SOME FACTORS AFFECTING URINARY CALCULUS FORMATION IN WEANED LAMBS

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This thesis has been composed by me, and it is a record of my own work.

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SUMMARY

The objective of undertaking this study was to examine the relationship between various dietary factors (physical form of the diet, dietary nitrogen level and source, different levels and combinations of calcium, magnesium and phosphorus) and urinary calculus formation in lambs weaned at 4 weeks of age.

The first experiment was an incomplete factorial utilising 64 lambs. The results obtained showed that neither pelleting the diet, dietary nitrogen level nor source initiated calculus formation. Pelleting the diet increased mineral digestibility and subsequently urinary mineral excretion was increased. Supplementation of the diet with urea or protein reduced the concentration of urinary minerals although phosphorus values for the urea-fed animals were much lower than those for the protein-fed animals.

The second experiment was a complete factorial utilising 64 lambs. The results indicated that supplements of both calcium and magnesium were likely to reduce calculus formation whereas phosphorus supplements enhanced urinary excretion of phosphorus. Calculus formation was not associated with any particular dietary combination of minerals but was more a function of time postweaning. There was a distinct time effect in relation to urinary mineral concentration postweaning: urinary mineral concentrations were very high (e.g. phosphorus, 95.6mg/100ml) in the immediate postweaning period (2 to 4 weeks) but decreased to comparatively low levels (e.g. phosphorus, 40.9mg/100ml) at 7 to 9 weeks postweaning. Calculi occurred when urinary mineral concentrations were at their highest in the immediate postweaning period. Autopsy findings indicated calculi were of renal origin and composed largely of magnesium phosphate.

The results of Experiment II indicated that the transitional period at weaning was important in relation to calculus formation. The water economy at this time was measured in Experiment III and it was found that water intake and urine output were at least halved postweaning. Also, during the preweaning period, water retention was a function of water intake whereas postweaning, it was under renal control. This control was imprecise over the immediate 10 day postweaning period and it appeared that this might be the time when calculus formation was initiated.

An attempt to supplement water intake via feeding tubes (Experiment IV) during the postweaning period was unsuccessful as lambs regurgitated the tubes. Another experiment (Experiment V) was undertaken in which additional water was supplied to lambs via cannulae inserted in the reticulorumen. The water economy of these lambs was compared with those weaned normally. The results obtained were similar to those in Experiment III although there was no evidence of water restriction, in the normally weaned lambs, causing renal deposits associated with calculus formation.

The results obtained and their implications in the aetiology and prevention of urolithiasis are discussed.

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1. INTRODUCTION

Despite the regular annual loss of about 20,000 hectares of agricultural land (Champion, 1974) agricultural output in the United Kingdom continues to rise. This is particularly true of crop production. In 1973/74 for example, United Kingdom farmers produced three times the tonnage of cereals that were produced in 1900 (Blaxter, 1976).

The continuing decline in land area is particularly important in relation to extensive systems of animal production. Most of the lamb produced in the United Kingdom comes from this type of system and despite the constraint of land area, the United Kingdom sheep breeding flock has expanded since 1972 and this has been reflected in the increased production of mutton and lamb (Annual Review of Agriculture, 1976). However, in contrast to the crop production situation, the United Kingdom is producing nearly 30% less mutton and lamb than immediately before the First World War (Blaxter, 1976) and consequently, in 1974, 43% of the mutton and lamb consumed in the United Kingdom was imported.

Obviously, there is scope for increased home production of lamb in the United Kingdom and this could best be achieved by improvements in flock management and reductions in lamb mortality (Food from our own Resources, 1975). Although lowland ewes constitute only 39% of the total ewe flock, they produce nearly half of all births (Howe, 1976). The increases in productivity, necessary to meet home demands, will probably come from this sector where improvements in flock management and new ideas can be readily introduced.

On lowland farms, lambs are usually sold off grass at about 15 weeks of age; the alternative to fattening lambs on grass would be to take them off the land and fatten them indoors.

The feeding of home grown cereals to obtain rapid liveweight gain in lambs has recently attracted a great deal of research effort (Andrews and Orskov, 1970; Orskov and Fraser, 1972; Orskov, Fraser and McHattie, 1974; Orskov, Duncan and Carnie, 1975) although few commercial units have been established along these lines. One of the major problems of this type of intensive sheep production is the high mortality that occurs among lambs. Mortalities of 13% (Zandstra, 1974), 3.15% (Lalov, Antonov, Popkhristov, Dimitrov and Andreev, 1971) and 4% (Poole, 1976) have been recorded in stocks of intensively fed lambs. In every case, the cause of death or reason for emergency slaughter was urolithiasis.

Research into the aetiology and prevention of urolithiasis in lambs has been confined largely to investigations involving store lambs kept under intensive systems of management and fed on concentrate diets. Factors such as dietary form, nitrogen and mineral content, have been identified as being associated with the occurrence of urolithiasis in these animals. None of these factors has been investigated with weaned lambs finished on concentrate diets for slaughter before 15 weeks of age. In view of this, and the possibility that lamb production units of this type will be developed in the United Kingdom it was considered important to investigate the relevance of some of these factors to the occurrence of urolithiasis amongst lambs reared in this manner.

2. REVIEW OF LITERATURE

Introductory Comment

Urolithiasis is virtually unknown in lambs at grass in the United Kingdom although it is a problem frequently encountered in intensive sheep production systems that rely heavily on concentrate feeding. Significant losses have been reported both in the United Kingdom (Wilson, 1976) and Eire (Poole, 1976). Most of the work which has been carried out in relation to urolithiasis in sheep has been in the United States. Much of the lamb produced in America is from store lambs fattened in large feedlots. This is in direct contrast to the extensive systems of lamb production employed in the United Kingdom. Urolithiasis has always been a problem in feedlots and thus virtually all the investigational work has been carried out using store lambs and not surprisingly, most of this work has been carried out in the United States. Therefore, the information which is reviewed in this literature survey is confined almost exclusively to that obtained using store lambs. Occasionally, reference is made to studies using mature sheep and other species.

2:1. Location and nature of ovine calculi

Calculi are normally found in the urinary bladder and the urethra of the male sheep although they are formed in the kidney. In spite of the fact that calculi may be present anywhere in the urinary tract, they are rarely found in the ureters. Renal calculi have been located in the tubules and interstitium(Davis, Scott, Crookshank and Spjut, 1969) and also in the collecting ducts (Beeson, Pence and Holm, 1943). Larger stones have been found in the pelvic areas of the kidney (Beeson et al. 1943) and bladder (Bauer, Matzke, Gränzer and Burgkart, 1971). Urethral calculi are only found in rams and wethers. As Hippocrates (cited by Van Leersum, 1928a)pointed out, "De aere, aquis et locis"- the female urethra is short and wide - and is therefore not likely to become obstructed.

Various chemical and physical types of calculi have been reported in the literature. They can be grouped into two broad categories: firstly, those found in lambs kept under extensive/range conditions and secondly, those found in lambs kept under intensive/housed conditions.

Stones reported to form under range conditions have been shown to contain silica (Forman and Sauer, 1962), or opal (Baker, Jones and Milne, 1961), or calcium carbonate (Sutherland, 1958) or oxalates (McIntosh, Pulsford, Spencer and Rosser, 1974). Furthermore, stones composed largely of organic substances have been reported to occur under these conditions. Examples of these stone-types are those containing isoflavone-related substances (Gardiner, Nairn and Meyer, 1966) and xanthin (Askew, 1956/7).

Apart from proteinaceous calculi which can form when stilboestrol is included in the diet (Marsh, 1961), the majority of calculi formed by sheep kept under intensive conditions are phosphatic amorphous aggregates (Cornelius, Moulton and McGowan, 1959; Packett and Coburn, 1965). The principal constituents of these aggregates are magnesium ammonium phosphate, magnesium and calcium phosphates (Cornelius, 1963; Packett and Hauschild, 1964; Hoar, Emerick and Embry, 1969; Udall, 1974).

2:2. Non-dietary factors associated with the formation of ovine calculi

2:2:1. Genetic Factors

There is no published evidence of genetic factors predisposing to urolithiasis in sheep in contrast to the established genetic control of cystinuria both in man (Garrod, 1908; Harris and Warren, 1953) and dogs (Tsan, Jones, Thornton, Levy, Gilmore and Wilson, 1972).

2:2:2. Age

The youngest age at which urolithiasis was recorded in lambs was in 3 to 4 week old crossbred Suffolk lambs housed indoors with their dams (Jones and Dawson, 1976). In weaned lambs, reared for early slaughter, urolithiasis may occur between 6 to 10 weeks of age and in store lambs between 6 months to 1 year. Urolithiasis occurs infrequently in adult sheep.

2:2:3. Sex

2:2:3:1. Male/Female

There is no difference in the incidence of renal calculi between males and females (Udall, 1959a, Emerick and Embry, 1964). Obstructive urolithiasis occurs in rams and wethers because the sigmoid flexure prevents the easy passage of calculous material. Consequently, the male urethra tends to become blocked in the region of the ischial arch.

2:2:3:2. Castrates

Belonje (1965) surmised that castration arrested the resolution of the adhesions and the subsequent development of the penis in ovines. The penis of an adult wether castrated at 3 to 6 weeks of age resembles that of a newborn lamb. The result of arrested penile development is a reduction in the size of the urethral lumen which increases the likelihood of blockage. Thus, wethers are more prone to urethral obstruction than rams. Work with calves (Marsh and Safford, 1957) demonstrated that a calf castrated at 7 months of age could pass a stone 13% greater in diameter than a calf castrated at 1 month of age whereas a bull could pass a stone 44% greater in diameter.

2:2:4. Infection

Bacterial and/or virus agents have not been incriminated in the formation of calculi in sheep. However, there is some controversial evidence in the literature relating to the use of broad spectrum antibiotics in sheep diets. Packett, Watkins and Kunkel (1958) showed that feeding a diet containing 22mg/kg of chlortetracycline to wether sheep significantly reduced the number of deaths due to calculus formation. On the other hand, Kunkel and Robbins (1959) failed to show that either oxytetracycline or chortetracyline at this level of inclusion reduced the incidence of renal calculi. The route of excretion of these antibiotics is largely via the bile and only a small amount passes via the kidney. In view of the dietary levels used, the amount excreted via the kidneys would not be enough to exert a bacteriostatic effect. Certainly, there would be some modification of the intestinal flora which could conceivably affect mineral digestibility although this is unlikely.

2:2:5. Endocrine Factors

2:2:5:1. Parathyroid

There is evidence to show that the activity of the parathyroids of the rat is increased during magnesium deficiency and that this results in renal calcification (Heaton and Anderson, 1965). Chown, Lee and Teal (1936) injected rats with parathyroid extract and obtained both intratubular and interstitial deposits of calcium in the kidneys. Injection of low doses of parathyroid hormone into pullets produced temporary reduction in urinary calcium excretion accompanied by a rise in phosphate excretion (Prashad and Edwards, 1973). However, Scott (1972) found no difference between concentrate and roughage diets in their effect on circulating parathyroid hormone levels in the blood of sheep. Although the parathyroids have been shown to produce renal changes which could be associated with calculus formation, there has been no definitive work to relate parathyroid function and the formation of calculi in ovines.

2:2:5:2. Antidiuretic Hormone (ADH)

Stacy (1969a) has implicated a postprandial antidiuresis with a resultant increased urine concentration following secretion of antidiuretic hormone by the posterior pituitary. He suggested that these postprandial changes, accompanied by a hypercalciuria and hypermagnesiuria and a pronounced fall in pH, were conducive to the formation of crystalline silica/calcite calculi. This aetiology would probably be inappropriate in the case of the formation of amorphous calcium/magnesium/phosphate aggregates.

2:2:5:3. Stilboestrol

Both Jordan (1953) and Udall and Jensen (1958) found that shortly after implanting lambs with stilboestrol, a large number died as a result of urolithiasis. Furthermore, Marsh (1961) supplied lambs with a diet containing 1mg/kg stilboestrol and obtained a 10% incidence of urolithiasis. However, Emerick and Embry (1964) either fed or implanted lambs with diethyl stilboestrol and found that diethyl stilboestrol had no effect on the incidence of urolithiasis. The probable explanation for Emerick and Embry's findings was that they used much lower levels of diethyl stilboestrol (3mg implants compared with 12 or 30mg).

A consistent finding amongst implanted groups of animals was that they grew faster and this would be associated with increased anabolic activity. The effect of stilboestrol on urolithiasis might be mediated via the parathyroid. Increased parathyroid activity would accelerate tissue turnover, releasing mucoprotein material (Said, 1969) which could form the plugs of precipitated mucoprotein found by Marsh (1961) occluding the urethra of lambs with urolithiasis.

2:2:6. Urinary Factors

2:2:6:1. Urine Stasis

Urine stasis is of little significance in the aetiology of ovine calculi although it has been shown to be important in the formation of calculi in recumbent fracture patients (Fett and Kane, 1946).

2:2:6:2. pH

Swingle and Cornelius (1962) found urine voided by lambs fed grain diets had a lower pH than that of urine from lambs fed roughage diets. Furthermore, Scott (1972) has shown quite clearly that sheep fed a roughage diet excreted an alkaline urine and when fed a concentrate diet, the urine was acid. It is apparent that calculi form in sheep when fed either concentrate or roughage diets and therefore it may be concluded that pH of the urine per se does not cause calculus formation. Additionally, Udall (1962) has demonstrated that the emphasis placed on urinary pH as an aetiological determinant was not warranted. He based his conclusion on an experiment in which lambs were fed sodium chloride at 1, 4 and 7% of the diet. Supplements of phosphoric acid (H_3PO_4) and potassium carbonate (K_2CO_3) were fed to produce respectively an acid-ash urine and an alkaline-ash urine. There was a marked reduction in the incidence of calculi in animals fed 4% or more of sodium chloride and an associated increase in urine volume. There were significant differences in urinary pH but no corresponding difference observed in the occurrence of renal calculi. Lastly, American research work has shown that urinary pH was similar in both animals that formed calculi and in those which did not (Bushman, Emerick and Embry, 1965a and b; Robbins, Kunkel and Crookshank, 1965).

2:2:6:3. Macromolecules

Changing sheep from a roughage-based diet to one consisting solely of concentrates produced an increase in the concentration of urinary macromolecules (Cornelius, Bishop, Berger and Pangborn, 1961; Swingle and Cornelius, 1962; Cornelius, 1963). This change in concentration was due almost entirely to a reduction in urine volume so that the daily output of urinary macromolecules was virtually the same on both diets. Swingle and Cornelius (1962) considered that this increase in concentration was possibly sufficient in itself to initiate urolithiasis. However, there is no evidence to support this view.

Of the urinary macromolecules, mucoprotein has attracted a lot of attention from research workers, probably because it is consistently found in the matrix of stones. Packett and Coburn (1965) showed that mucoprotein constituted 25% of amorphous stones. Udall (1959b) attempted to measure the predisposition to urolithiasis by estimating urinary mucoproteins. He found that the mucopolysaccharide part of the mucoprotein was correlated directly with the level of concentrate in the ration. Furthermore, the concentration of mucoproteins in the urine was inversely proportional to the dietary mineral level. Boyce and co-workers (Boyce, Garvey and Norfleet, 1954 and 1955; Boyce and Sulkin 1956) have emphasised the importance of urinary mucoprotein in the formation of calcigerous calculi in humans. These workers were able to demonstrate the calcium binding ability of mucoprotein. Furthermore, Keeler (1960 and 1963) has suggested that silica stones form in cattle by coprecipitation of silica and mucoprotein. However, there is no evidence to support the idea that phosphatic calculi occur in ovines due to the ion-binding properties of mucoprotein or by coprecipitation of metal ions and protein.

2:2:6:4. Volume

Feeding concentrates instead of roughages significantly reduces urine volume (Cornelius, et al., 1961; Cornelius 1963; Udall, Seger and Chen Chow 1965), e.g. in one experiment (Swingle and Cornelius, 1962) grain-fed sheep excreted only 40% of the daily urine volume that was produced on alfalfa diets. Similarly, animals with subcutaneous implants of stilboestrol produced less urine than animals without implants (Jordan, 1953). Nottle and Armstrong (1966) have suggested that concentration of urine might account for the difference in incidence of fatal silica urolithiasis. They stated that at least 600ml of urine should be excreted daily by sheep grazing silica-rich herbage.

Fewer calculi are likely to form in animals drinking a lot of water because they will probably be excreting a dilute urine. However, it does not follow that lambs excreting a dilute urine will be free of calculi. Bushman, Emerick and Embry (1968) found that 1% ammonium chloride in the diet was the most effective supplement in reducing the incidence of urolithiasis. Only 5% of lambs fed on this diet developed calculi although their urine volume was only about 1200ml/day. In contrast, an incidence of 35% was obtained with a diet containing 4% sodium chloride although this supplement produced the highest urine volume of 2704ml/day. Thus, although large urine volumes are beneficial in terms of calculus prevention, there are other factors involved.

