analyses were used to calculate the mineral content of the tissues on a fat-free basis.

Bones: The 3rd cervical vertebrae were carefully dissected from the 2nd and 4th vertebrae and these, together with the ribs and femurs, carefully cleaned with a scalpel of all extraneous tissue. All the bones were placed in holders and dried in an oven at 70°C for 7–10 days to constant mass. The cervical vertebrae and ribs were then deep frozen in dry ice and milled with dry ice as had been done with the muscle and organ samples. As the femurs were too large and their fat content too high to be put through the mill, these bones were crushed fine with a pestle and mortar. Before they were completely crushed, their mid-shaft cortical width was measured with a micrometer.

These fine-milled bone samples were again dried to constant mass at 103°C and then a subsample was ashed. After the ash content had been determined, the ash was dissolved in HCl and analysed for calcium, phosphorus and magnesium.

A further subsample was used to determine fat content by ether extraction in a Goldfish apparatus. The results of the fat analyses were used to calculate the mineral content of the bones on a fat-free basis. In addition the dry fat-free bone material was also ashed. After the ash content had been determined, the ash was dissolved in HCl and analysed for calcium, phosphorus and magnesium.

Rumen fluid: The samples were thoroughly mixed and a 10 ml specimen was dried, ashed, dissolved in HCl and analysed for calcium, phosphorus and magnesium.

Distilled water: All the distilled water used in these determinations was deionized glass distilled water with a resistance greater than 3 megaohm/cm.

Statistical methods: Standard deviations, correlations, regressions, analysis of variance and Tukeys D-test were calculated as outlined by Snedecor & Cochran (1967).

Results and Discussion

Feed

As can be seen in Table 2, the 3 rations used had 2 levels of calcium (0,22; 0,20 and 0,62%), 3 levels of phosphorus (0,05; 0,27 and 0,63%), and virtually the same levels of magnesium (0,12; 0,13 and 0,11%) and crude protein (7,28; 7,67 and 7,53%).

TABLE 2 The percentage calcium, phosphorus, magnesium and crude protein in the Low, Medium and High phosphate rations fed to the 3 groups of sheep

Ration constituent (%)	Low phosphate ration	Medium phosphate ration	High phosphate ration
Calcium	0,22	0,62	0,20
Phosphorus	0,05	0,27	0,63
Magnesium	0,12	0,13	0,11
Crude protein	7,28	7,67	7,53

Recent NRC standards (1968) recommend that sheep with a body mass of about 30 kg should receive diets containing 0,21% calcium, 0,19% phosphorus, 0,06% magnesium and 11% crude protein. The Low phosphate ration therefore contained the recommended amount of calcium, was very low in phosphorus but more than adequate in magnesium. The Medium phosphate ration contained much higher calcium, slightly higher phosphorus and more than adequate magnesium. The High phosphate ration, again, contained the recommended amount of calcium, was very high in phosphorus but had more than an adequate amount of magnesium. All 3 rations were low in crude protein but this was probably close to the levels which are consumed by sheep on natural pastures in this country (Du Toit, Louw & Malan, 1940; Van Schalkwyk, Lombard & Vorster, 1968).

Although the total food intake was similar in each of the 3 groups of animals, there may have been differences in the availability of the various components of each ration. This is a problem which will also be encountered under field conditions. There were wide ranges in calcium and particularly in phosphorus in the 3 diets. This meant that large differences would also have to be present between specimens taken from each group of animals if analyses were to be of practical diagnostic significance. Moreover, these sample differences would have to reflect the differences in the various rations.

Body mass

As the 3 groups of sheep had been selected on the basis of body mass, the group average for the Low

(28,1±3,2 kg), Medium (28,0±2,5 kg) and High phosphate (27,5±1,8 kg) groups was similar at the commencement of the experiment. The average was again similar for the Low (26,3±3,5 kg), Medium (26,3±3,0 kg) and High phosphate (25,4±1,8 kg) groups after the 4-week adaptation period. Thereafter, as shown in Fig. 1, there was a gradual but definite difference between the groups, with the Medium gaining mass faster than the High phosphate group, and the Low phosphate group actually slowly losing body mass during the experimental period. Thus, in the 23rd week of the experiment, the group average for the groups was Low (24,1±3,0 kg), High (33,3± 3,6 kg), Medium (38,8±4,2 kg). The sharp decrease in mass at the end of the experiment, when the group average was Low (19,5±3,0 kg), High (27,4±4,6 kg), Medium (35,1±4,2 kg), was the result of shearing the animals and withholding food and water for 24 h for critical mass determination. It is likely that the phosphorus intake affected body mass, at least in part, as it has been shown that both very low (Du Toit, Malan & Groenewald, 1932) and excessively high intakes of phosphorus (Wise, Ordoveza & Barrick, 1963; Young, Richards, Lofgreen & Luick, 1966) have a detrimental effect on performance in ruminants. Details of the values obtained for body mass appear elsewhere (Belonje, 1976b).

Calcium

Although the 3 rations had only 2 levels of calcium (Table 2), even after the 4-week adaptation period, the mean plasma calcium levels in the groups were at 3 different levels in an order which remained the same during 24 weeks (Fig. 2A). This order did not in fact reflect the dietary intake, as the 2 groups (Low and High phosphate) on similar calcium intakes of 0,2% bracketed the group (Medium phosphate) which had a calcium intake of 0,62% (Table 3). This difference between the groups was so pronounced by the 24th week that each mean value differed significantly ($P<0,01$) from the other. It has been shown that calcium intake affects the level of calcium in the blood (Benzie *et al*, 1955; McRoberts, Hill & Dalgarno, 1965a, b). In this study, however, the large variation in dietary phosphorus and consequent large differences in the levels of plasma inorganic phosphate (Table 3; Fig. 3) appeared to have affected the calcium levels in a reciprocal fashion ($r=-0,699$, $P<0,01$) to modify considerably this effect. It is obvious then that plasma levels of calcium cannot be used to indicate dietary intake without considering plasma inorganic phosphate at least, as in this experiment individual values for plasma calcium varied from 7,6–12,5 mg/100 ml on the same dietary intake of calcium but with vastly different intakes of phosphorus. Other factors such as the hormonal control of calcium (Simesen, 1970) and acid base balance (Belonje, 1976a) also make the interpretation of plasma calcium levels difficult. It is pleasing to note that in this study plasma protein levels which may affect total plasma calcium values (Berry, Gupta, Turner & Burns, 1973), were not significantly different between the groups (Table 3).