2:2:7. Feeding Response

Stacy and Brook (1964) reported that the renal response of sheep to food intake consisted of a reduced urine flow with a resultant increased concentration and lowered pH. They suggested that this was a reflection of a sudden shift of extracellular fluid into the gut at the onset of feeding. Further research by Stacy (see Stacy, 1969a, and b and c; Stacy and Wilson, 1970) clarified the mechanism of this response. According to these workers food intake appears to exert an effect on the functional characteristics of the ovine kidney, manifested by an increased renal clearance of calcium and magnesium thus producing a postprandial hypercalciuria and hypermagnesiuria. They considered that the hypercalciuria was probably causally related to the change in acid-base balance after feeding. The decreased renal tubular reabsorption of calcium that occurred in these experiments probably resulted from tubular processes. Excretion of inorganic phosphate was very low and these workers considered that the absence of this urinary buffer was probably an important factor contributing to the abrupt and considerable fall in urinary pH after the ingestion of food. These excretory changes reflected the renal response to intercompartmental shifts of body fluid following the potent stimulus to salivary secretion during eating. Stacy and coworkers speculated that these changes may facilitate calculus formation and in fact, analysis of some ovine calculi provided mineralogical evidence of sudden changes in the biological environment of the urinary tract. Stacy (1969a) found silica and calcite in adjacent layers, which was unusual, as silica precipitates at pH 5 to 6 when calcite would dissolve. These sudden changes demonstrated in pen-fed sheep could occur in episodic feeding, a behaviour which may be exhibited by sheep grazing naturally. However, this aetiology would be unlikely in sheep fed intensively as they would have unlimited access to food in order to maximise liveweight gains. Furthermore, the amorphous aggregates of phosphatic material found in intensively fed sheep would be unlikely to form in the organised manner suggested by Stacy.

2:3. Dietary factors associated with the formation of ovine calculi

2:3:1. Geographical, geological, environmental and seasonal factors.

Calculi composed of calcium carbonate were reported in animals grazing lush herbage in Queensland, Australia (Sutherland, 1958). In contrast, silica calculi were found in animals kept in the dry wheat-belt area of Western Australia (Department of Agriculture, Western Australia, 1949; Bennets, 1956). These different situations presumably are a reflection of different dietary mineral intakes resulting from the ingestion of different forages.

Soil mineral deficiences (phosphate, lime, molybdenum) have been linked with the occurrence of xanthin calculi in the area around Nelson, New Zealand (Easterfield, Rigg, Askew and Bruce, 1930; Askew, 1956/7).

Udall, et al., (1965) were able to show that urolithiasis was associated with the sudden onset of cold weather. Furthermore, Weaver (1971) demonstrated seasonal variations in ovine urinary constituents but was unable to relate this to the occurrence of urolithiasis. In contrast, Nottle and Armstrong (1966) were able to demonstrate that urinary silica concentrations were significantly greater in lambs during the Australian summer and autumn in areas of the country where silica urolithiasis was a problem. It appeared that the silica was more available in green succulent feeds than in the dry feeds fed during the winter and spring.

Isoflavone concentrations reach a peak in clovers (e.g. Trifolium subterraneum Dinninup) during early August and shortly after this, Australian pastures are dominated by clovers. Thus during September and October, ingestion of isoflavones is high and consequently, the urinary excretion of phenols is at its greatest at this time. This coincides with the onset of ovine urethral obstruction and it has been suggested (Parr, Steele, Gabbedy and Nottle, 1970) that sediments responsible for causing the obstructions are a direct result of the high level of urinary phenols.

2:3:2. Qualitative nature of the diet

Gardiner (1965) stated that feeding concentrate diets predisposed stone formation, due largely to the increased excretion of urinary calcium, magnesium, phosphorus, potassium and mucoprotein. The increase in excretion was emphasised by the associated reduction in water intake and therefore urine volume. The reduction in urine volume associated with the change from a roughage based diet to one of concentrates has already been mentioned (p.12).

Hjelle (1969) investigated the effect of the nature of the diet on urinary pH. In one experiment 83% of urine samples from hay-fed animals had a pH above 7 and concentrate supplementation increased the pH to 7.7. The mean urinary pH of samples taken from sheep at pasture and those fed silage was 6.96 and 6.49 respectively, with 63% and 40% of samples above pH7. Weaver (1971) collected urine samples by catheter from 20 ewes at pasture at 1 month intervals for 7 years. The mean urinary pH was 6.3 with a minimum of 4.8 and a maximum of 8.2. Values obtained in the May - July period were significantly higher than those obtained in the winter months from November to March.

Hoar, Emerick and Embry (1970b) found that feeding alfalfa hay reduced the urinary phosphate levels and suggested that this was a reason for the low incidence of urolithiasis in sheep fed on alfalfa diets. Scott (1972) investigated this matter further and found that sheep and calves fed a fibrous diet (53% straw and other components) excreted an alkaline urine, low in phosphate, whereas, when fed concentrate diets (80% barley and other components), the urine was acid and contained large amounts of phosphate. A high proportion of this phosphate was present as titratable $H_2PO_4^-$. The important inference to be drawn from Scott's work is that differences in phosphorus excretion were not related to differences in phosphorus intake. The amount of phosphorus reabsorbed by the renal tubule was consistently less in animals fed concentrate diets. This difference in tubular reabsorption appeared to be related to differences between diets in the amounts of phosphorus absorbed from the gut. Thus, digestibility of dietary phosphorus and factors affecting digestibility may play a significant part in the aetiology of urolithiasis.

2:3:3. Physical Form

Packett, et al., (1958) fed a mixed diet in a pelleted form to one group of wethers, and to another group in a meal form. Lambs fed the pelleted diet had more calculi than those fed the diet in the meal form. The authors concluded that pelleting a diet known to produce calculi was likely to increase the incidence of urolithiasis. Crookshank, Packett and Kunkel (1965) confirmed these findings and showed that lambs fed the pelleted calculus-inducing diet voided urine which contained more phosphorus than was present in the urine excreted by lambs fed the meal form of the diet. Furthermore, these authors showed that pelleting a diet, not normally associated with calculus formation, did not induce calculus formation. Thus, pelleting per se did not induce calculus formation although it appears that pelleting probably increased phosphorus digestibility and this would account for the elevated urinary phosphorus excretion. Consequently, this would enhance the possibility of calculus formation.

2:3:4. Water

Early work by Newsom, Tobiska and Osland (1943) indicated that lambs consuming the greatest amount of water were less likely to develop calculi. Various attempts have been made to increase water consumption based on the premise that excretion of a dilute urine would prevent calculus formation. Ørskov, Fraser and McDonald (1971) included 2% sodium chloride in a pelleted concentrate diet for lambs and found that the effect on water intake was small and not statistically significant. To be effective, 4% or more sodium chloride should be included in the diet (Udall, 1962). However, increasing water intake per se does not guarantee freedom from calculus formation. Udall (1974) concluded that the most effective control of urolithiasis in feedlot lambs was achieved by increasing the intake of chloride ion by using sodium or ammonium chloride and providing plenty of water. Examination of the literature indicates that, although augmented water intake will not prevent urolithiasis, it serves to reduce both the likelihood and severity of stone formation.

2:3:5. Nitrogen level and source

Packett, et al., (1958) compared the effect of a basal diet, containing 8% crude protein, with urea-supplemented and cottonseed meal-supplemented diets, both containing 11% crude protein; the calcium and phosphorus contents were the same in all diets - 0.4% and 0.2% respectively. Deaths due to calculus formation were significantly less ($p < 0.01$) amongst animals receiving the basal diet or the basal diet supplemented with urea compared with those receiving cottonseed meal-supplemented diets. Deaths in the urea-fed animals occurred about 80 days after the trial started whereas some of the cottonseed fed animals died after only 30 days. It appeared that increasing the nitrogen level of the diet increased the likelihood of death due to calculus formation although this was less likely when the source of nitrogen was not protein. Although, Packett, et al., (1958) did not account for the difference between urea-fed and cottonseed-fed animals. The explanation might be that the urea-fed animals drank more water and excreted dilute urine since Japanese work (Watanabe, Miyazaki and Kawashima, 1975) has shown that urine volume expressed on the basis of metabolic body size was significantly greater in urea-fed animals than in lambs fed soya.

2:3:6. Vitamins A and D

Packett and Coburn (1965) found that addition of vitamin D to a high-grain diet known to induce calculi, increased the incidence of urolithiasis in yearling wethers. In a further series of experiments, Packett, Lineberger and Jackson, (1968) found a very high incidence of urolithiasis (80%) in wether lambs fed a diet supplemented with vitamin D. These findings are not surprising in view of the established mode of action of this vitamin in increasing calcium availability.

Dutt and Sawhney (1969) found evidence of renal calculus formation in lambs deprived of vitamin A, although the low dietary levels of vitamin A supplied to these animals would be unlikely under normal husbandry conditions. Beeson, et al., (1943) concluded from their experiments that vitamin A had no effect on the formation of ovine calculi and in apparent agreement are the results of Lindley, Brugman, Cunha and Warwick (1949) who found that calculi occurred both in rams supplemented with vitamin A and in those deficient in A. However, Webb, Mitchell, Little and Schmitt (1968) have shown quite clearly that a vitamin A deficiency in sheep resulted in vast increases in urinary phosphorus excretion and a reduction in calcium excretion. The results of Lindley, Taysom, Ham and Schneider (1953) support this finding in that they showed that addition of vitamin A to the diet of sheep significantly reduced the urinary excretion of phosphorus by these animals, and increased urinary calcium excretion.

It seems clear from the available evidence, that vitamin A deficient sheep will be prone to urolithiasis in view of their elevated urinary phosphorus levels. Furthermore, Richards (1972) has demonstrated a phosphaturia in calves preceding clinical signs of a deficiency. This type of biochemical response has already been linked with calculus formation but has not been shown in sheep and could be of aetiological significance.

2:3:7. Minerals

2:3:7:1. Calcium

2:3:7:1:1. Quantity

Emerick and Embry (1963 and 1964) investigated the effect of dietary level of calcium on the development of urinary calculi in wethers and ewe lambs. In both experiments, increasing the dietary level of calcium using ground limestone from 0.4 to 0.9% of the diet reduced calculus formation. Similar results were obtained by Bushman, et al., (1965a and b) although calculus formation was not wholly prevented, these authors noted that serum and urine phosphorus levels were significantly reduced by increasing dietary calcium content. Schneider, Taysom and Ham (1952) ascribed the beneficial effect of additional dietary calcium in reducing calculus formation to a decrease in urinary phosphorus content.

2:3:7:1:2. Source

Robbins, et al., (1965) found that the incidence of urolithiasis in wether lambs increased when bonemeal was used as a source of calcium. This unexpected finding was probably due to the fact that the calcium in bonemeal was less available than in limestone and furthermore, the bonemeal was simultaneously supplying phosphorus.

Bushman (1968) determined the availability of calcium in calcium carbonate and dicalcium phosphate using as his criteria weight gain, bone ash and net retention in rats. No difference was found in the availability of calcium from either source.

Bushman, Embry and Emerick (1967) fed supplements of calcium chloride or calcium carbonate to wether lambs at 0.5 and 1.5% of a diet known to induce calculus formation. There was little difference in the effect of the supplements at the lower level of inclusion although at the 1.5% level the chloride supplement was much more effective than the carbonate in reducing incidence of urolithiasis. In another experiment, (Bushman, et al., 1968) a supplement of 1% calcium chloride was more effective than one of 2% calcium carbonate in reducing the incidence of urolithiasis. Based on the calcium retention and excretion data these authors concluded that variation in calcium excretion alone, without a concomitant reduction in urine pH was not important in the prevention of calculus formation. Later work by Hoar (1970) and Hoar, et al., (1970b) confirmed Bushman's findings that although limestone was the richest source of calcium (40% Ca) compared with the chlorides ($CaCl_2$-36.36% Ca, $CaCl_2.2H_2O$ - 27.39% Ca) it was not as effective in preventing calculus formation.

Gill, Finlayson and Vermeulen (1959) concluded that the principal mechanism whereby calcium carbonate reduced the incidence of urinary calculi in rats was by reducing phosphorus digestibility and hence urinary phosphorus excretion. The superiority of the chlorides of calcium in reducing calculus formation was probably due, in addition to reducing urinary phosphorus, to an increased urinary chloride excretion which may compete with phosphate for ion-binding sites. (Udall and Chen Chow, 1963) and reduce pH (Bushman, 1968).

2:3:7:2. Phosphorus

2:3:7:2:1. Quantity

Several authors (Lindley, *et al.*, 1953; Kunkel and Robbins, 1959; Cornelius, *et al.*, 1959; Cornelius, 1963) have shown that a diet containing $2\frac{1}{2}\%$ potassium phosphate (K_2HPO_4) consistently produced a high incidence of urolithiasis in sheep.

Emerick and Embry (1963, and 1964) showed that increasing the dietary level of phosphorus from 0.3 to 0.8% using disodium phosphate (Na_2HPO_4) significantly increased the incidence of urolithiasis. This effect of increasing dietary phosphorus concentration was confirmed by others (Bushman, *et al.*, 1965b; Hoar, *et al.*, 1969, 1970a and b; Hoar, 1970).

Work by Robbins, *et al.*, (1965) and Crookshank, Robbins and Kunkel, (1967) led to the conclusion that an increase in dietary phosphorus predisposed the formation of magnesium phosphate calculi. A multiple correlation coefficient of 0.87 was obtained (Robbins *et al.*, 1965) relating incidence of urolithiasis to length of feeding period. They suggested that the development of urolithiasis may be activated by high levels of serum phosphorus resulting from high dietary intakes. Elevated serum phosphorus could initiate the development of metabolic conditions resulting in renal retention of magnesium and increased urinary calcium and phosphorus excretion. However, I consider this to be an unlikely mechanism.

2:3:7:2:2. Source

The effect of various dietary phosphates on the incidence of urinary calculi in wether lambs has been investigated (Bushman, et al., 1965a). Supplements of either monosodium, disodium, sodium tripoly or dicalcium phosphate were compared. Dicalcium phosphate supplements were not associated with calculus formation whereas with the other phosphate sources the incidence was uniformly high and there appeared to be little difference between them.

Bushman (1968) measured the availability of phosphorus in disodium phosphate and in dicalcium phosphate using weight gain, bone ash and net retention as criteria in a trial using rats. The phosphorus appeared to be about one third more available in the disodium form than in the dicalcium form.

2:3:7:3. Calcium to Phosphorus ratio

Beeson, et al., (1943) found no calculi formed in wethers receiving diets with calcium: phosphorus ratios varying from 1:1.8 to 1:0.38. Weaver (1963) at Glasgow used a diet similar to one reported in the United States as being calculus-inducing to produce urolithiasis in a group of Blackface ram lambs. The diet failed to produce clinical symptoms in the animals and it was suggested that the ratio of calcium to phosphorus might have prevented the anticipated urolith formation. The phosphorus content was approximately the same in both diets whereas the calcium was approximately twice as much in the British diet. The calcium: phosphorus ratio in the American diet was 1:1.7 compared with 1.5:1 and 1.05:1 in the British diets. Bushman, et al., (1965b) suggested an optimum ratio of 2:1 or greater when high levels of phosphorus (0.55%) were offered. Hoar (1970) reported that a low ratio (0.5 to 1:1) did not promote calculus formation on a low phosphorus diet (0.28%). Increasing the level of dietary phosphorus to 0.47% necessitated a ratio greater than 2:1 when limestone supplied the calcium, although with calcium chloride only 1.2:1 was necessary. A ratio greater than 2.5:1 has been suggested as a means of preventing calculus formation (Bauer, et al., 1971). From the foregoing, a calcium: phosphorus ratio of at least 1.5:1 would seem desirable. Published requirements (Agricultural Research Council, 1965) indicate a ratio varying between 1.5 to 2:1 for lambs growing at the rate of 200g/day.

2:3:7:4. Magnesium

2:3:7:4:1. Quantity

Johnson, Palmer and Nelson (1940) recorded a 10% incidence of calculi (principally magnesium ammonium phosphate) in a flock of 2,600 wethers fed a diet rich in magnesium. More recently, an outbreak of urolithiasis amongst wether lambs was attributed to a high dietary level of magnesium (Nicholson, 1977). Calculi were amorphous and composed largely of magnesium (13.4%) and calcium (4.8%) with a trace of phosphate. The diet was supplemented with a high magnesium mineral supplement and was calculated to contain 4.4, 4.1 and 7.4g per kg dry matter of calcium, phosphorus and magnesium respectively.

Johnson et al., (1940) investigated the role of high dietary magnesium in the aetiology of urolithiasis. Feeding diets containing 1.5% magnesium to wether sheep produced a three fold increase in plasma magnesium, and slight crystal formation in the urine although no calculi were found. Similarly, Beeson, et al., (1943) could not produce calculi in wethers by feeding them magnesium-rich diets (0.4%). More recently, Bushman et al., (1965b) fed diets containing 0.18 and 0.38% magnesium to wether lambs. They observed that dietary magnesium was more effective than an equal amount of calcium in reducing urinary phosphorus although it was not more effective in reducing urolithiasis. Therefore, it seems that addition of magnesium to the diet is more likely to have a beneficial effect rather than a harmful one. Reduction of urinary phosphorus should reduce the possibility of calculus formation. The mode of action is probably one of interference at the absorptive site of phosphorus in the gut.

2:3:7:4:2. Source

There is no published information on the effect of magnesium source on urolithiasis.

2:3:7:5. Sodium Chloride

Elam, Ham and Schneider (1957) reported the beneficial effect of including 10% sodium chloride in the diet of wethers in preventing calculus formation. Similarly, force-feeding sodium chloride as an integral part (10%) of a pelleted diet prevented the formation of both renal and bladder calculi in sheep according to Udall (1959a).