Although plasma calcium levels did not indicate dietary intake, faecal calcium levels did (Table 4; Fig. 3). The Low phosphate group and High phosphate group which received 0,2% calcium had mean values of 0,48 and 0,47% calcium in the faeces while the Medium phosphate group which received 0,62% calcium had a mean of 1,6% calcium in the faeces. The same relationship was found in the faecal grab samples. The individual grab sample calcium levels were significantly correlated with the calcium levels in the individual pooled 4-day sample ($r=0,927$, $P<0,01$). This latter finding suggests that on a flock basis small samples of rectal faeces from a number of animals may be analysed or, even more simply, pooled, mixed and analysed for calcium to estimate the calcium intake. The more samples taken the better, so that the effect of single animals which may be abnormal may be neutralized. Furthermore, in order to make this method more critical, standards will have to be compiled of the effect of various levels of dietary calcium and other feed components on the level of calcium in the faeces. Even without such critical standards, however, the relationship between faecal calcium and phosphorus will be most useful in interpreting the levels of calcium found in the blood. This point will be discussed more fully under *Phosphorus* (*vide infra*).

The concentration of calcium in the urine (Table 5) reflects not dietary intake but plasma levels. On the other hand the mean daily output of calcium over 4 days clearly reflects dietary intake. However, in order to obtain daily urine specimens it would be necessary for the animals to carry harnesses and collection bottles which is considered to be unpractical under free-ranging conditions on natural pastures.

The calcium levels in the soft tissues (Table 6) did not appear to be affected by the calcium levels in the diet. No significant differences were found between the groups in the calcium content of dry left ventricle, gluteus medius, brain, liver and kidney expressed on a fat-free basis. The differences found between the groups in the calcium content of dry tongue are significant only at the 5% level and it is doubtful whether this analysis will have any practical value.

The calcium percentage in bone is affected by the calcium level in the diet (Tables 7, 8 and 9). The calcium percentages of the cervical vertebrae, ribs and femurs were always higher in the animals receiving the 0,62% calcium (Medium phosphate group) than the other 2 groups receiving 0,2% calcium. The position was similar when analyses were performed on dry fat-containing bone, dry fat-free bone or when the results were calculated on a fat-free basis. The deposition of calcium into bone is also dependent on the availability of phosphorus (Vaughan, 1970). The whole bone and fat-free bone analyses (Tables 7, 8 and 9) show that there was a higher percentage of calcium in the bones in the High phosphate group than in those of the Low phosphate group. This trend was not found in the calcium percentages in bone calculated to a fat-free basis. In fact, in the cervical and femur samples, the calcium content in the High phosphate group was actually lower than in the Low phosphate group. Nevertheless, the actual differences between the calcium content of the various bones by the different methods were so small that statistical significance does not necessarily indicate biological significance. In this experiment the levels of dietary calcium varied from the recommended 0,2% (Low and High phosphate rations) to 0,6% (Medium phosphate ration). Although calcium intakes below the recommended amount will result in lowered bone ash and calcium (McRoberts *et al.* 1965a), it is unlikely that free-ranging animals on natural pastures in this country will consume a diet containing less than 0,2 per cent calcium (Du Toit *et al.*, 1940). It is doubtful therefore whether bone calcium analyses are warranted or will be of any greater value than bone ash analyses as a diagnostic procedure for such animals.

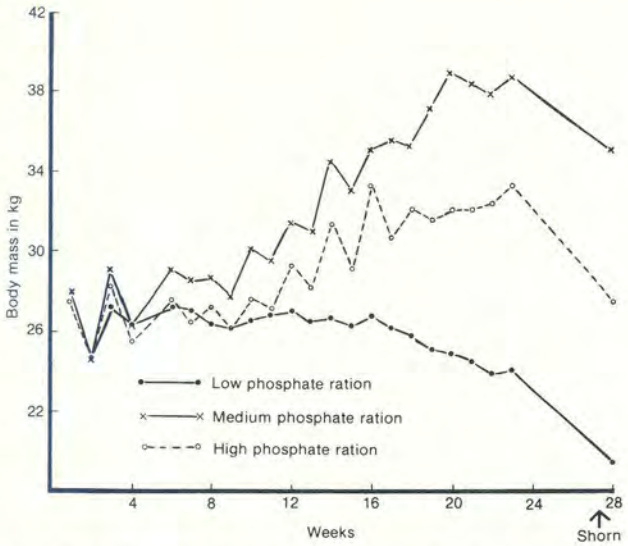

FIG. 1 The changes in body mass during the experimental period in the three groups of sheep receiving the Low, Medium and High phosphate rations

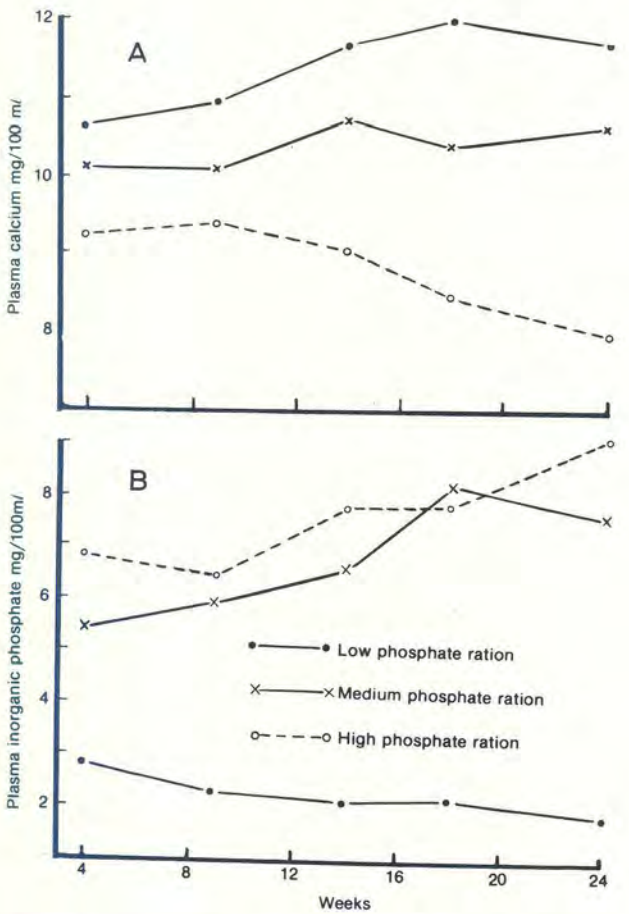

FIG. 2 The changes in the mean plasma levels of calcium (A) and inorganic phosphate (B) in the three groups of sheep receiving the Low, Medium and High phosphate rations

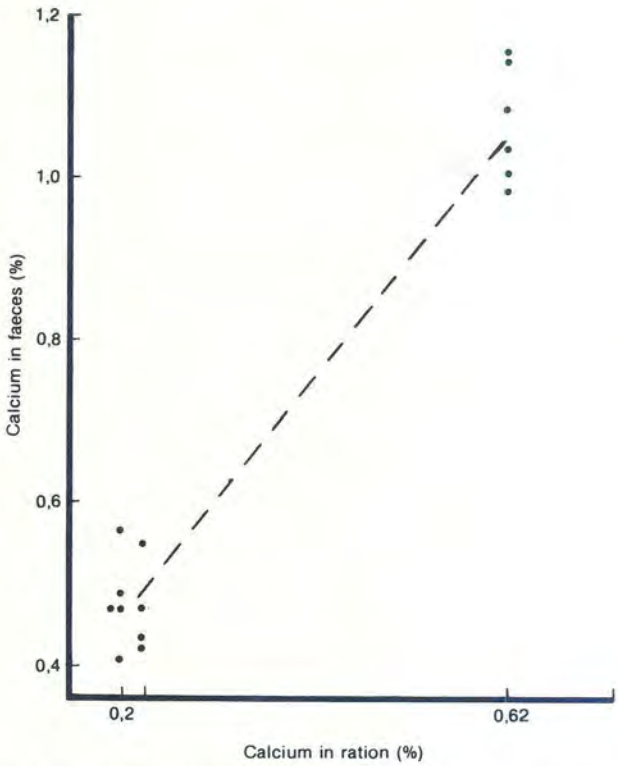

FIG. 3 The relationship between the percentage calcium in the ration and the percentage calcium in the faeces of individual sheep receiving the Low (0,22% calcium), Medium (0,62% calcium) and High phosphate (0,20% calcium) rations. (The dotted line joins the means)

TABLE 3 The means (±SD) of the plasma calcium, inorganic phosphate, magnesium and total protein levels during 24 weeks of the experimental period in the 3 groups of sheep receiving the Low, Medium or High phosphate rations

Plasma constituent	Weeks of experiment	Low phosphate group	Medium phosphate group	High phosphate group	Significance of differences between values in columns 1, 2 and 3		
		Column 1	Column 2	Column 3	Not significant	P<0,05	P<0,01
Calcium (mg/100 ml)..	4	10,65±0,29	10,11±0,60	9,25±0,25	1v2	2v3	1v3
	9	10,95±0,60	10,10±0,54	9,40±0,24	1v2:2v3	—	1v3
	14	11,73±0,72	10,72±0,77	9,05±0,50	1v2	2v3	1v3
	18	12,02±0,61	10,37±0,79	8,45±0,75	—	1v2	1v3:2v3
	24	11,70±0,51	10,60±0,59	7,96±0,25	—	—	1v2:1v3:2v3
Inorganic phosphate (mg/100 ml)	4	2,86±0,56	5,45±1,08	6,86±0,93	—	2v3	1v2:1v3
	9	2,31±0,85	5,95±0,75	6,47±1,05	2v3	—	1v2:1v3
	14	2,11±0,53	6,60±1,17	7,77±0,70	2v3	—	1v2:1v3
	18	2,18±0,33	8,24±1,39	7,80±0,77	2v3	—	1v2:1v3
	24	1,80±0,24	7,62±1,11	9,16±0,42	—	—	1v2:1v3:2v3
Magnesium (mg/100 ml)	4	2,39±0,05	2,37±0,14	2,36±0,14	1v2:1v3:2v3	—	—
	9	2,50±0,20	2,11±0,13	2,24±0,10	2v3	1v3	1v2
	14	2,72±0,36	2,48±0,18	2,45±0,25	1v2:1v3:2v3	—	—
	18	2,58±0,17	2,40±0,15	2,03±0,12	1v2	—	1v3:2v3
	24	2,25±0,16	1,93±0,08	1,84±0,15	2v3	—	1v2:1v3
Total plasma protein (g/100 ml)	4	6,07±0,44	5,80±0,34	5,83±0,23	1v2:1v3:2v3	—	—
	9	5,68±0,54	6,10±0,23	6,25±0,32	1v2:1v3:2v3	—	—
	14	5,95±0,42	5,98±0,22	6,05±0,24	1v2:1v3:2v3	—	—
	18	5,92±0,37	6,25±0,26	6,47±0,61	1v2:1v3:2v3	—	—
	24	6,10±0,37	6,23±0,22	6,16±0,17	1v2:1v3:2v3	—	—

There were 6 animals per group except in the 24th week when there were only 5 animals in the High phosphate group

TABLE 4 The mean percentage (±SD) calcium, phosphorus and magnesium in the 4-day composite and grab faecal samples and the correlation between the composite and grab samples in the 3 groups of sheep receiving the Low, Medium and High phosphate rations

Mineral determined in faeces (%)	Type of faecal sample	Low phosphate group	Medium phosphate group	High phosphate group	Correlation between 4-day and grab samples
Calcium	4-day	0,48±0,06	1,06±0,06	0,47±0,05	r=0,927
	Grab	0,47±0,09	1,08±0,17	0,57±0,10	
Phosphorus	4-day	0,15±0,02	0,57±0,13	1,21±0,40	r=0,963
	Grab	0,17±0,03	0,58±0,10	0,94±0,24	
Magnesium	4-day	0,17±0,02	0,21±0,01	0,31±0,03	r=0,898
	Grab	0,13±0,05	0,20±0,05	0,37±0,10	
No. of animals/group		5	6	5	

TABLE 5 The average concentration and mean daily output (±SD) of calcium, phosphorus and magnesium and the total excretion of hydroxyproline/24h/kg body mass ^{0,75} in the urine of the 3 groups of sheep receiving the Low, Medium and High phosphate rations

Urine component determined	Nature of determination	Low phosphate group	Medium phosphate group	High phosphate group
Calcium	Concentration (mg/ml)	0,24± 0,06	0,13± 0,07	0,06± 0,01
	Mean daily output (mg)	54,75±18,30	101,27± 42,49	45,81± 33,56
Phosphorus	Concentration (mg/ml)	0,03± 0,006	0,59± 0,85	1,59± 0,36
	Mean daily output (mg)	6,38± 1,08	817,20± 1 472,94	1 562,25± 1 566,23
Magnesium	Concentration (mg/ml)	1,03± 0,19	0,28± 0,16	0,24± 0,11
	Mean daily output (mg)	238,61±98,25	217,75± 94,51	227,50± 188,86
Hydroxyproline	(mg/24h/kg body mass ^{0,75})	1,82± 0,41	3,63± 0,48	3,62± 0,88
No. of animals/group		5	6	5

TABLE 6 Mean percentage (±SD) calcium, phosphorus and magnesium in dry and in fat-free muscle and organ samples from sheep receiving the Low, Medium and High phosphate rations