The relationship between sodium chloride intake, reduced incidence of urolithiasis and diuretic effects was investigated further, by Udall and Chen Chow (1963), to determine the precise mode of action of sodium chloride. They suggested that the beneficial effect of including sodium chloride in the diet was independant of urine pH and volume and thus of its diuretic effect. They hypothesised that there was interference with the formation of nucleation centres and that the mechanism was one of ion-competition for cationic binding sites on the matrix material. The labile characteristic of the anion probably determines the ability of the Cl^- ion to interfere and that the Cl^- ion, with its compactly distributed negative charge, binds in such a way as to displace the $PO4^=$ ion and thus inhibit formation of phosphatic calculi. However the disadvantage of this theory is that Udall and Chen Chow (1963) presuppose that matrix material is responsible for binding cations. This fact has not been established in ovines although workers in the human field (Boyce, et al., 1954 and 1955) are firmly convinced of the significance of ion-binding by matrix material, in the aetiology of calculous disease.

Udall, et al., (1965) compared a diet containing 4% sodium chloride with one containing equal total ash but low in salt. The salt-containing diet prevented calculus formation although the volume of urine produced by lambs fed the different diets was not significantly different.

Udall and Chen Chow (1965) attempted to confirm that the Cl^- ion was the active part of the salt molecule. They fed separate supplements of sodium carbonate, potassium carbonate and ammonium chloride to wether sheep to determine the effect of sodium, potassium and chloride on calculus formation. All diets had approximately the same ash content. More calculi were formed with increased intake and excretion of sodium and potassium whilst significantly fewer calculi were formed with the augmented excretion of chloride following chloride supplementation of the diet. However, the interpretation of this result is limited by the unknown effect of the ammonium ion. Notwithstanding this, Udall (1974) concluded that the most effective control of urolithiasis in feedlot lambs is achieved by increasing the chloride ion intake using either salt or ammonium chloride and by providing plenty of water.

Modlin (1967) introduced a new concept relating to the aetiology of renal stone in man. An examination of the sodium and calcium content of the urine voided by normal white and Bantu subjects and patients with renal stone revealed significant differences. Modlin (1967) found that urine from patients with calculi had a very low sodium: calcium ratio, compared with the normal Bantu; a population group in which renal stone rarely occurs. He was able to conclude from his results that the tendency to renal stone formation decreased with an increasing amount of sodium relative to calcium in the urine. This could well be another factor contributing to the effectiveness of dietary additions of sodium chloride in the prevention of urinary calculi.

2:3:7:6. Potassium

Research of the literature reveals two opposing views in relation to the role of potassium in calculus formation. Two groups of workers (Elam, Schneider and Ham, 1956; Bushman, 1968) found that addition of potassium to a diet fed to wethers increased the incidence of urolithiasis. In contrast Crookshank and co-workers (Robbins, et al., 1965; Crookshank, 1966) found that addition of potassium to the diet of wethers reduced the incidence of urolithiasis and increased urinary magnesium levels. They found that the degree of reduction was dependent upon the anion associated with the potassium cation. Not surprisingly, they found the chloride more effective than the phosphate. However, in view of more recent work (Hoar, 1970; Hoar, et al., 1970a) which examined the effect of additional dietary potassium on incidence of calculi and showed increased urinary mineral deposits it must be concluded that supplementary potassium is more likely to be detrimental to the well-being of lambs than of benefit.

2:4. Objectives of current study

From the foregoing literature review it is apparent that the factor consistently associated with calculus formation in store lambs is mineral imbalance. Thus, it follows that this should be the first area of study in relation to calculus formation in early weaned lambs. However, in view of the implication of concentrate diets and their physical form in the aetiology of calculus formation, a preliminary experiment was conducted to establish their significance in relation to the formation of calculi.

It was hoped that this study would contribute further to an understanding of the aetiology of calculus formation, with particular reference to early weaned lambs, by:

1. Measuring the effects of physical form of the diet, nitrogen level and source on the metabolism of the major minerals and mucopolysaccharide. At the same time, the effect on water economy was noted (Experiment I).

2. Examining the interrelationships between calcium, magnesium and phosphorus and measuring the effect of raising the dietary levels of these minerals on the metabolism of the major minerals, mucopolysaccharide and the water economy of lambs (Experiment II).

Following on from the observations made in Experiment II that lambs developed calculi within 4 weeks of weaning and that the incidence seemed unrelated to any particular combination of dietary minerals, further experiments were conducted to investigate the reason for this.

As the process of weaning was common to all the experimental lambs used in Experiment II and the major changeover at this time was from a liquid-based diet to a dry all-concentrate diet, the water economy of lambs at this time was investigated as a possible aetiological agent in the formation of calculi. The change in water economy at weaning was measured in Experiment III.

The results of Experiment III showed that a substantial change in water economy occurred postweaning. The significance of this change in relation to kidney structure and the formation of calculi was investigated in Experiments IV and V.

3. EXPERIMENTAL WORK

3:1. EXPERIMENT I

3:1:1. Introduction

Basic considerations in the formulation of a diet, apart from dry matter intake, are the animal's requirement for energy, protein, minerals and vitamins and the form in which these nutrients are supplied. The bulk of most diets is composed of foods supplying energy and protein. Animals respond immediately to changes in the supply of these nutrients and therefore they usually receive first consideration. To maximise liveweight gain, as much energy as possible should be consumed by the animal; for this to be achieved, cereal diets are normally fed.

The form in which cereals are fed to early weaned lambs has been investigated (Ørskov, 1973; Ørskov, Fraser and Gordon, 1974; Fraser and Ørskov, 1974; Ørskov, et al. 1974); the results indicate that feeding of whole grain to lambs does not reduce their performance. The only possible exception to this finding was the impaired performance of lambs fed whole oats. However, many producers still 'process' the cereal portion, together with other components, to produce pellets or cubes. Thus, in practice, lambs receive their diet either in the form of pellets or as a loose mixture of ingredients. It has been shown that pelleting a diet has an effect on its intake by animals (Wainman, Smith and Blaxter, 1971) and on calculus formation in lambs (Packett et al., 1958; Crookshank, et al., 1965).

In addition to energy, growing lambs require nitrogen to produce lean tissue. Conventional sources of nitrogen are expensive and in least-cost formulations it is desirable to use the cheapest source and lowest level of nitrogen that will support rapid growth rates. Recent work by Ørskov (Ørskov, McDonald, Fraser and Corse, 1971; Ørskov, Fraser and McDonald, 1972) has demonstrated an improved lamb performance by raising the crude protein in cereal-based diets from 11 to 15%. However, this effect was observed only when conventional protein sources were used: urea supplementation, beyond 12% crude protein in the dietary dry matter, produced no beneficial effect. Despite the undoubted productive advantage of feeding high protein diets, it has been shown (Packett, et al., 1958) that deaths due to urolithiasis were significantly greater among animals receiving diets containing 11% crude protein compared with those receiving lower protein diets (8% crude protein). Furthermore, replacement of cottonseed meal by urea in the 11% protein diets reduced the incidence of urolithiasis.

In view of the relative advantages and disadvantages of both urea and conventional nitrogen sources in terms of liveweight gain and their association with urolithiasis in lambs, an experiment was designed to examine the effect of the quantity and source of dietary nitrogen on calculus formation. Physical form of the diet was incorporated as a treatment in this experiment because of its implication in the aetiology of urolithiasis.

3:1:2. Materials and Methods

3:1:2:1. Plan of Investigation

Young lambs were used in this experiment because no work on urolithiasis has been reported in early weaned lambs.

These lambs were wethers, weaned at 4 weeks of age. Wethers were used because of the relative ease of separating urine and faeces. They were available locally throughout the year and 4 weeks was considered to be the youngest age at which lambs could be safely weaned. A 14 day period of adaptation was allowed so that animals could accustom themselves to treatment diets and metabolic crates. Metabolic trials were of 14 days duration so that variation would be reduced and more accurate measurements made. Two metabolic trials were conducted, at 6 (Trial A) and 11 (Trial B) weeks of age. Commercially, intensively fattened lambs are killed at 13 weeks of age and rather than rely on one 14 day trial between 4 and 13 weeks of age, two were carried out in view of the fact that animals would be changing from a monogastric to a ruminant state of digestion. Furthermore, there could be an age effect which might affect the measurements made. The accommodation was limited and in view of the fact that lambs were available throughout the year the experiment was divided into blocks or periods. A factorial design was considered suitable, incorporating four periods which were carried out at different times of the year. Thus, seasonal effects could be removed in the analysis of variance: an important factor, in view of the fact that heat-stressed sheep consume large amounts of water (Blaxter, Graham and Wainman, 1959).

Apart from taking diet and excreta samples, blood samples were taken because blood values often reflect physiological changes occurring within the body. Lamprecht, Darroch and

Crookshank (1969b) noted a relation between serum values and calculus formation. Autopsies were performed on all lambs at the end of each period to assess whether any pathological changes (both gross and microscopic) were present; despite the total absence of clinically apparent urolithiasis, Weaver (1963) found renal calculi in lambs at postmortem.

The minerals investigated (calcium, magnesium, phosphorus, sodium and potassium) were those considered most likely to be involved in calculus formation in lambs. The metabolic aspects studied were digestibility, urinary and serum concentrations, urinary excretion and retention of these minerals. These particular parameters were selected because they would provide an insight into the relationships between absorption, retention and excretion of these minerals. Mineral retention values quoted in the results were calculated as dietary intake minus losses in the faeces and urine; the scope of this experiment did not allow consideration of the other factors - such as endogenous losses and epithelial losses - relating to the measurement of retention. Water economy of lambs was measured because of its established significance in calculus formation.

Protein bound hexose values were measured in serum and urine. Romanowski (1965) pointed out when reviewing information pertinent to the biochemistry of urolith formation, that several workers have found a high correlation between calculus formation and the mucoprotein/glycoprotein content of urine. Patterson and Sweasey (1966) showed that bovine serum protein bound hexose levels correlated well with other carbohydrate constituents such as sialic acid and hexosamine.

Therefore, these authors (Patterson and Sweasey, 1968) considered that it was valid to express concentrations of glycoprotein fractions in terms of protein bound hexose. In the current study, protein bound hexose was considered to be indicative of glycoprotein.

3.1:2:2. Animals

Crossbred wether lambs averaging 9kg liveweight and aged about 28 days were purchased from a local sheep unit which produced lambs throughout the year. At this unit lambs were removed from their dams 48 hours after birth and introduced to ad lib cold milk substitute* (Appendix - Table 8.1:1) and kept in groups of twenty. Hay, water and creep feed (Appendix - Table 8.1:2) were freely available and animals were housed in purpose-built wooden sheds.

3.1:2:3. Diets

Diets were all-concentrate and ground through a 2mm screen and those that were pelleted were forced through a 4.8mm die. The raw materials used to formulate the diets were barley, extracted soya bean meal, white fish meal, feed grade urea, dibasic calcium phosphate, limestone, calcined magnesite and a combined vitamin/mineral intensive sheep supplement ** (Appendix - Table 8.1:3). Treatment diets (Appendix - Table 8.1:4) were formulated to the following specification:

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* Ewelac made by Volac Ltd., Croyden Old Farm, Wendy, Royston, Herts.

** Coopers Beta 212, Cooper Nutrition Products Ltd., Stepfield, Witham.

Treatment Number	Diet Composition	Crude Protein (% air dry)	Physical Form
1	Barley	10	Ground
2	Barley	10	Pelleted
3	Barley + Fishmeal/ Soya	18	Ground
4	Barley + Fishmeal/ Soya	18	Pelleted
5	Barley + Fishmeal/ Soya	14	Pelleted
6	Barley + Urea	14	Pelleted
7	Barley + Urea	18	Ground
8	Barley + Urea	18	Pelleted

Suitable mineral additions were made so that all diets contained 0.9% calcium, 0.6% phosphorus and 0.16% magnesium on an air dry basis. This combination of minerals will meet the animals requirement and furthermore it has been shown not to induce calculus formation.

3.1:2:4. Statistical Design

Three factors were investigated, two at two levels (physical form of the diet and nitrogen source) and one at three levels (level of nitrogen in the diet). A 2x2x3 complete factorial experiment would have given twelve treatment combinations as follows:-

Physical Form of diet	Ground						Pelleted					
Nitrogen Source	Urea			Protein			Urea			Protein		
Nitrogen Level	1	2	3	1	2	3	1	2	3	1	2	3
Treatment number	1	2	3	4	5	6	7	8	9	10	11	12

However, with the limited facilities available this number of factor level combinations was too large to be conveniently contained within a single experiment. Thus, certain combinations were omitted and a 2x2x2 incomplete factorial design was used, retaining those combinations considered most likely to yield meaningful results. The arrangement of factors was as follows:-

Physical Form of diet	Ground						Pelleted					
Nitrogen Source	Urea			Protein			Urea			Protein		
Nitrogen Level			3	1		3		2	3	1	2	3
Treatment number			7	1		3		6	8	2	5	4

There were eight treatments and eight animals allocated per treatment so that 64 lambs were used altogether. Only 16 animals could be accommodated at one time in metabolic crates so that the experiment was divided into four periods, 16 different animals being used in each period. Within a period or replicate, each treatment was imposed on two animals. Each replicate represented a block and inclusion of all treatments in each block enabled removal of block/seasonal effects in the analysis of variance.

Plate I

Multiple metabolic crate used to hold four lambs

3.1:2:5. Accommodation

Four loose boxes were available for experimental purposes and because there was insufficient room to accommodate four individual metabolic crates in each loose box, multiple crates were constructed. They were designed to hold four lambs and were made of dexion and varnished blockboard (Plate 1).

3.1:2:6. Experimental Procedure

3.1:2:6:1. Period

The lambs were weaned at 28 days of age immediately after purchase. Each group of 16 lambs was kept together in a loose box and given ad lib access to hay, water and the same creep feed as that supplied preweaning. The lambs were observed closely over the next 3 days for any signs of ill-health.

At 32 days of age the lambs were weighed and placed in metabolic crates. Experimental diets were randomly allocated to animals. Creep feed was replaced gradually with the appropriate experimental diet over a period of 3 to 4 days. No long roughage was provided. The prescribed diet and deionised water were available ad lib, throughout the period.

After the treatment diets had been introduced the animals were allowed to equilibriate over a period of 7 days before the first metabolic trial (Trial A); this was begun when the lambs were 42 days of age and lasted 14 days. For the following 21 days the lambs remained in their respective metabolic crates and continued to receive their treatment diets. At 77 days of age the second 14 day metabolic trial (Trial B) was begun.

Three days after the completion of Trial B, the animals were killed, weighed and subjected to postmortem examination. This concluded one period which began when the animals were 28 days of age and lasted for 66 days. Animals remained in the metabolic crates throughout each period except for temporary removal during each metabolic trial to facilitate routine daily procedures.

3.1:2:6:2. Metabolic Trial

Animals were weighed at the beginning and end of each metabolic trial. Animals were fitted with faecal collection harnesses to prevent contamination of the urine and these had to be emptied every morning. At the beginning of each period enough food was weighed out in 1kg amounts and sealed in plastic bags to last the whole of the period. Food was available ad lib. and replenished when necessary. Food refusals were rare and when they occurred residues were removed, analysed and appropriate corrections made to the intake figures. At the end of each trial, food residues were removed, analysed and correction made in the intake calculations. Fresh deionised water was provided daily. Metabolic crates and equipment were cleaned daily with deionised water.

3:1:2:7. Sampling Procedures

3:1:2:7:1. Feed/Feed Residues

Treatment diets were sampled at the beginning of each period and feed residues were collected and sampled at the end of the first and second weeks of each trial. These samples were dried and then analysed.

3:1:2:7:2. Faeces

Total faeces excreted daily were collected in plastic bags retained in the harness. These bags were replaced daily. The fresh weight of faeces excreted was noted and roughly 30% taken for dry matter determination. Thus 14 faeces samples were obtained for each animal per trial. The faeces samples from days 1 to 7 inclusive were bulked on the basis of 10% of total daily dry matter output. Samples from days 8 to 14 inclusive were bulked on the same basis.

3:1:2:7:3. Urine

Urine voided by each lamb passed through the mesh floor of the metabolism cage on to a plastic chute and into a collecting vessel placed beneath each chute. Each vessel was fitted with a funnel plugged with non-absorbent cotton wool to prevent entry of any solids. Urine volume was measured daily for each animal and a 10% mixed sample taken and stored at 4°C in an airtight plastic bottle. Samples were bulked every 48 hours so that seven composite samples (2 x 24 hour 10% samples) were obtained for each animal in each metabolic trial. These composite samples were analysed immediately for protein bound hexose and the remainder of the seven samples bulked together in a plastic bag, acidified and kept at -20°C for mineral analysis.

3:1:2:7:4. Blood

Blood was collected from each animal on alternate days throughout each metabolic trial providing seven samples/animal/trial. Lambs were removed from the metabolic crates each morning for changing faecal collection bags and during this time they were blood sampled. Samples were obtained by left jugular venipuncture using 10 ml untreated vacutainers and 20G 40mm needles.* Immediately following sampling, blood samples were centrifuged at 1000G for 10 minutes. The serum was taken off and stored at -20°C after aliquots had been taken for immediate protein bound hexose estimation.

* Becton Dickinson (U.K.) Ltd., York House, Empire Way, Wembley, Middlesex, HA9 0PS.

3:1:2:7:5. Autopsy

Animals were killed using a captive bolt, bled and weighed. Carcases were placed in dorsal recumbency. A midline incision was made through the skin from the mandible to the pelvis and the skin reflected leaving the penis to the left side. The oesophagus and rectum were ligated and the alimentary tract removed. The pelvis was sectioned through the left and right arches and the floor of the pelvis dissected free taking care to preserve the penis intact. The kidneys, ureters, bladder and penis were removed from the abdomen for examination.