Type of specimen	Element (%)	In dry tissue				In fat-free tissue			
		Low phosphate group (1)	Medium phosphate group (2)	High phosphate group (3)	Significance of differences between groups	Low phosphate group (1)	Medium phosphate group (2)	High phosphate group (3)	Significance of differences between groups
Tongue...	Calcium.....	0,03±0,01	0,04±0,02	0,02±0,004	P<0,05(2)v(3)	0,04±0,01	0,05±0,02	0,03±0,005	NS
	Phosphorus..	0,68±0,05	0,70±0,04	0,71±0,04	NS	0,86±0,06	0,88±0,03	0,91±0,05	NS
	Magnesium..	0,08±0,01	0,08±0,01	0,09±0,02	NS	0,10±0,01	0,11±0,01	0,11±0,03	NS
Left Ventricle	Calcium.....	0,02±0,005	0,03±0,01	0,03±0,01	NS	0,03±0,00	0,03±0,02	0,03±0,01	NS
	Phosphorus..	0,91±0,12	0,90±0,03	0,93±0,06	NS	1,11±0,07	1,14±0,03	1,08±0,04	NS
	Magnesium..	0,09±0,01	0,09±0,004	0,10±0,01	NS	0,11±0,01	0,11±0,00	0,12±0,005	NS
Gluteus Medius	Calcium.....	0,03±0,01	0,02±0,004	0,02±0,005	NS	0,03±0,01	0,02±0,004	0,02±0,005	NS
	Phosphorus..	0,78±0,03	0,72±0,06	0,78±0,05	NS	0,84±0,03	0,89±0,06	0,90±0,06	NS
	Magnesium..	0,09±0,004	0,09±0,009	0,10±0,004	NS	0,10±0,01	0,11±0,01	0,12±0,01	P<0,01(1)v(3)
Brain.....	Calcium.....	0,05±0,04	0,03±0,01	0,03±0,005	NS	0,09±0,07	0,05±0,01	0,04±0,005	NS
	Phosphorus..	1,40±0,09	1,28±0,13	1,38±0,05	NS	2,43±0,23	2,08±0,35	2,43±0,10	NS
	Magnesium..	0,07±0,005	0,07±0,02	0,08±0,01	NS	0,12±0,01	0,12±0,03	0,13±0,02	NS
Liver.....	Calcium.....	0,02±0,004	0,02±0,01	0,02±0,00	NS	0,03±0,01	0,03±0,01	0,02±0,004	NS
	Phosphorus..	1,10±0,07	1,18±0,05	1,16±0,03	NS	1,30±0,16	1,35±0,08	1,40±0,17	NS
	Magnesium..	0,07±0,01	0,06±0,01	0,06±0,004	NS	0,08±0,02	0,07±0,01	0,07±0,01	NS
Kidney...	Calcium.....	0,06±0,01	0,07±0,02	0,06±0,02	NS	0,07±0,01	0,08±0,02	0,08±0,03	NS
	Phosphorus..	1,18±0,10	1,17±0,06	1,19±0,11	NS	1,39±0,06	1,41±0,05	1,47±0,10	NS
	Magnesium..	0,09±0,01	0,08±0,004	0,09±0,01	NS	0,11±0,01	0,10±0,004	0,11±0,01	NS
No. of animals/group....		6	5	5		6	5	5	

NS=Not significant

TABLE 7 Analyses (mean ±SD) of total dry 3rd cervical vertebrae in sheep receiving the Low, Medium and High phosphate rations

Determinations	Low phosphate group	Medium phosphate group	High phosphate group	Significance of differences between values in Columns 1, 2 and 3		
	Column 1	Column 2	Column 3	Not significant	P<0,05	P<0,01
Total dry bone mass (g)	9,95± 2,02	18,84±3,23	13,96±2,88	1v3:2v3	—	1v2
Bone mass (g/kg body mass)	0,52± 0,07	0,53±0,06	0,51±0,05	1v2:1v3:2v3	—	—
Total bone ash mass (g)	3,06± 0,60	8,58±1,63	5,46±1,23	—	1v3	1v2:2v3
Ash mass (g/kg body mass)	0,16± 0,03	0,24±0,03	0,20±0,01	—	1v3:2v3	1v2
Ash (%)	31,13± 4,52	45,57±3,61	39,09±2,28	—	—	1v2:1v3:2v3
Fat (%)	38,63±10,27	19,27±5,59	26,35±3,22	2v3	1v3	1v2
Calcium (%)	11,75± 1,49	16,48±1,09	14,15±0,98	—	1v3:2v3	1v2
Phosphorus (%)	5,68± 0,78	8,55±0,59	7,36±0,56	—	2v3	1v2:1v3
Magnesium (%)	0,19± 0,03	0,37±0,03	0,33±0,02	2v3	—	1v2:1v3
Ratio of calcium: phosphorus	2,07± 0,03	1,93±0,05	1,92±0,05	2v3	—	1v2:1v3
Correlation between ash and fat	All samples r=−0,963					
DRY BONE: FAT-FREE BASIS						
Ash (%)	50,97± 3,54	56,42±0,83	53,05±1,02	1v3:2v3	—	1v2
Calcium (%)	19,28± 1,26	20,42±0,66	19,20±1,42	1v2:1v3:2v3	—	—
Phosphorus (%)	9,30± 0,49	10,60±0,17	9,99±0,41	—	1v3:2v3	1v2
Magnesium (%)	0,32± 0,03	0,45±0,03	0,45±0,02	2v3	—	1v2:1v3
DRY DEFATTED BONE						
Ash (%)	50,02± 0,85	54,84±6,55	53,68±0,74	1v2:1v3:2v3	—	—
Calcium (%)	19,22± 0,24	21,69±0,54	19,98±0,37	—	1v3	1v2:2v3
Phosphorus (%)	8,67± 0,10	10,42±0,24	9,92±0,45	—	2v3	1v2:1v3
Magnesium (%)	0,34± 0,02	0,48±0,03	0,48±0,04	2v3	—	1v2:1v3
Ratio of calcium: phosphorus	2,22± 0,04	2,08±0,03	2,02±0,06	2v3	—	1v2:1v3
No. of animals/group	6	6	5			

TABLE 8 Analyses (mean ±SD) of total dry 7th rib in sheep receiving the Low, Medium and High phosphate rations

Determinations	Low phosphate group	Medium phosphate group	High phosphate group	Significance of differences between values in Columns 1, 2 and 3		
	Column 1	Column 2	Column 3	Not significant	P<0,05	P<0,01
Total dry bone mass (g)	4,13±1,07	9,59±1,31	6,64±1,08	—	—	1v2:1v3:2v3
Bone mass (g/kg body mass)	0,21±0,04	0,27±0,02	0,24±0,01	1v2:2v3	—	1v2
Total bone ash mass (g)	1,65±0,41	4,99±0,78	3,08±0,56	—	—	1v2:1v3:2v3
Ash mass (g/kg body mass)	0,08±0,02	0,14±0,01	0,11±0,01	1v3	—	1v2:2v3
Ash (%)	40,12±3,71	52,03±3,43	46,27±2,03	—	1v3:2v3	1v2
Fat (%)	23,67±6,70	13,39±4,20	20,39±3,27	1v3:2v3	—	1v2
Calcium (%)	15,40±1,46	18,76±1,21	16,25±2,78	1v3:2v3	1v2	—
Phosphorus (%)	6,69±0,58	8,99±0,60	7,75±0,77	—	1v3:2v3	1v2
Magnesium (%)	0,23±0,03	0,42±0,04	0,40±0,02	2v3	—	1v2:1v3
Ratio of calcium: phosphorus	2,30±0,05	2,09±0,07	2,09±0,23	2v3	1v2:1v3	—
Correlation between ash and fat	All samples r = −0,903					
DRY BONE: FAT-FREE BASIS						
Ash (%)	52,60±2,61	60,04±1,72	58,11±0,74	2v3	—	1v2:1v3
Calcium (%)	20,18±0,94	21,66±0,87	20,37±3,12	1v2:1v3:2v3	—	—
Phosphorus (%)	8,77±0,25	10,38±0,36	9,73±0,78	2v3	1v3	1v2
Magnesium (%)	0,30±0,02	0,48±0,04	0,50±0,01	2v3	—	1v2:1v3
DRY DEFATTED BONE						
Ash (%)	53,16±2,48	59,47±1,04	57,09±0,18	2v3	—	1v2:1v3
Calcium (%)	19,74±1,11	22,71±1,34	22,06±0,62	2v3	—	1v2:1v3
Phosphorus (%)	9,34±0,67	10,73±0,40	10,47±0,32	2v3	—	1v2:1v3
Magnesium (%)	0,32±0,02	0,48±0,03	0,51±0,02	2v3	—	1v2:1v3
Ratio of calcium: phosphorus	2,11±0,07	2,12±0,09	2,11±0,09	1v2:1v3:2v3	—	—
No. of animals/group	6	6	5			

TABLE 9 Analyses (mean ±SD) of whole dry femurs in sheep receiving the Low, Medium and High phosphate rations

Determinations	Low phosphate group	Medium phosphate group	High phosphate group	Significance of differences between values in Columns 1, 2 and 3		
	Column 1	Column 2	Column 3	Not significant	P<0,05	P<0,01
Total dry bone mass (g)	65,86±17,48	108,59±14,71	88,72±13,03	1v3:2v3	—	1v2
Bone mass (g/kg body mass)	3,40± 0,36	3,10± 0,23	3,26± 0,21	1v2:1v3:2v3	—	—
Cortex width (mm)	1,24± 0,28	3,06± 0,28	2,36± 0,16	—	—	1v2:1v3:2v3
Total bone ash mass (g)	15,29± 3,75	39,31± 6,75	27,56± 6,21	—	2v3	1v2:1v3
Ash mass (g/kg body mass)	0,80± 0,13	1,12± 0,12	1,00± 0,08	2v3	1v3	1v2
Ash (%)	23,49± 3,69	36,11± 1,93	30,82± 2,66	—	2v3	1v2:1v3
Fat (%)	51,50± 6,59	38,23± 3,00	46,06± 4,02	1v3	2v3	1v2
Calcium (%)	9,16± 1,27	14,15± 1,08	12,20± 1,30	—	2v3	1v2:1v3
Phosphorus (%)	4,08± 0,58	6,45± 0,33	5,54± 0,55	—	2v3	1v2:1v3
Magnesium (%)	0,16± 0,03	0,28± 0,01	0,25± 0,02	2v3	—	1v2:1v3
Ratio of calcium: phosphorus	2,24± 0,04	2,18± 0,07	2,20± 0,05	1v2:1v3:2v3	—	—
Correlation between ash and fat	All samples r = −0,885					
DRY BONE: FAT-FREE BASIS						
Ash (%)	48,41± 3,25	58,44± 1,13	57,12± 1,93	2v3	—	1v2:1v3
Calcium (%)	18,92± 1,08	22,89± 1,09	18,83± 1,32	1v3	—	1v2:2v3
Phosphorus (%)	8,43± 0,49	10,44± 0,18	10,27± 0,61	2v3	—	1v2:1v3
Magnesium (%)	0,34± 0,02	0,45± 0,02	0,45± 0,03	2v3	—	1v2:1v3
DRY DEFATTED BONE						
Ash (%)	47,28± 2,22	58,37± 0,62	55,14± 1,58	—	2v3	1v2:1v3
Calcium (%)	18,73± 1,18	21,80± 0,63	20,19± 0,90	1v3	2v3	1v2
Phosphorus (%)	7,93± 0,39	10,09± 0,20	9,34± 0,56	—	2v3	1v2:1v3
Magnesium (%)	0,32± 0,03	0,43± 0,03	0,43± 0,03	2v3	—	1v2:1v3
Ratio of calcium: phosphorus	2,36± 0,04	2,16± 0,04	2,16± 0,04	2v3	—	1v2:1v3
No. of animals/group	6	6	5			

That the calcium content of the rumen fluid (Table 10) did not reflect dietary intake under these conditions is contrary to the findings of other workers (Annison & Lewis, 1962). The concentration of calcium in rumen fluid is, however, subject to a number of factors (Nel & Moir, 1968) and these, together with the present findings, exclude the use of this parameter for the assessment of dietary calcium intake.

TABLE 10 The mean (±SD) calcium, phosphorus and magnesium content of rumen fluid from sheep receiving the Low, Medium and High phosphate rations

Element (mg/100 ml)	Low phosphate group	Medium phosphate group	High phosphate group
Calcium...	8,78±2,20	7,03± 4,15	4,66± 1,18
Phosphorus	46,61±8,05	72,98±16,84	92,43±18,11
Magnesium	3,24±0,91	2,05± 1,10	3,35± 0,55
No. of animals/group	6	6	5

Phosphorus

The diets contained 3 very different levels of phosphorus, namely, 0,05, 0,27 and 0,63% (Table 2). From the 4th week there was always a significant difference (P<0,01) between the plasma inorganic phosphate (Table 3; Fig. 2B) of the Low phosphate group and either the Medium phosphate or High phosphate group. The differences between the latter 2 groups were not significant (P<0,01) until the 24th week of the experiment. From these results it would appear that a low dietary phosphate is associated with a low plasma inorganic phosphate. This fall occurred rapidly as the values for inorganic phosphate were already very low immediately after the animals had completed the changeover to the experimental diet by the 4th week. This finding agrees with similar findings of numerous workers (Du Toit *et al.*, 1930; Rossouw, 1930; Du Toit *et al.*, 1932; Ewer, 1951; McRoberts *et al.*, 1965a; Young *et al.*, 1966; Nel & Moir, 1974). It is important, however, that for the greatest part of the experiment the plasma inorganic phosphate of the High phosphate group which received

0,63% dietary phosphorus was not significantly higher than that of the Medium phosphate group which received 0,27%. Moreover, although the inorganic phosphate of the High phosphate group was not significantly higher than that in the Medium phosphate group, the effect of the high intake of dietary phosphorus had a profound effect on calcium. There was a highly significant decrease in plasma calcium in the High phosphate group although dietary calcium intake was adequate. It would appear, then, that, although plasma inorganic phosphate reflects fairly accurately low intakes of phosphorus, the same cannot be said for high intakes. This stresses once again the difficulties associated with the interpretation of blood values. However, as our natural pastures are invariably low in phosphorus (Du Toit *et al.*, 1940) plasma inorganic phosphate determinations remain valuable indicators of phosphorus intake under free-range conditions.