Ureters, bladder and penile urethra

These organs were incised along their entire length and examined for the presence of calculous material. Any material recovered was washed with deionised water and kept for analysis.

Kidneys

Both kidneys were incised along their longitudinal axis for macroscopic examination for the presence of calculous material and samples taken from the cortex, medulla and pelvic areas for microscopic examination.

3:1:2:8. Analytical Methods

The number of samples acquired per animal during one metabolic trial was as follows:-

Description of Sample	Number
Serum	7
Urine	7 (48 hr samples)
Urine	1 (bulk)
Faeces	2 (7 day bulk samples)
Diet	1
Feed residues	varies

Protein bound hexose was determined on fresh samples of urine and serum to prevent the possibility of hexose being hydrolysed from the protein, leading to underestimates. Dry matter determinations were carried out on fresh faecal and dietary samples to provide accurate information for calculating water balances.

3:1:2:8:1. Feed/Feed Residue/Faeces

(i) Dry Matter (D.M.)

Fresh samples were placed in weighed tin foil dishes, the dishes were reweighed and placed in a 100°C oven for 48 hours. After cooling in a desiccator the dishes were weighed and sample dry matter calculated.

After drying, samples were ground through a 1.6 mm screen so that representative samples could be taken for analysis.

(ii) Ash

Approximately 1g of dried sample was weighed accurately into a weighed silica basin and then placed in a muffle furnace pre-set at 550°C. After 24 hours the sample and basin were placed in a desiccator to cool and then reweighed.

(iii) Minerals

About 10ml of 12 M HCl was added to the ash and the mixture evaporated to dryness on a water bath. About 10ml of 2M HCl was added to the basin and the contents filtered through a Whatmans No. 1 filter paper into a 100ml volumetric flask, followed by washings of deionised water. The flask was made up to the mark with deionised water when cool.

The calcium and magnesium content of the ash solution was determined by atomic absorption using a Pye Unicam SP90A Series II atomic absorption spectrophotometer. Sodium and potassium levels were determined by flame emission using the same spectrophotometer. In the determination of calcium lanthanum chloride was added to overcome the suppression of the calcium absorption by phosphate ions.

Phosphorus was determined colorimetrically using the technique of Fiske and Subbarow (1925) as modified by Gomori (1942).

Reagents used were 1% metol in 3% sodium metabisulphite and molybdate solution made up of 7.5g ammonium molybdate in 600 ml distilled water plus 200 ml 5M sulphuric acid. The test was set up as follows:-

Water Blank - 8 ml deionised water
Standard - 3 ml " " + 5 ml standard
Test - 7 ml " " + 1 ml ash solution

One ml of molybdate solution was added to each tube followed by 1 ml metol solution. The contents of each tube were mixed and set aside in the dark for 30 minutes to allow maximum colour development and then the absorbances read using a Pye Unicam SP600 at 680nm.

3:1:2:8:2. Urine

(i) Minerals

Calcium and magnesium were determined using a Pye Unicam SP90A Series II spectrophotometer. Sodium and potassium were estimated by flame emission and calcium and magnesium by atomic absorption. Lanthanum chloride was included in samples for calcium estimation to prevent phosphorus suppressing calcium absorption.

Inorganic phosphorus was estimated in the manner described in 3:1:2:8:1 (iii).

(ii) Protein-bound Hexose

The method of Winzler (1955) was used and absorbances read at 540nm using a Pye Unicam SP600 spectrophotometer.

3:1:2:8:3. Serum

(i) Minerals

Calcium and magnesium were estimated by atomic absorption using a Pye Unicam SP90A Series II spectrophotometer. Disodium EDTA was added to prevent phosphate suppressing both calcium and magnesium absorption.

Sodium and potassium were estimated by flame emission using the same apparatus.

Phosphorus was determined using the same technique as that described in 3:1:2:8:1 (iii).

(ii) Protein bound Hexose

The same method was used as described in 3:1:2:8:2 (ii).

(iii) Urea

A modification of the method of Archer and Robb (1925) was used. The protein precipitant was sodium hydroxide and zinc sulphate instead of sodium tungstate and sulphuric acid, and the urease was ground up in alcohol instead of water.

3:1:2:8:4. Renal Tissue

Histochemistry

Portions of kidney were fixed in 10% formol saline and embedded in paraffin in vacuo. The material was sectioned at 8µ and stained with haematoxylin and eosin. In addition, the following histochemical tests were carried out using Culling's (1963) techniques:

(1) von Kossa to identify calcium salts (2) periodic acid-Schiff (PAS) to identify mucopolysaccharide and (3) alcian green to identify acidic mucopolysaccharides.

3:1:2:8:5. Calculous Material

The material was dried in a 100°C oven after washing with deionised water and then crushed and ground using a pestle and mortar. Qualitative analysis was performed using an Oxford Stone Analysis Set*. This kit was designed to show the presence of carbonate, cystine, phosphate, oxalate, urate, magnesium, calcium and ammonia.

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* Boehringer Corporation (London) Ltd., Bilton House, 54, Uxbridge Road, Ealing, London, W5 2TZ.

3:1:2:9. Statistical Analysis

Only a limited combination of factors could be compared using the analysis of variance because the experiment was designed as an incomplete factorial. The following orthogonal comparisons were made:-

(1) Ground and pelleted diets (treatments 1, 3 & 7 vs. 2, 4 & 8)

(2) Basal and urea supplemented diets (treatments 1 & 2 vs. 7 & 8)

(3) Basal and protein supplemented diets (treatments 1 & 2 vs. 3 & 4)

(4) Protein and urea supplemented diets (treatments 3, 4 & 5 vs. 6, 7 & 8)

Furthermore, two possible interactions were investigated:

(1) Interaction between urea supplementation and physical form of the diet (treatments 1 & 8 vs. 2 & 7)

(2) Interaction between protein supplementation and physical form of the diet (treatments 1 & 4 vs. 2 & 3)

The analysis of variance was performed by computer at the ERCC* using an EDEX4** program which also calculated missing values. The procedure for calculating main effects and interactions was the same as that outlined in Appendix 8:3 using data from Experiment II.

The following non-orthogonal comparisons were investigated using 't' tests:

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* Edinburgh Regional Computing Centre, The King's Buildings, Mayfield Road, Edinburgh, EH9 3JZ.

** generated by ARC Unit of Statistics, University of Edinburgh, James Clerk Maxwell Building, The King's Buildings, Mayfield Road, Edinburgh, EH9 3JZ.

(1) Between the basal diet and the low protein supplemented diet (treatments 2 vs. 5)

(2) Between the low protein and high protein supplemented diets (treatments 5 vs. 4)

(3) Between the basal diet and the low urea supplemented diet (treatments 2 vs. 6)

(4) Between the low urea and high urea supplemented diets (treatments 6 vs. 8).

Thus it was possible to investigate the effects of low level supplementation of either urea or protein on the various parameters measured and to determine any difference between the effects of low and high levels of supplementation.

In the results section of this thesis the levels of significance used are *, ** and *** which denote $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively.

3:1:3. Results

3:1:3:1. Mineral Metabolism

3:1:3:1:1. Calcium

Pelleting

Animals fed on pelleted diets retained significantly more calcium in trial A and had a significantly higher urine concentration and excretion of calcium in trial B than those fed ground diets (Table 1). The urinary excretion and concentration of calcium was consistently higher in animals fed pelleted diets.

Urea

Urea supplementation of the basal diet significantly reduced urinary calcium concentration in trial A (Table 2).

Comparison of the 14% crude protein, urea supplemented, pelleted diet (LUD) with the 10% crude protein, basal pelleted diet (BD) showed that urinary excretion of calcium was significantly less ($p<0.05$) during both trials in animals receiving LUD. The LUD also significantly reduced urinary calcium concentration ($p<0.05$) in trial B (appendix 8.2:1).

Protein

Calcium digestibility and retention was higher and urinary calcium lower in the lambs fed on the 18% crude protein, protein supplemented, pelleted diet (HPD) than in those fed the B.D. These effects were significant and observed in both trials (Table 3).

Urine calcium concentration was significantly less ($p<0.05$) during trial B in animals receiving 14% crude protein, protein supplemented, pelleted diet (LPD) compared with those

TABLE 1

THE EFFECT OF PELLETING ON SOME ASPECTS OF CALCIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			GROUND (a)	PELLETED (b)		
Digestibility (%)	A	24	26.3	31.4	2.80	NS
	B	"	28.3	31.0	3.14	NS
Urinary Excretion (mg/14 days)	A	"	636	742	150	NS
	B	"	828	1226	181	*
Retention (mg/14 days)	A	"	15514	20374	2307	*
	B (c)	"	23673	29015	2787	NS
Urinary Concentration (mg/100ml)	A	"	20.1	21.8	3.47	NS
	B	"	12.4	19.8	2.53	**
Serum Concentration (mg/100ml)	A	"	11.6	11.6	0.11	NS
	B	"	12.2	12.1	0.13	NS

(a) Treatments 1, 3 and 7
(b) Treatments 2, 4 and 8
(c) One missing value

TABLE 2

THE EFFECT OF UREA SUPPLEMENTATION ON SOME ASPECTS OF CALCIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			BASAL DIET (a)	BASAL DIET + UREA (b)		
Digestibility (%)	A	16	24.6	25.5	4.05	NS
	B	"	24.9	29.6	4.10	NS
Urinary Excretion (mg/14 days)	A	"	767	694	188	NS
	B	"	1254	1000	230	NS
Retention (mg/14 days)	A	"	15219	13901	3265	NS
	B (c)	"	23079	22544	3888	NS
Urinary Concentration (mg/100ml)	A	"	28.4	21.6	4.86	*
	B	"	23.7	16.0	3.76	NS
Serum Concentration (mg/100ml)	A	"	11.7	11.5	0.13	NS
	B	"	12.2	12.2	0.16	NS

(a) Treatments 1 and 2
(b) Treatments 7 and 8
(c) One missing value

TABLE 3

THE EFFECT OF PROTEIN SUPPLEMENTATION ON SOME ASPECTS OF CALCIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			BASAL DIET (a)	BASAL DIET + PROTEIN (b)		
Digestibility (%)	A	16	24.6	36.4	3.43	**
	B	"	24.9	34.5	3.85	*
Urinary Excretion (mg/14 days)	A	"	767	607	184	NS
	B	"	1254	827	222	NS
Retention (mg/14 days)	A	"	15219	24713	2825	**
	B (c)	"	23079	33409	3413	**
Urinary Concentration (mg/100ml)	A	"	28.4	12.8	4.24	***
	B	"	23.7	8.6	3.10	***
Serum Concentration (mg/100ml)	A	"	11.7	11.7	0.14	NS
	B	"	12.2	12.1	0.17	NS

(a) Treatments 1 and 2
(b) Treatments 3 and 4
(c) One missing value

fed BD (appendix 8:2:2).

Protein/Urea comparison

Animals receiving protein supplements had significantly higher serum calcium values in trial A and retained significantly more calcium in trial B than those receiving urea supplements (Table 4).

Interactions

There were no significant interactions between treatments in relation to calcium metabolism (Table 5).

3:1:3:1:2. Phosphorus

Pelleting

Serum phosphorus was significantly higher during trial B in those animals fed pelleted diets compared to those receiving ground diets (Table 6).

Animals fed pelleted diets excreted consistently more phosphorus in their urine and although the effect was not statistically significant, it may be important in relation to calculus formation.

Urea

Addition of urea to the basal diet significantly reduced urinary phosphorus concentration (trials A and B) and phosphorus retention in trial A only (Table 7).

Urinary phosphorus excretion was significantly less in trials A ($p < 0.01$) and B ($p < 0.05$) in those animals receiving the LUD compared with those fed the BD (appendix 8:2:1).

Protein

Supplementary protein significantly reduced urine phosphorus concentration in both trials and significantly increased phosphorus retention in trial B (Table 8).

TABLE 4

A COMPARISON OF THE EFFECT OF PROTEIN VERSUS UREA SUPPLEMENTATION ON SOME ASPECTS OF CALCIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			BASAL DIET + PROTEIN (a)	BASAL DIET + UREA (b)		
Digestibility (%)	A	24	33.7	28.4	3.16	NS
	B	"	33.3	30.1	3.32	NS
Urinary Excretion (mg/14 days)	A	"	595	591	153	NS
	B	"	883	869	189	NS
Retention (mg/14 days)	A	"	22776	18419	2539	NS
	B (c)	"	32451	24511	2826	**
Urinary Concentration (mg/100ml)	A	"	14.4	19.5	3.94	NS
	B	"	10.5	14.9	3.16	NS
Serum Concentration (mg/100ml)	A	"	11.8	11.5	0.10	*
	B	"	12.2	12.2	0.13	NS

(a) Treatments 3, 4 and 5
(b) Treatments 6, 7 and 8
(c) One missing value

TABLE 5

THE INTERACTION BETWEEN TREATMENTS IN RELATION TO SOME ASPECTS OF CALCIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

MEASUREMENTS	TRIAL	n	INTERACTIONS		SE		SIGNIFICANCE	
			(1) PROTEIN/ PELLETING (a)	(2) UREA/ PELLETING (b)	(1)	(2)	(1)	(2)
Digestibility (%)	A	16	-5.5168	1.8658	3.4327	4.0484	NS	NS
	B	"	-0.9854	0.0178	3.8501	4.1005	"	"
Urinary Excretion (mg/14 days)	A	"	208.11	112.800	184	188	"	"
	B	"	-185.52	-159.22	221	230	"	"
Retention (mg/14 days)	A	"	-2946.2	921.6	2825.4	3264.3	"	"
	B (c)	"	-3837.0	- 62.3	3413.2	3887.3	"	"
Urinary Concentration (mg/100ml)	A	"	2.8715	2.0935	4.2440	4.8600	"	"
	B	"	-3.3345	-3.8890	3.0955	3.7546	"	"
Serum Concentration (mg/100ml)	A	"	-0.0450	0.0800	0.1430	0.1370	"	"
	B	"	0.0600	0.1350	0.1700	0.1690	"	"

(a) Treatments 1 and 4 vs. 2 and 3
(b) Treatments 1 and 8 vs. 2 and 7
(c) One missing value

TABLE 6

THE EFFECT OF PELLETING ON SOME ASPECTS OF PHOSPHORUS METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			GROUND (a)	PELLETED (b)		
Digestibility (%)	A	24	49.0	50.2	3.73	NS
	B	"	46.1	52.3	3.74	NS
Urinary Excretion (mg/14 days)	A	"	5510	5619	1019	NS
	B	"	9904	11304	1826	NS
Retention (mg/14 days)	A	"	16061	17359	1806	NS
	B (c)	"	23246	28319	2797	NS
Urinary Concentration (mg/100ml)	A	"	155.4	180.5	27.26	NS
	B	"	130.6	178.3	23.60	NS
Serum Concentration (mg/100ml)	A	"	8.8	9.0	0.19	NS
	B	"	9.1	9.9	0.24	**

(a) Treatments 1, 3 and 7
(b) Treatments 2, 4 and 8
(c) One missing value

TABLE 7

THE EFFECT OF UREA SUPPLEMENTATION ON SOME ASPECTS OF PHOSPHORUS METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			BASAL DIET (a)	BASAL DIET + UREA (b)		
Digestibility (%)	A	16	48.7	51.5	4.57	NS
	B	"	48.9	43.8	4.62	NS
Urinary Excretion (mg/14 days)	A	"	6008	4236	1215	NS
	B	"	11271	7175	2161	NS
Retention (mg/14 days)	A	"	17898	13598	2086	*
	B (c)	"	24377	19485	3687	NS
Urinary Concentration (mg/100ml)	A	"	221.7	145.7	34.05	*
	B	"	210.7	112.7	26.57	***
Serum Concentration (mg/100ml)	A	"	9.1	8.6	0.21	NS
	B	"	9.7	9.2	0.29	NS

(a) Treatments 1 and 2
(b) Treatments 7 and 8
(c) One missing value

TABLE 8

THE EFFECT OF PROTEIN SUPPLEMENTATION ON SOME ASPECTS OF PHOSPHORUS METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			BASAL DIET (a)	BASAL DIET + PROTEIN (b)		
Digestibility (%)	A	16	48.7	48.5	4.57	NS
	B	"	48.9	54.9	4.58	NS
Urinary Excretion (mg/14 days)	A	"	6008	6449	1249	NS
	B	"	11271	13366	2237	NS
Retention (mg/14 days)	A	"	17898	18635	2212	NS
	B (c)	"	24377	33487	3426	*
Urinary Concentration (mg/100ml)	A	"	221.7	136.4	33.39	*
	B	"	210.7	140.1	28.91	*
Serum Concentration (mg/100ml)	A	"	9.1	9.0	0.23	NS
	B	"	9.7	9.6	0.30	NS

(a) Treatments 1 and 2
(b) Treatments 3 and 4
(c) One missing value

The LPD significantly increased ($p < 0.05$) the urinary excretion of phosphorus in trial B (appendix 8:2:2).

Protein/Urea Comparison

Urinary excretion of phosphorus and phosphorus retention were significantly less during both trials in animals fed urea supplemented diets compared with those fed protein supplemented diets. This effect is interesting in that it is contrary to that seen with calcium and that calculi found under intensive conditions are composed largely of phosphorus rather than of calcium. These changes were probably a reflection of reduced phosphorus digestibility in lambs fed the urea supplemented diets. During trial B, phosphorus digestibility was significantly less in those animals fed these diets (Table 9).

Interactions

There were no significant interactions between treatments in relation to phosphorus metabolism (Table 10).

3:1:3:1:3. Magnesium

Pelleting

Magnesium retention was significantly greater in animals fed pelleted diets compared with those fed ground diets during trial B (Table 11).

Urea

Addition of urea to the basal diet significantly reduced the urinary concentration and excretion of magnesium during both trials (Table 12).

The LUD significantly reduced ($p < 0.05$) urinary excretion of magnesium in trial B compared with the BD. Magnesium re-

TABLE 9

A COMPARISON OF THE EFFECT OF PROTEIN VERSUS UREA SUPPLEMENTATION ON SOME ASPECTS OF PHOSPHORUS METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			BASAL DIET + PROTEIN (a)	BASAL DIET + UREA (b)		
Digestibility (%)	A	24	50.6	49.5	3.70	NS
	B	"	56.4	46.8	3.44	**
Urinary Excretion (mg/14 days)	A	"	6578	3920	909	**
	B	"	13688	6896	1444	***
Retention (mg/14 days)	A	"	19444	15238	1648	*
	B (c)	"	33685	23077	2461	***
Urinary Concentration (mg/100ml)	A	"	160.9	141.9	29.09	NS
	B	"	160.6	123.6	24.40	NS
Serum Concentration (mg/100ml)	A	"	9.0	8.7	0.18	NS
	B	"	9.8	9.4	0.23	NS

(a) Treatments 3, 4 and 5
(b) Treatments 6, 7 and 8
(c) One missing value

TABLE 10

THE INTERACTION BETWEEN TREATMENTS IN RELATION TO SOME ASPECTS OF PHOSPHORUS METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

MEASUREMENTS	TRIAL	n	INTERACTIONS		SE		SIGNIFICANCE	
			(1) PROTEIN/ PELLETING (a)	(2) UREA/ PELLETING (b)	(1)	(2)	(1)	(2)
Digestibility(%)	A	16	-2.8503	-0.2299	4.5711	4.5719	NS	NS
	B	"	-2.6019	-1.8532	4.5808	4.6187	NS	NS
Urinary Excretion (mg/14 days)	A	"	-392.71	466.74	1249	1215	NS	NS
	B	"	-668.04	-42.45	2237	2161	NS	NS
Retention (mg/14 days)	A	"	828.5	1482.6	2211.5	2085.7	NS	NS
	B(c)	"	-2783.4	-1521.4	3426.9	3686.1	NS	NS
Urinary Concentration (mg/100ml)	A	"	-11.3255	4.3500	33.3851	34.0450	NS	NS
	B	"	3.1685	-7.1060	28.9055	26.5729	NS	NS
Serum Concentration (mg/100ml)	A	"	-0.0250	0.420	0.2360	0.2160	NS	NS
	B	"	-0.0150	-0.070	0.3050	0.2950	NS	NS

(a) Treatments 1 and 4 vs. 2 and 3
(b) Treatments 1 and 8 vs. 2 and 7
(c) One missing value

TABLE 11

THE EFFECT OF PELLETING ON SOME ASPECTS OF MAGNESIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			GROUND (a)	PELLETED (b)		
Digestibility (%)	A	24	48.8	52.3	1.76	NS
	B	"	48.7	52.4	1.98	NS
Urinary Excretion (mg/14 days)	A	"	3262	3572	363	NS
	B	"	4719	5061	426	NS
Retention (mg/14 days)	A	"	3418	4192	563	NS
	B (c)	"	4865	6699	652	**
Urinary Concentration (mg/100ml)	A	"	95.8	107.7	14.29	NS
	B	"	66.7	80.2	7.54	NS
Serum Concentration (mg/100ml)	A	"	2.7	2.7	0.05	NS
	B	"	2.9	2.9	0.07	NS

(a) Treatments 1, 3 and 7
(b) Treatments 2, 4 and 8
(c) One missing value

TABLE 12

THE EFFECT OF UREA SUPPLEMENTATION ON SOME ASPECTS OF MAGNESIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			BASAL DIET (a)	BASAL DIET + UREA (b)		
Digestibility (%)	A	16	49.8	49.0	2.21	NS
	B	"	48.8	48.5	2.60	NS
Urinary Excretion (mg/14 days)	A	"	3835	2822	412	*
	B	"	5853	4252	500	**
Retention (mg/14 days)	A	"	3498	3019	737	NS
	B (c)	"	4696	4048	1029	NS
Urinary Concentration (mg/100ml)	A	"	135.4	87.0	17.71	**
	B	"	103.5	66.1	11.09	**
Serum Concentration (mg/100ml)	A	"	2.8	2.7	0.06	NS
	B	"	2.9	2.9	0.08	NS

(a) Treatments 1 and 2
(b) Treatments 7 and 8
(c) One missing value

tention was significantly higher ($p < 0.05$) in animals fed the LUD compared with those receiving the 18% crude protein, urea supplemented, pelleted diet (HUD) during trial B (appendix 8:2:1).

Protein

Magnesium retention was higher and urinary magnesium concentration lower in the lambs fed on the HPD than in those fed the BD. These effects were significant and observed in both trials. The increase in magnesium digestibility and reduction in urinary magnesium excretion brought about by feeding extra protein were significant only in trial B (Table 13).

Lambs fed the HPD retained significantly more ($p < 0.01$) magnesium than those fed the LPD in trial A. The urinary magnesium concentration in lambs fed the LPD was significantly less ($p < 0.05$) in trial B compared with the urine from lambs fed the BD. (appendix 8:2:2).

Protein/Urea comparison

Animals fed protein supplemented diets retained significantly more magnesium in trial B than those fed urea supplemented diets (Table 14).

Interactions

There were no significant interactions between treatments in relation to magnesium metabolism (Table 15).

3:1:3:1:4. Sodium

Pelleting

Sodium digestibility, urinary and serum sodium concentrations were all significantly greater during trial B in those animals fed pelleted diets compared with those fed ground

TABLE 13

THE EFFECT OF PROTEIN SUPPLEMENTATION ON SOME ASPECTS OF MAGNESIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			BASAL DIET (a)	BASAL DIET + PROTEIN (b)		
Digestibility (%)	A	16	49.8	52.8	2.16	NS
	B	"	48.8	54.4	2.43	*
Urinary Excretion (mg/14 days)	A	"	3835	3593	444	NS
	B	"	5853	4564	522	*
Retention (mg/14 days)	A	"	3498	4898	689	*
	B (c)	"	4696	8602	798	***
Urinary Concentration (mg/100ml)	A	"	135.4	82.8	17.50	**
	B	"	103.5	50.8	9.23	***
Serum Concentration (mg/100ml)	A	"	2.8	2.8	0.06	NS
	B	"	2.9	2.9	0.09	NS

(a) Treatments 1 and 2
(b) Treatments 3 and 4
(c) One missing value

TABLE 14

A COMPARISON OF THE EFFECT OF PROTEIN VERSUS UREA SUPPLEMENTATION ON SOME ASPECTS OF MAGNESIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			BASAL DIET + PROTEIN (a)	BASAL DIET + UREA (b)		
Digestibility (%)	A	24	51.4	49.4	1.75	NS
	B	"	53.6	50.2	2.03	NS
Urinary Excretion (mg/14 days)	A	"	3650	3017	344	NS
	B	"	4749	4159	446	NS
Retention (mg/14 days)	A	"	4261	3653	590	NS
	B (c)	"	7849	5299	716	***
Urinary Concentration (mg/100ml)	A	"	94.2	102.0	15.71	NS
	B	"	58.3	70.5	10.07	NS
Serum Concentration (mg/100ml)	A	"	2.7	2.7	0.05	NS
	B	"	2.9	2.9	0.07	NS

(a) Treatments 3, 4 and 5
(b) Treatments 6, 7 and 8
(c) One missing value

TABLE 15

THE INTERACTION BETWEEN TREATMENTS IN RELATION TO SOME ASPECTS OF MAGNESIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

MEASUREMENTS	TRIAL	n	INTERACTIONS		SE		SIGNIFICANCE	
			(1) PROTEIN/ PELLETING (a)	(2) UREA/ PELLETING (b)	(1)	(2)	(1)	(2)
Digestibility (%)	A	16	0.3771	-0.3806	2.1559	2.2107	NS	NS
	B	"	-1.4272	-1.7489	2.4286	2.5969	"	"
Urinary Excretion (mg/14 days)	A	"	-58.81	-121.7	444	412	"	"
	B	"	-308.81	-71.28	522	500	"	"
Retention (mg/14 days)	A	"	930.0	120.6	688.5	738.7	"	"
	B(c)	"	-385.9	-491.1	798.8	1029.2	"	"
Urinary Concentration (mg/100ml)	A	"	-1.0315	-12.6750	17.5018	17.7092	"	"
	B	"	-1.2310	-4.6745	9.2320	11.0939	"	"
Serum Concentration (mg/100ml)	A	"	0.0150	-0.0550	0.0670	0.0640	"	"
	B	"	0.0200	0.1500	0.0920	0.0880	"	"

(a) Treatments 1 and 4 vs. 2 and 3
(b) Treatments 1 and 8 vs. 2 and 7
(c) One missing value

diets (Table 16). However, these findings are probably only of mathematical significance, having little physiological meaning.

Urea

There was no significant difference between the effect of the basal and that of the urea supplemented diet on the parameters measured (Table 17).

The LUD significantly reduced ($p < 0.05$) the amount of sodium retained compared with those animals fed the BD during trial A. Comparison of the effect of the LUD with the HUD in trial A showed that urinary sodium concentration was significantly less ($p < 0.05$) in animals fed the HUD (appendix 8:2:1).

Protein

Protein supplements significantly increased urinary sodium excretion during both trials although this was probably a reflection of an increased sodium intake arising from the inclusion of soya in the diet (Table 18).

The LPD significantly increased ($p < 0.05$) sodium digestibility in trial A compared with the BD. Sodium retention was significantly less ($p < 0.05$) in animals fed the HPD compared with the LPD in trial B (appendix 8:2:2).

Protein/Urea Comparison

Urinary sodium excretion was significantly less in animals fed urea supplements compared with those receiving additional protein (Table 19). This effect was seen in both trials and was not surprising in view of the sodium contributed by the soya to the protein supplemented diets.

TABLE 16

THE EFFECT OF PELLETING ON SOME ASPECTS OF SODIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			GROUND (a)	PELLETED (b)		
Digestibility (%)	A	24	87.0	89.5	1.26	NS
	B	"	85.7	89.9	1.30	**
Urinary Excretion (mg/14 days)	A	"	10727	10557	940	NS
	B	"	20265	21697	1952	NS
Retention (mg/14 days)	A	"	2712	4093	920	NS
	B (c)	"	2540	2171	1262	NS
Urinary Concentration (mg/100ml)	A	"	302.8	335.5	40.82	NS
	B	"	256.4	323.9	25.67	*
Serum Concentration (mg/100ml)	A	"	358.7	357.8	1.45	NS
	B	"	353.6	356.5	1.33	*

(a) Treatments 1, 3 and 7
(b) Treatments 2, 4 and 8
(c) One missing value

TABLE 17

THE EFFECT OF UREA SUPPLEMENTATION ON SOME ASPECTS OF SODIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			BASAL DIET (a)	BASAL DIET + UREA (b)		
Digestibility (%)	A	16	87.1	88.4	1.55	NS
	B	"	86.2	88.2	1.63	NS
Urinary Excretion (mg/14 days)	A	"	9959	8961	1254	NS
	B	"	17736	18160	2876	NS
Retention (mg/14 days)	A	"	3317	2890	1132	NS
	B (c)	"	3093	2882	1554	NS
Urinary Concentration (mg/100ml)	A	"	366.5	287.5	49.27	NS
	B	"	319.3	275.7	31.13	NS
Serum Concentration (mg/100ml)	A	"	357.8	358.8	1.79	NS
	B	"	354.7	355.5	1.62	NS

(a) Treatments 1 and 2
(b) Treatments 7 and 8
(c) One missing value

TABLE 18

THE EFFECT OF PROTEIN SUPPLEMENTATION ON SOME ASPECTS OF SODIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			BASAL DIET (a)	BASAL DIET + PROTEIN (b)		
Digestibility (%)	A	16	87.1	89.1	1.54	NS
	B	"	86.2	88.6	1.60	NS
Urinary Excretion (mg/14 days)	A	"	9959	13005	1152	*
	B	"	17736	27046	2391	***
Retention (mg/14 days)	A	"	3317	4002	1127	NS
	B (c)	"	3093	1092	1545	NS
Urinary Concentration (mg/100ml)	A	"	366.5	303.4	49.99	NS
	B	"	319.3	275.3	31.44	NS
Serum Concentration (mg/100ml)	A	"	357.8	357.9	1.77	NS
	B	"	354.7	355.2	1.62	NS

(a) Treatments 1 and 2
(b) Treatments 3 and 4
(c) One missing value

TABLE 19

A COMPARISON OF THE EFFECT OF PROTEIN VERSUS UREA SUPPLEMENTATION ON SOME ASPECTS OF SODIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			BASAL DIET + PROTEIN (a)	BASAL DIET + UREA (b)		
Digestibility (%)	A	24	89.9	88.5	1.24	NS
	B	"	89.9	89.2	1.40	NS
Urinary Excretion (mg/14 days)	A	"	12802	9735	887	***
	B	"	26199	18178	1906	***
Retention (mg/14 days)	A	"	4069	2831	897	NS
	B (c)	"	1534	3673	1257	NS
Urinary Concentration (mg/100ml)	A	"	339.3	336.6	41.16	NS
	B	"	299.2	288.5	25.94	NS
Serum Concentration (mg/100ml)	A	"	356.3	358.6	1.40	NS
	B	"	354.7	355.7	1.30	NS

(a) Treatments 3, 4 and 5
(b) Treatments 6, 7 and 8
(c) One missing value

Interactions

There were no significant interactions between treatments in relation to sodium metabolism (Table 20).

3:1:3:1:5. Potassium

Pelleting

Digestibility and urinary concentration of potassium were significantly greater in animals fed pelleted diets compared with those fed ground diets during trial B (Table 21).

Urea

Addition of urea to the BD produced no significant differences amongst the parameters measured (Table 22). Similarly, there were no significant differences between measurements made in animals receiving either the intermediate level of urea supplementation or the BD or the HUD (appendix 8:2:1).

Protein

Animals receiving additional protein digested significantly more and excreted significantly more potassium in the urine than those animals fed the basal diet. These effects were apparent in both metabolic trials (Table 23).

In trial A, the LPD significantly increased ($p < 0.05$) potassium digestibility and urinary excretion of potassium by lambs compared with the BD. The HPD significantly increased ($p < 0.05$) the urinary potassium excretion by lambs in trial B compared with the LPD (appendix 8:2:2).

Protein/Urea Comparison

Apart from potassium digestibility, the parameters measured were all significantly lower in animals fed the urea supplemented diets (Table 24).

TABLE 20

THE INTERACTION BETWEEN TREATMENTS IN RELATION TO SOME ASPECTS OF SODIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	INTERACTIONS		SE		SIGNIFICANCE	
			(1) PROTEIN/ PELLETING (a)	(2) UREA/ PELLETING (b)	(1)	(2)	(1)	(2)
Digestibility (%)	A	16	-0.3220	1.0322	1.5401	1.5523	NS	NS
	B	"	-3.3132	-2.6585	1.5953	1.6291	"	"
Urinary Excretion (mg/14 days)	A	"	-867.28	-418.91	1152	1254	"	"
	B	"	-2406.52	-545.57	2391	2876	"	"
Retention (mg/14 days)	A	"	929.0	-842.5	1127.0	1132.4	"	"
	B (c)	"	-2169.0	-2090.0	1545.4	1584.6	"	"
Urinary Concentration (mg/100ml)	A	"	7.7310	-19.2625	49.9910	49.2735	"	"
	B	"	-3.8185	-27.0750	31.4351	31.1314	"	"
Serum Concentration (mg/100ml)	A	"	1.885	0.4500	1.7770	1.7950	"	"
	B	"	-0.940	-0.690	1.6240	1.6220	"	"

(a) Treatments 1 and 4 vs. 2 and 3
(b) Treatments 1 and 8 vs. 2 and 7
(c) One missing value

TABLE 21

THE EFFECT OF PELLETING ON SOME ASPECTS OF POTASSIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			GROUND (a)	PELLETED (b)		
Digestibility (%)	A	24	84.4	86.1	1.21	NS
	B	"	86.1	89.1	1.21	*
Urinary Excretion (mg/14 days)	A	"	11401	11119	1484	NS
	B	"	16495	21272	2617	NS
Retention (mg/14 days)	A	"	18943	19173	1802	NS
	B (c)	"	23761	25797	3269	NS
Urinary Concentration (mg/100ml)	A	"	304.1	352.0	51.13	NS
	B	"	255.3	308.7	25.26	*
Serum Concentration (mg/100ml)	A	"	22.1	21.8	0.30	NS
	B	"	20.2	20.5	0.27	NS

(a) Treatments 1, 3 and 7
(b) Treatments 2, 4 and 8
(c) One missing value

TABLE 22

THE EFFECT OF UREA SUPPLEMENTATION ON SOME ASPECTS OF POTASSIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			BASAL DIET (a)	BASAL DIET + UREA (b)		
Digestibility (%)	A	16	82.3	85.4	1.64	NS
	B	"	86.3	86.6	1.56	NS
Urinary Excretion (mg/14 days)	A	"	9334	7525	2193	NS
	B	"	15688	18235	4137	NS
Retention (mg/14 days)	A	"	17932	16955	2343	NS
	B (c)	"	23386	21726	4118	NS
Urinary Concentration (mg/100ml)	A	"	331.4	248.9	62.44	NS
	B	"	266.3	256.8	32.99	NS
Serum Concentration (mg/100ml)	A	"	21.9	21.7	0.37	NS
	B	"	20.6	20.1	0.31	NS

(a) Treatments 1 and 2
(b) Treatments 7 and 8
(c) One missing value

TABLE 23

THE EFFECT OF PROTEIN SUPPLEMENTATION ON SOME ASPECTS OF POTASSIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			BASAL DIET (a)	BASAL DIET + PROTEIN (b)		
Digestibility (%)	A	16	82.3	88.0	1.48	***
	B	"	86.3	89.9	1.48	*
Urinary Excretion (mg/14 days)	A	"	9334	16921	1818	***
	B	"	15688	31559	3205	***
Retention (mg/14 days)	A	"	17932	22289	2207	NS
	B (c)	"	23386	29227	4004	NS
Urinary Concentration (mg/100ml)	A	"	331.4	403.9	62.63	NS
	B	"	266.3	322.9	30.93	NS
Serum Concentration (mg/100ml)	A	"	21.9	22.3	0.36	NS
	B	"	20.6	20.6	0.33	NS

(a) Treatments 1 and 2
(b) Treatments 3 and 4
(c) One missing value

TABLE 24

A COMPARISON OF THE EFFECT OF PROTEIN VERSUS UREA SUPPLEMENTATIONS ON SOME ASPECTS OF POTASSIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			BASAL DIET + PROTEIN (a)	BASAL DIET + UREA (b)		
Digestibility (%)	A	24	88.3	88.5	1.24	NS
	B	"	90.0	87.9	1.24	NS
Urinary Excretion (mg/14 days)	A	"	15996	7135	1284	***
	B	"	27924	17587	2887	***
Retention (mg/14 days)	A	"	21721	15907	1650	**
	B (c)	"	30515	23164	3081	*
Urinary Concentration (mg/100ml)	A	"	423.7	300.4	47.37	*
	B	"	317.5	259.3	24.84	*
Serum Concentration (mg/100ml)	A	"	22.4	21.7	0.28	*
	B	"	20.8	20.1	0.24	**

(a) Treatments 3, 4 and 5
(b) Treatments 6, 7 and 8
(c) One missing value

Interactions

There were no significant interactions between treatments in relation to potassium metabolism (Table 25).