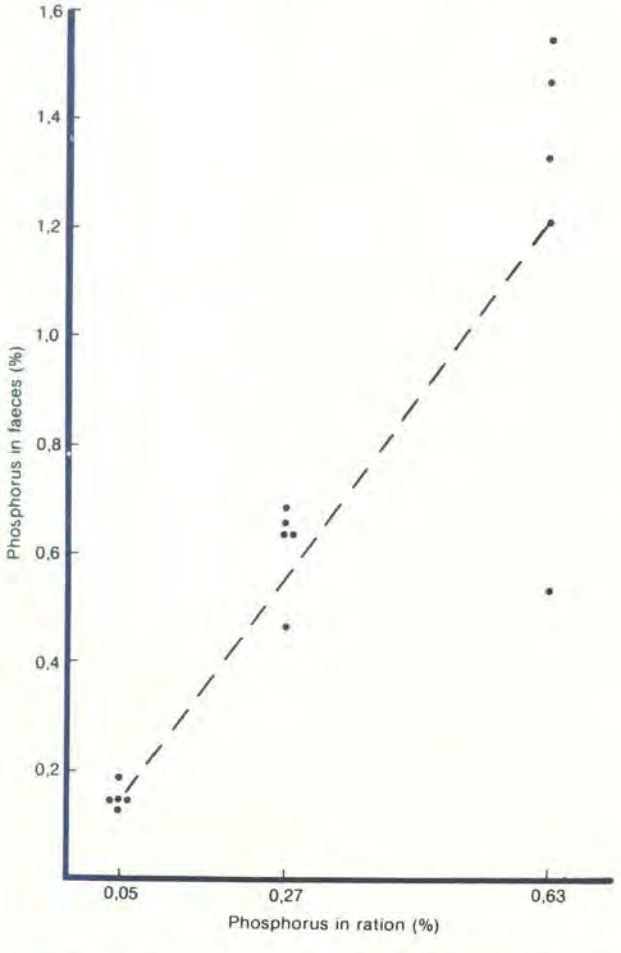

FIG. 4 The relationship between the percentage phosphorus in the ration and the percentage phosphorus in the faeces of individual sheep receiving the Low (0,05% phosphorus), Medium (0,27% phosphorus) and High phosphate(0 ,63 % phosphorus) rations. (The dotted line joins the means)

As in the case of calcium, faecal phosphorus reflected dietary phosphorus levels (Table 4; Fig. 4), a relationship found in both the 4-day composite sample and the grab samples. In fact, the individual grab sample phosphorus values correlated highly significantly ($P<0,01$ $r=0,963$) with the phosphorus levels in the individual pooled 4-day samples. This finding indicates that, as in the case of calcium, small samples of the rectal faeces from a number of free-ranging animals may be most useful in assessing the level of phosphorus in the diet. Moreover, the relationship between calcium and phosphorus in the faeces will aid in the interpretation of the blood values of calcium and phosphorus.

Although both the mean concentrations and mean daily output of phosphorus in the urine rose as the dietary phosphorus intake increased (Table 5), it will be noticed that the variation within the groups is very high, particularly in the Medium and High phosphate groups. Although urine analyses are unpractical, an interesting and important observation was made in the Medium and High phosphate groups. In the former group the actual concentrations of phosphorus in the urine were 0,03; 0,06; 0,07; 0,07; 1,31 and 2,00 mg/ml and the mean daily output, in the same order, 34,16; 34,12; 34,84; 43,84; 1 048,06; 3 708,20 mg. As can be seen from this ranking, the last 2 sheep had a very much higher concentration and daily output of phosphorus than the other 4 and it was these 2 sheep that had the lowest phosphorus percentages in the faeces (Fig. 4). Similarly, in the High phosphate group, the concentrations of phosphorus in the urine were 1,07; 1,46; 1,54; 1,94 and 1,93 mg/ml and the mean daily output, in the same order, 130,40; 1 604,17; 565,95; 1 355,20 and 4 154,56 mg. Again, the last sheep, which had almost the highest phosphorus concentration but by far the highest daily output, was the animal with the lowest phosphorus concentration in the faeces in this group (Fig. 4). It would appear then that, compared with the other sheep, these 3 animals absorbed more phosphorus from the digestive tract and then excreted it in the urine. Meyer (1972) also noted the anomalous condition where certain sheep excrete large amounts of phosphorus in the urine. In his examples he found this to be independent of vitamin D or magnesium. Nevertheless, these examples stress the importance of collecting a large number of faecal samples in a flock and pooling the samples for analyses to eliminate the effect of individuals who differ markedly from the norm.

The values for phosphorus in the soft tissues either on a dry whole basis or expressed on a dry fat-free basis (Table 6) gave no indication of phosphorus intake. In the 6 tissues examined there were no significant differences between the groups.

The phosphorus content of bone follows the calcium content very closely as is seen particularly in the calcium: phosphorus ratio (Tables 7, 8 and 9). Although calcium is more dominant, since it is under the influence of parathyroid hormone and calcitonin which act on bone (Copp, 1965), phosphorus has a modifying effect on calcium deposition as was mentioned in the discussion on bone calcium. Calcium and phosphorus together form the largest part of bone mineral and the ratio between the 2 is virtually constant. The analysis of bone for phosphorus is therefore not warranted and, as was mentioned under calcium, bone ash analysis should suffice to give an indication of bone mineralization as affected by both calcium and phosphorus levels.

The phosphorus content of rumen fluid gave a good indication of phosphorus intake (Table 10). This is in agreement with the findings of Tomas (1965) and Nel & Moir (1974) but differs from those of Clark (1953). As there is a direct relationship between serum inorganic phosphate and rumen phosphorus (Tomas, 1965), it is probably more convenient to determine plasma levels, though rumen fluid determinations may be used as a useful adjunct where necessary.

Magnesium

As the magnesium levels of the 3 rations were for all practical purposes equal, variations between the groups would reflect the compounded effect of the availability of this mineral and the interrelationship between magnesium and other dietary components. As this experiment was designed mainly to test diagnostic methods for calcium and phosphorus and not to determine the interrelationships between elements, mention will only be made of the findings of the analyses for magnesium.