3:1:3:2. Water Metabolism

3:1:3:2:1. Water Intake

Lambs consumed less water when offered pelleted dietsthan when offered ground ones; the same pattern was observed when basal diets were supplemented with urea. However, lambs fed protein supplemented diets consumed more water than when fed the basal diet alone and furthermore, more water was consumed by lambs fed protein supplemented diets compared with urea supplemented diets (Table 26).

Increasing the level of crude protein in the diet by adding protein stimulated an increase in water intake although addition of urea produced the opposite effect (Table 27).

TABLE 25

THE INTERACTION BETWEEN TREATMENTS IN RELATION TO SOME ASPECTS OF POTASSIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

MEASUREMENTS	TRIAL	n	INTERACTIONS		SE		SIGNIFICANCE	
			(1) PROTEIN/ PELLETING (a)	(2) UREA/ PELLETING (b)	(1)	(2)	(1)	(2)
Digestibility %	A	16	1.0707	2.3137	1.4815	1.6429	NS	NS
	B	"	0.3594	2.2656	1.4836	1.5582	"	"
Urinary Excretion (mg/14 days)	A	"	-99.27	185.64	1818	2193	"	"
	B	"	-1106.17	471.62	3205	4137	"	"
Retention (mg/14 days)	A	"	2732.5	1792.2	2207.9	2343.9	"	"
	B (c)	"	-1133.6	-126.4	4003.7	4118.4	"	"
Urinary Concentration (mg/100ml)	A	"	29.2250	-12.9935	62.6250	62.4360	"	"
	B	"	40.6835	-4.2125	30.9308	32.9966	"	"
Serum Concentration (mg/100ml)	A	"	-0.060	-0.040	0.3670	0.3740	"	"
	B	"	0.1500	0.2050	0.3310	0.3180		

(a) Treatments 1 and 4 vs. 2 and 3
(b) Treatments 1 and 8 vs. 2 and 7
(c) One missing value

TABLE 26

THE EFFECT OF PHYSICAL FORM OF THE DIET AND NITROGEN SUPPLEMENTATION ON WATER INTAKE (ml/24 hr) BY WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

COMPARISONS (Values in brackets represent treatments)		TRIAL	n	1		2	
1	VERSUS 2			MEAN	SE	MEAN	SE
Ground Diets (1, 3 & 7)	Pelleted Diets (2, 4 & 8)	A	24	862.1	62.45	808.1	49.10
		B	"	1596.4	113.58	1476.0	85.33
Basal Diet (1 & 2)	Basal Diet + Urea (7 & 8)	A	16	809.7	63.44	686.4	49.12
		B	"	1411.2	115.71	1368.8	90.86
Basal Diet (1 & 2)	Basal Diet + Protein (3 & 4)	A	16	809.7	63.44	1009.1	68.46
		B	"	1411.2	115.71	1828.5	130.00
Basal Diet (3, 4 & 5)	Basal Diet + Urea (6, 7 & 8)	A	24	951.5	57.35	701.2	43.74
		B	"	1734.0	110.89	1342.5	90.69

TABLE 27

The effect of treatments on mean daily water intake (ml/24 hr) by wether lambs during two 14 day metabolic trials begun at six (trial A) and eleven (trial B) weeks of age.

	Treatment	n	Trial A	SD	Trial B	SD
1	10% CP ground	8	836.5	271.2	1436.3	443.3
2	10% CP pelleted	"	782.8	250.5	1386.2	511.0
3	18% CP ground	"	1058.6	347.0	1952.1	615.1
4	18% CP pelleted	"	959.7	186.2	1704.8	407.3
5	14% CP pelleted	"	836.4	275.5	1544.9	573.8
6	14% CP pelleted (urea)	"	731.2	258.0	1290.0	600.7
7	18% CP ground (urea)	"	691.2	473.4	1400.7	474.1
8	18% CP pelleted (urea)	"	681.6	217.7	1336.9	236.5

3:1:3:2:2. Urine Volume

Protein supplementation of the diet significantly increased urine output by animals during both trials and these lambs excreted significantly more urine than those receiving urea supplements (Table 28).

There were no significant differences between the different levels of each supplement in their effects on urine production (appendices 8:2:1, 8:2:2).

3:1:3:2:3. Water Retention

Protein supplementation of the diet significantly increased the water retained by lambs during trial A and these animals retained significantly more water than those fed urea supplemented diets during this trial (Table 29).

TABLE 28

THE EFFECT OF PHYSICAL FORM OF THE DIET AND NITROGEN SUPPLEMENTATION ON THE VOLUME OF URINE (ml/24hr) VOIDED BY WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

COMPARISONS (Values in brackets represent treatments)		TRIAL	n	MEANS		SED	SIGNIFICANCE
1 VERSUS	2			1	2		
Ground Diets (1, 3 & 7)	Pelleted Diets (2, 4 & 8)	A	24	295.2	265.9	26.43	NS
		B	24	651.3	550.3	60.74	NS
Basal Diet (1 and 2)	Basal Diet + Urea (7 and 8)	A	16	234.0	238.2	39.77	NS
		B	16	513.2	505.3	87.53	NS
Basal Diet (1 and 2)	Basal Diet + Protein (3 and 4)	A	16	234.0	369.5	32.37	***
		B	16	513.2	784.0	74.40	***
Basal Diet + Protein (3, 4 & 5)	Basal Diet + Urea (6, 7 & 8)	A	24	328.5	232.2	27.89	***
		B	24	700.7	487.1	61.22	***

TABLE 29

THE EFFECT OF PHYSICAL FORM OF THE DIET AND NITROGEN SUPPLEMENTATION ON WATER RETAINED (ml/24hr) BY WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

COMPARISONS (Values in brackets represent treatments)		TRIAL	n	MEANS		SED	SIGNIFICANCE
1 VERSUS	2			1	2		
Ground Diets (1, 3 & 7)	Pelleted Diets (2, 4 & 8)	A B	24 24	448.4 804.0	449.5 821.6	38.44 68.58	NS NS
Basal Diet (1 and 2)	Basal Diet + Urea (7 and 8)	A B	16 16	432.8 770.9	373.0 751.0	49.44 87.73	NS NS
Basal Diet (1 and 2)	Basal Diet + Protein (3 and 4)	A B	16 16	432.8 770.9	541.1 916.6	47.08 83.99	* NS
Basal Diet + Protein (3, 4 & 5)	Basal Diet + Urea (6, 7 & 8)	A B	24 24	535.0 904.3	387.0 775.6	32.51 67.53	*** NS

There were no significant differences between the different levels of each supplement in their effects on water retention (appendices 8:2:1, 8:2:2).

3:1:3:2:4. Interactions

There were no significant interactions between treatments in their effect on urine volume or water retention (Table 30).

3:1:3:3 Protein bound hexose Metabolism

3:1:3:3:1. Urinary Concentration

Protein supplements significantly reduced the urinary concentration of hexose during both trials whilst urea supplements only produced a significant reduction in trial A (Table 31).

There were no significant differences between the levels of each supplement in their effects on urinary concentration of hexose (appendices 8:2:1, 8:2:2) and there were no significant interactions between treatments in their effect on urinary concentration of hexose (appendix 8:2:3).

3:1:3:3:2. Urinary Excretion

Protein supplementation of the diet significantly increased excretion of protein bound hexose by lambs during both trials and these animals excreted significantly more hexose than those fed urea supplements during each trial (Table 32).

There were no significant differences between the levels of each supplement in their effects on urinary excretion of hexose (appendices 8:2:1, 8:2:2) and there were no significant interactions between treatments in their effect on urinary excretion of hexose (appendix 8:2:3).

TABLE 30

THE EFFECT OF INTERACTION BETWEEN PHYSICAL FORM OF THE DIET AND LINEAR ADDITIONS OF PROTEIN OR UREA ON SOME ASPECTS OF WATER METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	INTERACTIONS (1)	INTERACTIONS (2)	SE		SIG	
			PROTEIN/ PELLETING	UREA/ PELLETING	(1)	(2)	(1)	(2)
Water Retention (ml/24 hr)	A	16	-5.000	-1.530	47.078	49.435	NS	NS
	B	"	-45.655	-21.315	83.989	87.728	"	"
Urine Volume (ml/24 hr)	A	"	-31.790	11.090	32.366	39.768	"	"
	B	"	-62.390	23.410	74.396	87.533	"	"

TABLE 31

THE EFFECT OF PHYSICAL FORM OF THE DIET AND NITROGEN SUPPLEMENTATION ON THE URINARY CONCENTRATION OF PROTEIN-BOUND HEXOSE (mg/100ml) IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

COMPARISON (Values in brackets represent treatments)		TRIAL	n	MEANS		SED	SIGNIFICANCE
1	VERSUS 2			1	2		
Ground Diets (1, 3 & 7)	Pelleted Diets (2, 4 & 8)	A	24	39	38	2.7	NS
		B	24	27	29	2.0	NS
Basal Diet (1 and 2)	Basal Diet + Urea (7 and 8)	A	16	43	36	3.4	*
		B	16	30	29	2.6	NS
Basal Diet (1 and 2)	Basal Diet + Protein (3 and 4)	A	16	43	36	3.3	*
		B	16	30	25	2.5	*
Basal Diet + Protein (3, 4 and 5)	Basal Diet + Urea (6, 7 and 8)	A	24	38	38	2.9	NS
		B	24	26	30	2.0	NS

TABLE 32

THE EFFECT OF PHYSICAL FORM OF THE DIET AND NITROGEN SUPPLEMENTATION ON THE URINARY EXCRETION OF PROTEIN BOUND HEXOSE (mg/24hrs) IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

COMPARISONS (Values in brackets represent treatments)		TRIAL	n	MEANS		SED	SIGNIFICANCE
1 VERSUS	2			1	2		
Ground Diets (1, 3 & 7)	Pelleted Diets (2, 4 & 8)	A	24	187.6	176.7	10.27	NS
		B	24	304.4	281.0	18.19	NS
Basal Diet (1 and 2)	Basal Diet + Urea (7 and 8)	A	16	171.6	156.3	14.43	NS
		B	16	258.9	279.2	27.48	NS
Basal Diet (1 and 2)	Basal Diet + Protein (3 and 4)	A	16	171.6	218.6	12.57	***
		B	16	258.9	355.4	22.28	***
Basal Diet + Protein (3, 4 & 5)	Basal Diet + Urea (6, 7 & 8)	A	24	207.6	157.4	8.65	***
		B	24	334.1	266.5	18.58	***

3:1:3:3:3. Serum Concentration

There were no significant treatment effects (Table 33 and appendices 8:2:1, 8:2:2) nor any significant interactions between treatments in their effect on serum protein bound hexose (appendix 8:2:3).

3:1:3:4. Autopsy Findings

During period 3 one animal receiving treatment 6 diet demonstrated clinical signs of urolithiasis during the second metabolic trial. Blood urea was estimated and was found to be 89.3mg/100ml. Catheterisation of the penile urethra was attempted to dislodge the obstruction. This procedure was unsuccessful and the animal killed (see appendix 8:4 for autopsy report). A small amount of milky-white granular material was recovered from the bladder but unfortunately there was insufficient sample to allow quantitative analysis. Qualitative analysis revealed the presence of magnesium, phosphorus and traces of calcium and ammonia.

Examination of the remaining 63 animals at autopsy failed to demonstrate the presence of calculous material. There were no apparent macroscopic or microscopic abnormalities in the kidneys or urinary tracts of these animals apart from a subacute interstitial nephritis and low-grade pyelonephritis in kidneys taken from one animal which had been successfully treated for urolithiasis. This animal was receiving treatment 4 diet and had demonstrated clinical signs of urethral obstruction between metabolic trials. Blood urea was estimated at 284.4mg/100ml. The penile urethra was successfully catheterised. Daily blood urea values taken after this were

TABLE 33

THE EFFECT OF PHYSICAL FORM OF THE DIET AND NITROGEN SUPPLEMENTATION ON THE SERUM CONCENTRATION OF PROTEIN BOUND HEXOSE (mg/100ml) OF WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

COMPARISONS (Values in brackets represent treatments) 1	VERSUS 2	TRIAL	n	MEANS 1	2	SED	SIGNIFICANCE
Ground Diets (1, 3 & 7)	Pelleted Diets (2, 4 & 8)	A	24	146	143	3.8	NS
		B	24	152	151	4.8	NS
Basal Diet (1 and 2)	Basal Diet + Urea (7 and 8)	A	16	148	146	3.9	NS
		B	16	152	152	4.9	NS
Basal Diet (1 and 2)	Basal Diet + Protein (3 and 4)	A	16	148	141	3.9	NS
		B	16	152	151	4.9	NS
Basal Diet + Protein (3, 4 & 5)	Basal Diet + Urea (6, 7 & 8)	A	24	143	146	3.8	NS
		B	24	152	153	4.8	NS

as follows; 161.7, 61.1, 34, 37.2, 35.5 and 43.1 mg/100ml. It was assumed that this animal was fully recovered and it was included in the second metabolic trial.

3:1:4. Discussion

3:1:4:1. Pelleting

Pelleting produced consistent effects on calcium, magnesium, phosphorus, sodium and potassium although in many cases these effects were nonsignificant. Essentially, pelleting increased the digestibility of these minerals and the subsequent increases in mineral excretion and concentration observed were probably a reflection of this.

Various workers (Packett, et al., 1958; Crookshank, et al., 1965) have indicated that pelleting a diet known to produce calculi increased the incidence of urolithiasis amongst wether lambs. Slinger, Summers, Pepper, Bentley and Cisneros (1966) showed that the pelleting of diets for poultry produced significant increases in the bone ash of these birds, and increased phosphorus availability. Although Crookshank, et al., (1965) found that the phosphorus concentration of the urine excreted by wether lambs fed pelleted diets was increased they did not relate this finding to total urinary phosphorus excretion. The results presented in this thesis show that pelleting a diet consistently increased phosphorus digestibility in animals consuming the diet and that furthermore, urine concentration and excretion of phosphorus was increased. Data have been presented by several authors (Emerick, Embry and Olson, 1959; Packett and Hauschild, 1964; Bushman et al., 1965a and b) to indicate that urinary phosphorus level is probably the most important measurable factor predisposing the development of phosphatic calculi in store lambs. In view of this the relationship between pelleting a diet and the development of urinary calculi in lambs seems established.

Pelleting the diet produced nonsignificant increases in the magnesium measurements made in this study although Crookshank, <u>et al.</u>, (1965) showed that feeding wethers pelleted diets reduced the urinary magnesium concentration. Furthermore, these workers demonstrated a reduction in urine potassium concentration which is contrary to the findings reported in this thesis. The effects of pelleting demonstrated by these authors were seen only when diets known to induce calculi were pelleted. Pelleting diets unlikely to induce calculus formation in store lambs had no effect on the measurements made. In view of this, and the inability of Crookshank and his co-authors to present reproducible results, their findings in relation to the effect of pelleting diets on calculus formation remain open to question.

A study of the incidence of urinary calculi in humans (Modlin, 1967) has indicated that they occur most frequently in urine containing a low ratio of sodium to calcium. During Trial A, there was little difference in this ratio between animals fed ground (15.1:1) or pelleted diets (15.4:1). However, during trial B, a much lower ratio was obtained in the urine from animals fed pelleted diets (16.4:1) compared to those fed ground diets (20.7:1).

3:1:4:2. Nitrogen source and level

Replacement of cottonseed meal with urea as a nitrogen supplement has been shown to reduce the incidence of urolithiasis in wethers (Packett, <u>et al.</u>, 1958). Watanabe <u>et al.</u>, (1975) found no difference between urinary calcium and phosphorus levels of sheep fed either urea or soya protein supplements. Although both protein and urea supplements tended to reduce urinary concentration of minerals, phosphorus values obtained in this study for the urea-fed animals were generally lower than those obtained for the protein-fed lambs. Thus, calculus formation would seem to be less likely in urea-fed animals, in view of the lower urinary mineral levels induced, compared with animals fed diets containing conventional nitrogen sources. Furthermore, urea supplementation of the basal diet increased the urinary sodium:calcium ratio from 11.8:1 to 13.3:1 in trial A and from 13.5:1 to 17.2:1 in trial B. Comparison of the ratios in urine from both urea and protein supplemented lambs showed that the ratio was greater in those animals receiving additional protein although undoubtedly this would be a reflection of their greater sodium intake.

Price, Brown, Menvielle and Smith (1972) have shown that increasing the level of urea in purified diets for wether lambs, produced a significant linear increase in water intake by these lambs; this was reflected in an increased urine output. Although no statistical comparisons were made for water intake in the present study, it was obvious that there was no increase in water intake associated with dietary increments of urea. As a consequence of this, urine volumes were similar for lambs fed either the basal diet or the urea supplemented diets. Thus urea supplements did not increase the volume of urine voided by these lambs. Similarly, feeding urea to rats has been shown to cause a reduction in urine volume (Gamble, McKhann, Butler and Tuthill, 1934). Watanabe et al., (1975) considered that urea exerted a diuretic effect in sheep based on their findings that feeding a diet to sheep containing 2.25% urea produced a 31% increase in urine volume. They considered that this effect of urea was responsible for the prevention of urolithiasis during experiments conducted by other workers (Kawashmia, Saita, Niyama and Uesaka, 1973) using diets containing urea. The increased water intake and subsequent increase in urine output observed in sheep by Watanabe, et al., (1975) may have been a response to total nitrogen in the diet rather than urea per se as the urea diet contained 16.8% crude protein compared with the soya diet which only contained 14.1%.

High protein feeds are associated with higher water intakes in ruminants than low protein foods (Sykes 1955). This effect was apparent from the results reported in this thesis although the increases observed could have been due in part to the higher sodium content of these diets. Comparisons of isonitrogenous diets (Price, et al., 1972) in their effect on water intake by lambs has shown that those animals fed diets supplemented with soya protein consumed about 50% more water than those fed urea-supplemented diets. Furthermore, a similar comparison in the current study showed that water intake was much greater in those lambs fed protein rather than urea supplements. This effect cannot be explained on the basis of dietary sodium content as Orskov, et al., (1971) noted that even 2% sodium chloride in the diet did not significantly increase water intake. Possibly, this increased intake was a reflection of a greater metabolic demand for water associated with the increased nitrogen retention on the protein supplemented diets. Additionally, comparison of the volume of urine voided by lambs fed isonitrogenous diets in the current study showed that those animals fed urea supplemented diets excreted significantly less urine than those fed protein supplements. These results do not support the view that urea prevents calculus formation by a diuretic action. However, the results do show that both urea and protein supplements tend to reduce the concentration of those urinary minerals associated with the development of urolithiasis in lambs and therefore may be beneficial in the prevention of calculus formation.

The confusion in terminology and inability to precisely classify the mucoproteins of serum and urine makes comparison of results a procedure fraught with difficulty. Lockey, Anderson and MacLagen, (1956) found that serum and urine mucoprotein values were within normal limits in human cases of renal calculi: Cornelius, et al., (1959) showed that serum mucoprotein values were increased in wethers fed a diet known to induce calculus formation. In the current study, there was no measurable effect on serum protein bound hexose. Levels of dietary nitrogen had a distinct effect on urinary protein bound hexose concentration. It was reduced by additional nitrogen, although *in toto*, more was excreted, particularly by those animals fed protein supplements. This could be a reflection of increased tissue turnover rate induced by an increased growth rate (Udall, 1959b). However, this augmented excretion rate could not be related to calculus formation.

The lack of calculi cases during this experiment and the absence of both histological and histochemical changes in the kidneys indicated that the treatments employed may be considered as unlikely to induce calculus formation. However, a longer period of time may have resulted in the formation of calculi due to changes in mineral excretion patterns.

3:2. EXPERIMENT II

3:2:1. Introduction

It has been shown that increasing the calcium level of the diet reduces the incidence of urolithiasis amongst wether lambs averaging 29kg liveweight (Bushman, 1968). The same author demonstrated that increasing the level of dietary phosphorus had the opposite effect. The evidence relating to the effect of supplementary dietary magnesium on calculus formation is equivocal. Ørskov (1975) stresses that magnesium should not be given in excess to lambs whilst Beeson et al., (1943) found that diets rich in magnesium did not induce calculus formation in wether lambs within a period of 160 days. Bushman (1968) showed magnesium was more effective than calcium in reducing urinary phosphorus concentration and was thus likely to reduce the incidence of urolithiasis.

The current experiment was conducted (i) to investigate the effect of different dietary combinations of calcium, magnesium and phosphorus on calculus formation in early weaned lambs and (ii) to compare the results obtained with these lambs with published values obtained with store lambs.

In view of the results obtained in Experiment I, the experimental diets were ground and formulated to contain 160g crude protein per kg dry matter using soya as a protein source.

3:2:2. Materials and Methods

3:2:2:1. Plan of Investigation

This was essentially the same as described in 3:1:2:1. and in addition, renal tissue and bone were to be analysed.

3:2:2:2. Animals

The source, type of lamb and method of rearing were the same as that described in 3:1:2:2.

3:2:2:3. Diets

Diets were all-concentrate and ground through a 2mm screen. Raw materials used to formulate the diets were barley, extracted soya bean meal, calcined magnesite, limestone, calcium phosphate* (appendix 8:1:3:3.) and a combined vitamin/mineral intensive sheep supplement** (appendix 8:1:3:2.).

Treatment diets (appendix 8:1:5.) were formulated to the following specification:

Treatment Number		% air dry diet Calcium	Phosphorus	Magnesium
1	(Control diet)	0.4	0.4	0.2
2		0.4	0.75	0.2
3		0.4	0.4	0.4
4		0.8	0.4	0.2
5		0.8	0.4	0.4
6		0.4	0.75	0.4
7		0.8	0.75	0.2
8		0.8	0.75	0.4

The lower levels of calcium and phosphorus were based on ARC (1965) recommendations. The lower level of magnesium was set at more than twice the recommended level to ensure that any deleterious effects due to magnesium supplements would be obvious.

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* Ibex)
** Coopers Beta 102 T.E.) Cooper Nutrition Products Ltd., Stepfield, Witham.

3:2:2:4. Statistical Design

Sixtyfour lambs were used in a 2^3 factorially designed experiment involving two levels of calcium, two levels of magnesium and two levels of phosphorus. Animals were allocated to treatment in the manner described in 3:1:2:4. and the procedures replicated as described in that section.

3:2:2:5. Accommodation

This was identical to that described in 3:1:2:5.

3:2:2:6. Experimental Procedure

This was the same as that outlined in 3:1:2:6.

3:2:2:7. Sampling Procedures

This was the same as that outlined in 3:1:2:7. and in addition, during the third period of the experiment, bone samples were taken from each of the two animals fed treatment diets 1, 2, 6 and 7. The femur and humerus from the left side of each animal was dissected free of tissue, x-rayed and then placed in a 100°C oven for 1 week to dry. Each bone was taken, crushed with a hammer and returned to the oven for further drying.

3:2:2:8. Analytical Methods

3:2:2:8:1. Feed/Feed Residues/Faeces.

The methods used were the same as described in 3:1:2:8:1.

3:2:2:8:2. Urine

The same methods were used as described in 3:1:2:8:2.

3:2:2:8:3. Serum

The methods used were the same as described in 3:1:2:8:3.

3:2:2:8:4. Renal Tissue

The methods used were the same as described in 3:1:2:8:4 and in addition, bilateral samples of cortex and medulla were taken from animals at the end of the fourth period and dried in a 100°C oven for 48 hours and the dried samples treated as in 3:1:2:8:1. (ii) and (iii).

3:2:2:8:5. Calculous Material

The methods used were the same as described in 3:1:2:8:5. and in addition, the following quantitative techniques were employed.

(i) Minerals

(a) Chemical Methods

A known amount (preferably 1g) of the dried material was ashed in a muffle furnace at 550°C. After cooling in a desiccator, the ash was weighed and the proportion of inorganic material was calculated. The ash was treated in the same way as described in 3:1:2:8:1 (iii) and analysed in a similar fashion to determine calcium, magnesium and phosphorus content.

In many instances only small amounts of material were recovered for analytical purposes (< 500mg) and therefore chemical analysis was inappropriate. Furthermore, this form of analysis did not indicate the precise chemical form of the stone components and thus alternative semi-quantitative techniques were tried.

(b) Physical Methods

X-ray Crystallography

Powdered samples were mixed with a small amount of acetone and then pipetted onto a glass slide. The mixture was spread evenly over the slide. The acetone evaporated leaving a thin layer of sample spread over the slide. An x-ray generator set at 36kV and 20mA directed a monochromatic x-ray beam at the sample. The diffracted beam was measured using a scintillation counter mounted on a goniometer rotating at 2^{o} theta per minute. A discriminator unit was used to remove background radiation. A continuous chart record of the x-ray diffraction pattern was obtained from theta angles of 4^{o} to 48^{o}. Crystalline samples produce peaks at specific theta angle positions. Using a reference manual (United States Department of Commerce, 1950) the crystal lattice spacing may be obtained from the theta angle readings. Mineral constituents of calculi may be then identified by comparing the crystal lattice spacing values with those contained in the 'Index to the x-ray powder data file', (American Society for Testing Materials, (A.S.T.M.) 1959)).

X-ray fluorescence

Samples found to be amorphous and non-crystalline in nature were analysed by x-ray fluorescence. A disadvantage of the technique was that a minimum quantity of 100mg of dried calculous material was required. One part by weight of sample was mixed with one part of lanthanum oxide and eight parts of lithium tetraborate. The mixture was fused at 1000°C, the melt was crushed and briquetted into a disc. This disc was compared to others of known composition, prepared in the same way, using a Phillips automatic x-ray spectrometer, model PW 1212.

(ii) Organic Material

(a) Nitrogen

Nitrogen was determined as ammonia using Nessler's reagent by the method described by Vogel (1962) and directly as follows: 500mg of dried material were weighed into a small beaker. 10ml of 2M HCl was added and the mixture warmed to dissolve the calculous material. The solution was quantitatively transferred to a 50ml graduated flask and made up to the mark when cool. The contents of the flask were mixed and 10ml of the contents pipetted into a Markham distillation apparatus. Distillation was carried out for 15 minutes. The distillate was collected in a receiver containing 5ml of $0.1MH_2SO4$ containing a drop of methyl red indicator. The contents of the receiver were back-titrated with 0.04M NaOH. This procedure allowed the determination of nitrogen in 100mg of calculous material.

(b) Protein bound hexose

Dried, ground calculous material (5 to 20mg) was dissolved in 1ml of 0.1M NaOH and the same procedure was carried out as described in 3:1:2:8:2 (ii).

(c) Hydroxyproline

The Neuman and Logan method (1950) as modified by Leach (1960) was used to estimate hydroxyproline following hydrolysis of calculous material with HCl.

3:2:2:8:6. Bone

The dried, crushed remains of each bone were fat extracted for 24 hr using petroleum ether (boiling point 40°C - 60°C) in a Soxhlet apparatus. After fat extraction, the bone was ground into small pieces using a pestle and mortar and then ashed and analysed for minerals as outlined in 3:1:2:8:1 (ii) and (iii).

3:2:2:9. Statistical Analysis

Analysis of variance was performed using a Genstat* program at the E.R.C.C.** and missing values were calculated using the Rothamsted/Genstat method*. A sample analysis of variance calculation using urine volume data from metabolic trial A is shown in appendix 8:3.

* Statistics Department, Rothamsted Experimental Station, Harpenden, Hertfordshire.

** The Edinburgh Regional Computing Centre, The King's Buildings, Mayfield Road, Edinburgh, EH9 3JZ.

3:2:3. Results

There were considerable differences between metabolic trial A and trial B in the values of all the parameters measured with the exception of serum measurements.

3:2:3:1. Mineral Metabolism

3:2:3:1:1. Calcium

(i) Calcium level (Table 34)

When the diet containing the higher level of calcium was fed urinary excretion of calcium was reduced whilst digestibility, retention and urinary concentration were increased. These effects were significant except in trial A for urinary excretion and concentration of calcium and in trial B for calcium digestibility. In trial A serum calcium was significantly higher although in trial B it was lower in lambs fed the high calcium diet.

(ii) Magnesium level (Table 35)

When the diet containing the higher level of magnesium was fed calcium digestibility was significantly increased in trial A although values for the other measurements were reduced. Serum calcium was significantly reduced in both trials in lambs fed the diet containing the high level of magnesium.

(iii) Phosphorus level (Table 36)

The excretion and concentration of calcium in the urine was increased in lambs fed the high level of phosphorus. The effect was significant in trial B. Calcium digestibility was significantly reduced in lambs fed the high level of phosphorus in trial A and associated with this was a

TABLE 34

THE EFFECT OF TWO DIETARY LEVELS OF CALCIUM (0.4 and 0.8% of air dry diet) ON SOME ASPECTS OF CALCIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			0.4% CALCIUM	0.8% CALCIUM		
Digestibility (%)	A(a)	32	19.1	33.2	3.02	***
	B(b)	"	31.2	31.3	2.94	NS
Urinary Excretion (mg/14 days)	A	"	110	101	33.8	NS
	B	"	369	216	62.0	*
Retention (mg/14 days)	A(a)	"	5120	18230	1520	***
	B(b)	"	11600	23000	2130	***
Urinary Concentration (mg/100ml)	A	"	2.4	2.7	0.88	NS
	B	"	0.8	1.7	0.17	***
Serum Concentration (mg/100ml)	A	"	10.98	11.27	0.112	*
	B(b)	"	11.02	10.83	0.128	NS

(a) Two missing values
(b) Eleven missing values

TABLE 35

THE EFFECT OF TWO DIETARY LEVELS OF MAGNESIUM (0.2 and 0.4% of air dry diet) ON SOME ASPECTS OF CALCIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			0.2% MAGNESIUM	0.4% MAGNESIUM		
Digestibility (%)	A(a)	32	22.4	29.9	3.02	*
	B(b)	"	32.1	30.4	2.94	NS
Urinary Excretion (mg/14 days)	A	"	130	82	33.8	NS
	B	"	305	280	62.0	NS
Retention (mg/14 days)	A(a)	"	11760	11580	1520	NS
	B(b)	"	17900	16700	2130	NS
Urinary Concentration (mg/100ml)	A	"	3.1	2.0	0.88	NS
	B	"	1.3	1.3	0.17	NS
Serum Concentration (mg/100ml)	A	"	11.32	10.94	0.112	**
	B	"	11.23	10.62	0.128	***

(a) Two missing values
(b) Eleven missing values

TABLE 36

THE EFFECT OF TWO DIETARY LEVELS OF PHOSPHORUS (0.4 and 0.75% of air dry diet) ON SOME ASPECTS OF CALCIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			0.4% PHOSPHORUS	0.75% PHOSPHORUS		
Digestibility (%)	A(a)	32	31.4	20.9	3.02	**
	B(b)	"	30.5	32.0	2.94	NS
Urinary Excretion (mg/14 days)	A	"	83	128	33.8	NS
	B	"	178	406	62.0	***
Retention (mg/14 days)	A(a)	"	13710	9630	1520	**
	B(b)	"	16000	18500	2130	NS
Urinary Concentration (mg/100ml)	A	"	2.2	2.9	0.88	NS
	B	"	1.1	1.5	0.17	*
Serum Concentration (mg/100ml)	A	"	11.29	10.97	0.112	**
	B	"	11.12	10.73	0.128	**

(a) Two missing values
(b) Eleven missing values

significant reduction in calcium retention. Serum calcium was signficantly reduced in both trials in lambs fed the diet containing the high level of phosphorus.

(iv) Interactions

Digestibility

There was asignificant interaction between calcium and magnesium in trial A (Table 37a). Both calcium and magnesium at the higher dietary level increased calcium digestibility but when combined in the diet their effects were not additive. (Table 37b).

Table 37b

The effect of the interaction between dietary calcium and magnesium on mean calcium digestibility (%) measured in lambs during trial A.

		Magnesium (%)	
		0.2	0.4
Calcium (%)	0.4	11.8	26.4
	0.8	32.9	33.5

Urinary Excretion

There were no significant interactions (Table 38).

Retention

There were no significant interactions (Table 39).

Urinary Concentration

There was a significant interaction between magnesium and phosphorus in trial B (Table 40a). Both phosphorus and magnesium at the higher dietary level increased urinary calcium concentration but their combined effect was less than that produced by the high level of phosphorus. (Table 40b).