Plasma magnesium values (Table 3) were significantly higher ($P<0,01$) in the Low phosphate group than in the other 2 groups during Week 24. In fact, in all the plasma samples analysed, there was a significant negative correlation between plasma magnesium and plasma inorganic phosphate ($P<0,01$, $r=-0,465$). There was also a negative correlation between plasma calcium and plasma inorganic phosphate (*vide supra*). It is thus not surprising that in these animals there was a significant positive correlation between plasma calcium and plasma magnesium ($P<0,01$, $r=0,489$).

In the faecal samples (Table 4) it is clear that as phosphorus increased, so magnesium increased in both the 4-day and grab samples. This effect is so marked that in the individual 4-day samples there is a significant positive correlation between the faecal phosphorus and faecal magnesium levels ($P<0,01$, $r=0,822$). It would appear from the above that increasing dietary phosphate interfered in some way with the net absorption of magnesium resulting in increased faecal excretion and decreased plasma levels.

The magnesium concentration in the urine (Table 5) was a reflection of plasma magnesium levels which is a well-established finding (Chicco, Ammerman, Hillis & Arrington, 1972; Gardner, 1973). Although the Low phosphate group, which had the highest plasma magnesium levels, also had the highest mean daily output of magnesium, the variation within groups was wide and the differences between the groups small.

The magnesium content of the bones showed an interesting pattern (Tables 7, 8 and 9). Whereas the magnesium content of bones of the Medium and High phosphate groups were either the same or nearly so, the content in the Low phosphate group was always much lower. That the Low phosphate group had the lowest faecal magnesium percentage (Table 4) and the highest plasma magnesium levels (Table 3) suggest that the magnesium in this group was being absorbed and was available in the blood and body fluids. Why the magnesium was not deposited in the bone in this group is puzzling as bone magnesium has been shown to be related to plasma magnesium and bone analyses are used to indicate magnesium status (Smith, 1959). Blaxter & Sharman (1955) have suggested that a more critical method to assess bone magnesium as a diagnostic procedure is to determine the ratio of calcium: magnesium. The average calcium: magnesium ratios of whole and fat-free bone were 47,1 and 44,0 in the Medium and High phosphate groups respectively, while this ratio in the Low phosphate group was 60,4 which again exemplifies the lack of magnesium deposition in these bones. Field, Suttle & Nisbet (1975) also found that diets low in phosphorus caused a marked fall in the bone magnesium content in Blackface lambs. Although this anomaly is not clear at present it shows that care must be taken in assessing magnesium status from bone magnesium levels if dietary phosphorus levels are not known.

The magnesium level in the rumen fluid (Table 10) was almost equal in the Low and High phosphate groups while it was lower in the Medium phosphate group.

Other bone parameters

As has been mentioned above, both the calcium and phosphorus percentages in bone increased in the order Low phosphate <High phosphate <Medium phosphate groups. The same sequence is found for total dry bone mass in the cervical vertebrae (Table 7), ribs (Table 8) and femurs (Table 9). This sequence is again found for body mass (Fig. 1) and, although there are differences between the groups with regard to total dry bone mass, these differences are minimized once the variation in total body mass is removed and bone mass is expressed per unit body mass. Similarly, in all 3 types of bone there are significant differences (at least $P<0,05$) between the 3 groups in total ash mass. The significance of these differences are again decreased once body mass is taken into account.

Significant differences (at least $P<0,05$) were also found in the percentage ash of the various bones between the 3 groups, and these differences were of a higher order than the differences between bone ash mass/kg body mass. Although Benzie *et al.* (1955) showed that ash mass is a more sensitive measure of resorption than ash percentage, in the present experiment where there were large differences in body mass between the groups, total ash mass may not be valid. In this case ash percentage of dry whole bone appears to be a better measure than ash mass/kg body mass.

If the ash percentages, either calculated to a fat-free basis or actually determined on fat-free bone (Tables 7, 8 and 9), are compared with the ash percentages on whole dry bone mentioned above, it appears that the latter gives a better indication of differences between the groups and may therefore be a better method for assessing the degree of bone mineralization during diagnostic procedures. Moreover, an important finding in this experiment was the significant ($P<0,01$) negative correlation between bone fat percentage and whole dry bone ash percentage. The correlation coefficient between the two is $-0,963$ for cervical vertebrae, $-0,903$ for ribs and $-0,885$ for femurs (Fig. 5, 6 and 7). From this finding it is reasonable to assume that, as bone is resorbed, fat is deposited, and *vice versa,* and this relationship is particularly noticeable in cancellous bone such as the cervical vertebrae. However, once fat is not in intimate contact with the bone structure and a certain amount is concentrated in a marrow area, then this relationship will be less significant, as was seen in the ribs. Finally, when only a certain amount of fat is in contact with the bone structure and a great deal is concentrated in a marrow cavity, as in the femur, this relationship will be even less significant. Previous work in this department (McDonald & Belonje, 1975) showed a similar correlation between fat and ash percentage in cervical vertebrae but this was only over a range of about 7% fat and 5% ash in 6 experimental animals. In the present study this was extended to a range of about 38% fat and 22% ash in 17 animals. This finding has an important practical application in diagnostic procedures. It means that, by determining the fat percentage of dry bone (particularly spongy bone such as the cervical vertebrae), one is able to arrive at a reasonable estimation of the ash percentage of that bone which again reflects directly the degree of mineralization of the bone.

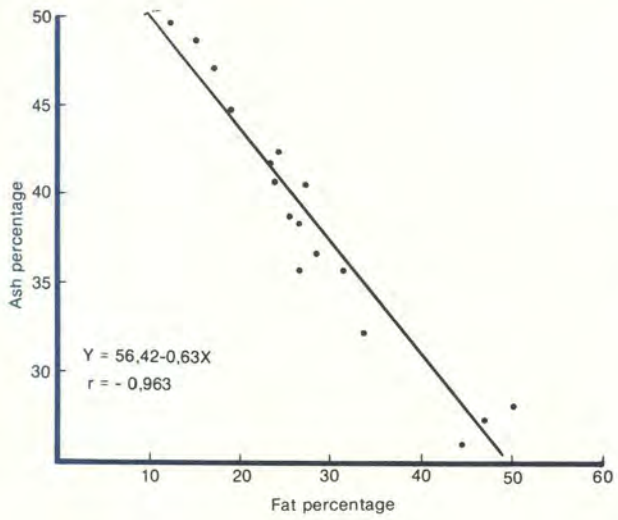

FIG. 5 The relationship between fat percentage and ash percentage in dry cervical vertebrae in the sheep receiving the Low, Medium and High phosphate diets

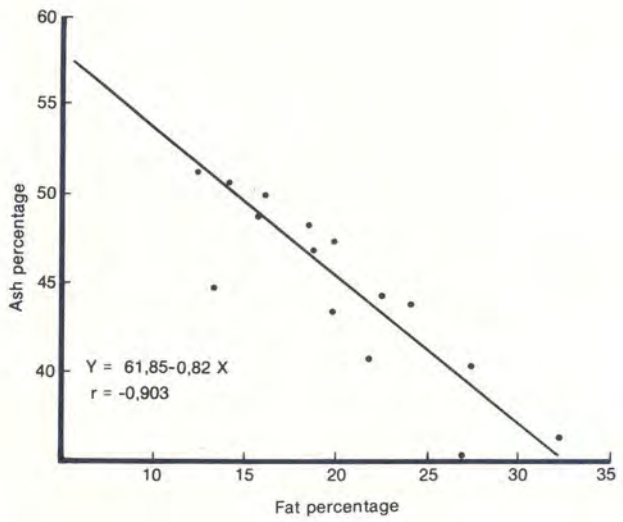

FIG. 6 The relationship between fat percentage and ash percentage in dry ribs in the sheep receiving the Low, Medium and High phosphate diets

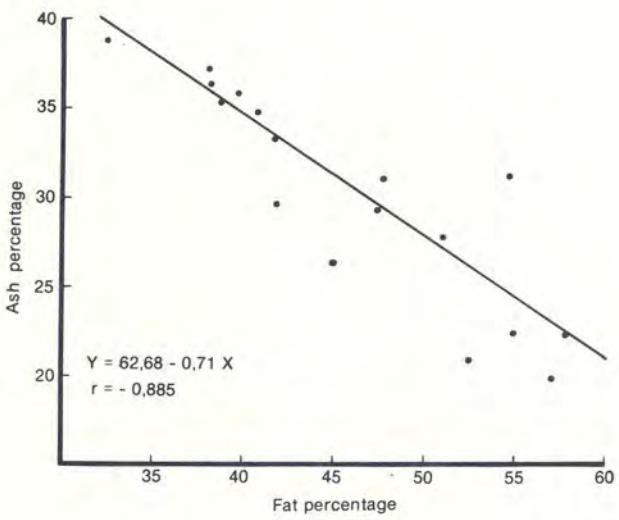

FIG. 7 The relationship between fat percentage and ash percentage in dry femurs in the sheep receiving the Low, Medium and High phosphate diets

TABLE 11 Mean percentage (±SD) ash, protein, fat, water and energy in the bodies of the 3 groups of sheep receiving the Low, Medium and High phosphate rations as determined from tritiated water space

Body constituent	Low phosphate group	Medium phosphate group	High phosphate group	Significance of differences between values in Columns 1, 2 and 3		
	Column 1	Column 2	Column 3	Not significant	P<0,05	P<0,01
Ash (%)	4,93±0,49	3,71±0,14	4,32±0,14	—	1v3:2v3	1v2
Protein (%)	16,26±0,65	15,31±0,22	16,22±0,42	1v3	1v2:2v3	—
Fat (%)	5,25±4,88	12,32±1,73	5,42±3,18	1v3	1v2:2v3	—
Water (%)	74,25±4,27	68,05±1,52	74,10±2,79	1v3	1v2:2v3	—
Energy (Mcal/kg)	1,36±0,40	1,97±0,14	1,38±0,27	1v3	—	1v2:2v3
No. of animals/group	5	6	5			

A final observation on the bones was that there was a significant difference (P<0,01) between the width of the femoral cortices (Table 9) in the 3 groups of sheep. As cortical width was closely correlated to ash percentage (P<0,01, r=0,879), this measurement may be useful as an indication of the degree of mineralization of the femur.

Urinary hydroxyproline excretion

The urinary hydroxyproline excretion (Table 5) was interesting. The mean excretion of hydroxyproline did not differ between the Medium phosphate and High phosphate groups but there was a very significant (P<0,01) reduction in the Low phosphate group. Hydroxyproline excretion being a measure of bone turnover (Vaughan, 1970), demonstrates that the animals on the Low phosphate ration had not only the least mineralized bone but also a far lower bone turnover rate than the other 2 groups.

Body composition

Body composition calculated from tritiated water space (Table 11) in the living animal was useful only in showing the partition of the various constituents of the body. The order of body ash percentage was in fact the reverse of the order of ash percentage found in the bones and therefore body ash determined by this method cannot be used as an indication of bone mineralization.

Conclusions and Recommendations

It would appear that plasma analyses for calcium are not reliable indicators of dietary intake. Furthermore, while low plasma inorganic phosphate levels indicate a low level of phosphorus intake, a very high phosphorus intake does not necessarily result in plasma inorganic phosphate levels higher than those in animals which consume recommended levels of phosphorus.

In the ruminant. the faeces are the principal pathway for the excretion of both calcium (Otto 1932; Hansard, Comar & Plumlee, 1952; Nel & Moir, 1974) and phosphorus (Young *et al.*, 1966; Scott, 1970; Reveron, Topps & Gelman, 1974). For this reason the calcium and phosphorus contents of the faeces must reflect the dietary intake. In order to diminish the effect of animals which may be abnormal it is suggested that small samples of rectal faeces should be collected from a large number of animals in a flock for analysis. These analyses will then give an indication of the relationship between dietary calcium and phosphorus which will aid in the interpretation of the plasma levels of calcium and inorganic phosphate. Furthermore, the faecal levels of calcium and phosphorus which represent the minimum required intake could be established. If a pooled faecal sample from a flock contained less than this minimum, supplementation would be indicated. A more sophisticated method would be to correlate dietary and faecal concentrations over a wide range. From this one would be able to predict actual intake from faecal concentration as has been done for phosphorus in beef cattle by Cohen (1974).

The variation in the levels of calcium and phosphorus in the urine, the fact that these concentrations reflect only plasma levels and the necessity for taking 24 h samples to determine daily output preclude the use of urine as a diagnostic aid.

Bone ash analyses are probably the most practical indicators of mineral status. To determine bone ash, it is suggested that whole dry bone be used without prior fat extraction. On the other hand, as fat and ash have been shown in this study to be so closely correlated in cancellous bone, a fat analysis could be performed on dry whole bone and then ash percentage calculated from fat percentage. When animals cannot be slaughtered, the rib biopsy technique described by Little (1972) may be used to collect bone specimens. It is of interest to note that, whereas alkaline phosphatase is not a good indicator of deranged skeletal metabolism in sheep (Young *et al.*, 1966; Simesen, 1970; Reveron *et al.*, 1974), the present study indicates that hydroxyproline may be so. At present hydroxyproline determinations are limited, since a 24 h urine specimen is required, but plasma analyses may prove to be useful.

Neither body composition, determined from tritiated water space, nor the analysis of tissues such as brain, liver, kidney, tongue, cardiac muscle or the gluteus medius for calcium and phosphorus give any indication of either the mineral status or the dietary intake of calcium and phosphorus of the sheep.

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