TABLE 37a

THE EXTENT OF INTERACTION BETWEEN MINERALS ON DIGESTIBILITY (%) OF CALCIUM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A(a)	32	-7.0	3.02	*
	B(b)	"	4.0	2.94	NS
Calcium/Phosphorus	A(a)	"	2.8	3.02	NS
	B(b)	"	-0.9	2.94	NS
Magnesium/Phosphorus	A(a)	"	5.1	3.02	NS
	B(b)	"	0.1	2.94	NS
Calcium/Magnesium/Phosphorus	A(a)	"	-2.8	3.02	NS
	B(b)	"	-1.8	2.94	NS

(a) Two Missing Values
(b) Eleven Missing Values

TABLE 38

THE EXTENT OF INTERACTION BETWEEN MINERALS ON URINARY EXCRETION (mg/14 days) OF CALCIUM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A	32	13	33.8	NS
	B	"	80	62.0	NS
Calcium/Phosphorus	A	"	8	33.8	NS
	B	"	111	62.0	NS
Magnesium/Phosphorus	A	"	11	33.8	NS
	B	"	67	62.0	NS
Calcium/Magnesium/Phosphorus	A	"	0	33.8	NS
	B	"	68	62.0	NS

TABLE 39

THE EXTENT OF INTERACTION BETWEEN MINERALS ON RETENTION (mg/14 days) OF CALCIUM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A (a)	32	2710	1520	NS
	B (b)	"	800	2130	NS
Calcium/Phosphorus	A (a)	"	2480	1520	NS
	B (b)	"	200	2130	NS
Magnesium/Phosphorus	A (a)	"	210	1520	NS
	B (b)	"	600	2130	NS
Calcium/Magnesium/Phosphorus	A (a)	"	-150	1520	NS
	B (b)	"	2330	2130	NS

(a) Two missing values
(b) Eleven missing values

TABLE 40a

THE EXTENT OF INTERACTION BETWEEN MINERALS ON URINARY CONCENTRATION (mg/100ml) OF CALCIUM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A	32	-1.08	0.88	NS
	B	"	-0.15	0.17	NS
Calcium/Phosphorus	A	"	0.84	0.88	NS
	B	"	0.28	0.17	NS
Magnesium/Phosphorus	A	"	0.30	0.88	NS
	B	"	0.42	0.17	*
Calcium/Magnesium/Phosphorus	A	"	-0.77	0.88	NS
	B	"	0.17	0.17	NS

Table 40b

The effect of the interaction between dietary magnesium and phosphorus on mean urinary calcium concentration (mg/100 ml) measured in lambs during trial B.

		Phosphorus (%)	
		0.4	0.75
Magnesium (%)	0.2	0.920	1.741
	0.4	1.319	1.301

Serum Concentration

There were significant interactions between calcium and magnesium in trial A and between magnesium and phosphorus in trial B. (Table 41a). In trial A the higher level of dietary calcium increased serum calcium and the higher level of dietary magnesium had the reverse effect. When combined in the diet at their upper levels these minerals interacted to reduce serum calcium.

In trial B, both magnesium and phosphorus at the higher dietary level reduced serum calcium although their combined effect was greater than either mineral on its own. (Table 41b).

Table 41b

The effect of the interactions between dietary calcium and magnesium in trial A and dietary magnesium and phosphorus in trial B on mean serum calcium concentration (mg/100ml) measured in lambs.

			Magnesium (%)	
			0.2	0.4
Trial A	Calcium(%)	0.4	11.066	10.913
		0.8	11.577	10.971
Trial B	Phosphorus(%)	0.4	11.566	10.688
		0.75	10.903	10.558

TABLE 41a

THE EXTENT OF INTERACTION BETWEEN MINERALS ON SERUM CONCENTRATION (mg/100ml) OF CALCIUM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A	32	0.23	0.113	*
	B(a)	"	0.11	0.128	NS
Calcium/Phosphorus	A	"	0.13	0.113	NS
	B(a)	"	0.01	0.128	NS
Magnesium/Phosphorus	A	"	-0.09	0.113	NS
	B(a)	"	-0.27	0.128	*
Calcium/Magnesium/Phosphorus	A	"	0.11	0.113	NS
	B(a)	"	-0.03	0.128	NS

(a) Eleven Missing Values

3:2:3:1:2. Phosphorus

(i) Calcium Level (Table 42)

Phosphorus digestibility was significantly reduced by the higher level of dietary calcium during trial B and urinary excretion of phosphorus was significantly reduced in both trials. Urinary phosphorus concentration was significantly reduced in trial A and retention was significantly increased in trial B by the higher level of dietary calcium.

(ii) Magnesium level (Table 43)

The higher dietary level of magnesium significantly reduced the urinary excretion of phosphorus in both trials and the urinary concentration in trial A.

(iii) Phosphorus Level (Table 44)

The higher dietary level of phosphorus significantly increased all the values measured with the exception of serum phosphorus in trial A.

(iv) Interactions

Digestibility

There were significant interactions between calcium and magnesium in trial A and between calcium, magnesium and phosphorus in trial B (Table 45a). Both calcium and magnesium at the higher dietary level increased phosphorus digestibility although their combined effect was less than either mineral on its own during trial A.

During trial B at the low level of dietary phosphorus, calcium or magnesium at their upper dietary levels were individually very effective in reducing phosphorus digestibility. When combined at the upper levels, these minerals were not so

TABLE 42

THE EFFECT OF TWO DIETARY LEVELS OF CALCIUM (O.4 and O.8% of air-dry diet) ON SOME ASPECTS OF PHOSPHORUS METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			O.4% CALCIUM	O.8% CALCIUM		
Digestibility (%)	A(a)	32	39.6	44.1	2.89	NS
	B(b)	"	46.2	38.6	2.93	*
Urinary Excretion (mg/14 days)	A(a)	"	5157	3171	775.4	*
	B(c)	"	12917	6141	1003.2	***
Retention (mg/14 days)	A(a)	"	9830	12230	1670	NS
	B(b)	"	10570	14480	1620	*
Urinary Concentration (mg/100ml)	A(a)	"	122.8	68.4	12.47	***
	B(c)	"	37.3	44.5	9.25	NS
Serum Concentration (mg/100ml)	A	"	9.17	9.27	0.292	NS
	B(b)	"	9.60	9.44	0.223	NS

(a) Two missing values
(b) Eleven missing values
(c) Eight missing values

TABLE 43

THE EFFECT OF TWO DIETARY LEVELS OF MAGNESIUM (0.2 and 0.4% of air-dry diet) ON SOME ASPECTS OF PHOSPHORUS METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			0.2% MAGNESIUM	0.4% MAGNESIUM		
Digestibility (%)	A(a)	32	41.2	42.5	2.89	NS
	B(b)	"	44.3	40.5	2.93	NS
Urinary Excretion (mg/14 days)	A(a)	"	4949	3379	775.4	*
	B(c)	"	11539	7520	1003.2	***
Retention (mg/14 day)	A(a)	"	11480	10570	1670	NS
	B(b)	"	11790	13260	1620	NS
Urinary Concentration (mg/100ml)	A(a)	"	111.6	79.6	12.47	*
	B(c)	"	44.4	37.4	9.25	NS
Serum Concentration (mg/100ml)	A	"	9.03	9.41	0,292	NS
	B(b)	"	9.33	9.71	0.223	NS

(a) Two missing values
(b) Eleven missing values
(c) Eight missing values

TABLE 44

THE EFFECT OF DIETARY LEVELS OF PHOSPHORUS (0.4 and 0.75% of air-dry diet) ON SOME ASPECTS OF PHOSPHORUS METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			0.4% PHOSPHORUS	0.75% PHOSPHORUS		
Digestibility (%)	A(a)	32	38.4	45.3	2.89	*
	B(b)	"	31.6	53.2	2.93	***
Urinary Excretion (mg/14 days)	A(a)	"	1498	6830	775.4	***
	B(c)	"	2506	16553	1003.2	***
Retention (mg/14 days)	A(a)	"	7390	14660	1670	***
	B(b)	"	7560	17490	1620	***
Urinary Concentration (mg/100ml)	A(a)	"	33.7	157.5	12.47	***
	B(c)	"	12.5	69.3	9.25	***
Serum Concentration (mg/100ml)	A	"	8.98	9.46	0.292	NS
	B(b)	"	9.16	9.88	0.223	**

(a) Two missing values
(b) Eleven missing values
(c) Eight missing values

TABLE 45a

THE EXTENT OF INTERACTION BETWEEN MINERALS ON DIGESTIBILITY (%) OF PHOSPHORUS IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A(a)	32	-6.6	2.89	*
	B(b)	"	2.1	2.93	NS
Calcium/Phosphorus	A(a)	"	-2.9	2.89	NS
	B(b)	"	-2.4	2.93	NS
Magnesium/Phosphorus	A(a)	"	-1.0	2.89	NS
	B(b)	"	-2.8	2.93	NS
Calcium/Magnesium/Phosphorus	A(a)	"	1.4	2.89	NS
	B(b)	"	8.5	2.93	**

(a) Two Missing Values
(b) Eleven Missing Values

effective. At the upper level of dietary phosphorus, neither magnesium nor calcium was very effective at their upper levels in reducing the digestibility of phosphorus. However, when these two minerals were combined at their upper dietary levels they were much more effective in reducing phosphorus digestibility (Table 45b).

Table 45b.

The effect of the interactions between dietary calcium and magnesium in trial A and dietary calcium, magnesium and phosphorus in trial B on mean phosphorus digestibility (%) measured in lambs.

Trial A

		Magnesium (%)	
		0.2	0.4
Calcium (%)	0.4	35.7	43.6
	0.8	46.7	41.5

Trial B

	Magnesium (%)	0.2		0.4	
	Phosphorus (%)	0.4	0.75	0.4	0.75
Calcium(%)	0.4	40.0	58.3	28.4	58.1
	0.8	24.3	54.7	33.8	41.7

Urinary Excretion

There were significant interactions between magnesium and phosphorus in trial A, between calcium and phosphorus and between calcium, magnesium and phosphorus in trial B (Table 46a). In trial A, both magnesium and phosphorus at the higher dietary level increased urinary phosphorus excretion although when combined the effect of phosphorus was much reduced.

TABLE 46a

THE EXTENT OF INTERACTION BETWEEN MINERALS ON URINARY EXCRETION (mg/14 days) OF PHOSPHORUS IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A(a)	32	1088	775.4	NS
	B(b)	"	832	1003.2	NS
Calcium/Phosphorus	A(a)	"	1541	775.4	NS
	B(b)	"	5455	1003.2	***
Magnesium/Phosphorus	A(a)	"	2006	775.4	*
	B(b)	"	1966	1003.2	NS
Calcium/Magnesium/Phosphorus	A(a)	"	1174	775.4	NS
	B(b)	"	3842	1003.2	***

(a) Two Missing Values
(b) Eight Missing Values

In trial B, the higher dietary level of calcium reduced phosphorus excretion whilst the higher dietary level of phosphorus had the reverse effect. When the higher dietary levels of these minerals were combined the effect of phosphorus was reduced by about half. In the second order interaction, both the upper dietary levels of calcium and magnesium independently reduced urinary phosphorus excretion although when these levels were combined they were not so effective in reducing excretion at the lower level of dietary phosphorus inclusion. However, at the upper level of dietary phosphorus, the reduction caused by the upper levels of magnesium or calcium acting independently was not as great as the reduction obtained when the upper levels of these minerals were combined (Table 46b).

Table 46b

The effect of interactions between dietary magnesium and phosphorus in trial A, and between calcium and phosphorus and calcium, magnesium and phosphorus in trial B on mean urinary phosphorus excretion (mg/14 days) measured in lambs.

			Phosphorus (%)	
			0.4	0.75
Trial A	Magnesium (%)	0.2	1280	8618
		0.4	1716	5041
Trial B	Calcium (%)	0.4	3167	22668
		0.8	1845	10437

	Magnesium (%)	0.2		0.4	
	Phosphorus (%)	0.4	0.75	0.4	0.75
Calcium (%)	0.4	5699	23323	635	22012
	0.8	1366	15767	2323	5108

Retention

The interaction between calcium, magnesium and phosphorus in trial B was significant (Table 47a). Both magnesium and calcium at the higher dietary level reduced phosphorus retention at the lower level of dietary phosphorus although when combined phosphorus retention was increased. At the higher level of dietary phosphorus the upper levels of magnesium and calcium separately and together increased phosphorus retention (Table 47b).

Table 47b

The effect of interactions between dietary calcium, magnesium and phosphorus on mean phosphorus retention (mg/14 days) measured in lambs during trial B.

	Magnesium (%)	0.2		0.4	
	Phosphorus (%)	0.4	0.75	0.4	0.75
Calcium (%)	0.4	7800	11590	5750	17140
	0.8	5980	21800	10690	19450

Urinary Concentration

There were significant interactions between calcium and phosphorus and between magnesium and phosphorus in trial A (Table 48a). Both upper dietary levels of calcium and magnesium reduced the effect of high dietary phosphorus on urinary phosphorus concentration (Table 48b).

TABLE 47a

THE EXTENT OF INTERACTION BETWEEN MINERALS ON RETENTION (mg/14 days) OF PHOSPHORUS IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A(a)	32	1770	1670	NS
	B(b)	"	280	1620	NS
Calcium/Phosphorus	A(a)	"	1190	1670	NS
	B(b)	"	-2350	1620	NS
Magnesium/Phosphorus	A(a)	"	1640	1670	NS
	B(b)	"	- 140	1620	NS
Calcium/Magnesium/Phosphorus	A(a)	"	1500	1670	NS
	B(b)	"	3670	1620	*

(a) Two Missing Values
(b) Eleven Missing Values

TABLE 48a

THE EXTENT OF INTERACTIONS BETWEEN MINERALS ON URINARY CONCENTRATION (mg/100ml) OF PHOSPHORUS IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A(a)	32	4.75	12.47	NS
	B(b)	"	5.30	9.25	NS
Calcium/Phosphorus	A(a)	"	52.55	12.47	***
	B(b)	"	-6.40	9.25	NS
Magnesium/Phosphorus	A(a)	"	30.05	12.47	*
	B(b)	"	-0.60	9.25	NS
Calcium/Magnesium/Phosphorus	A(a)	"	8.90	12.47	NS
	B(b)	"	9.85	9.25	NS

(a) Two Missing Values
(b) Eleven Missing Values

Table 48b

The effect of interactions between dietary calcium and phosphorus and between magnesium and phosphorus on urinary phosphorus concentration (mg/100ml) measured in lambs during trial A.

		Phosphorus (%)	
		0.4	0.75
Calcium (%)	0.4	34.6	211.0
	0.8	32.7	104.0
Magnesium (%)	0.2	34.7	188.6
	0.4	32.7	126.5

Serum Concentration

There were no significant interactions (Table 49).

3:2:3:1:3. Magnesium

(i) Calcium Level (Table 50)

The higher dietary level of calcium significantly increased the retention and urinary concentration of magnesium during both trials. The urinary excretion of magnesium was significantly reduced in trial B by high dietary calcium. Magnesium digestibility was increased in both trials by high levels of dietary calcium but the effect was only significant in trial A.

(ii) Magnesium Level (Table 51)

The higher dietary level of magnesium significantly increased magnesium retention and serum concentration during both trials and urinary excretion and concentration in trial B.

(iii) Phosphorus Level (Table 52)

The higher dietary level of phosphorus significantly reduced urinary excretion and concentration of magnesium during both trials. Magnesium digestibility was significantly

TABLE 49

THE EXTENT OF INTERACTION BETWEEN MINERALS ON SERUM CONCENTRATION (mg/100ml) OF PHOSPHORUS IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A	32	-0.26	0.292	NS
	B(b)	"	-0.27	0.223	NS
Calcium/Phosphorus	A	"	-0.46	0.292	NS
	B(b)	"	-0.38	0.223	NS
Magnesium/Phosphorus	A	"	0.32	0.292	NS
	B(b)	"	-0.04	0.223	NS
Calcium/Magnesium/ Phosphorus	A	"	0.21	0.292	NS
	B(b)	"	-0.34	0.223	NS

(b) Eleven missing values

TABLE 50

THE EFFECT OF TWO DIETARY LEVELS OF CALCIUM (0.4 and 0.8% of air-dry diet) ON SOME ASPECTS OF MAGNESIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			0.4 % CALCIUM	0.8% CALCIUM		
Digestibility (%)	A(a)	32	39.0	50.4	2.07	***
	B(b)	"	43.2	46.8	2.42	NS
Urinary Excretion (mg/14 days)	A	"	3624	3635	365.8	NS
	B	"	8629	6146	775.6	**
Retention (mg/14 days)	A(a)	"	4100	6880	830	**
	B(b)	"	2840	7880	980	***
Urinary Concentration (mg/100 ml)	A	"	85.0	110.5	10.52	*
	B	"	24.7	60.9	5.95	***
Serum Concentration (mg/100 ml)	A	"	3.79	3.60	0.154	NS
	B(b)	"	3.85	3.82	0.123	NS

(a) Two missing values
(b) Eleven missing values

TABLE 51

THE EFFECT OF TWO DIETARY LEVELS OF MAGNESIUM (0.2 and 0.4% of air-dry diet) ON SOME ASPECTS OF MAGNESIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			0.2% MAGNESIUM	0.4% MAGNESIUM		
Digestibility (%)	A(a)	32	43.2	46.2	2.07	NS
	B(b)	"	46.2	43.8	2.42	NS
Urinary Excretion (mg/14 days)	A	"	3331	3928	365.8	NS
	B	"	6443	8332	775.6	*
Retention (mg/14 days)	A(a)	"	3250	7730	830	***
	B(b)	"	2510	8210	980	***
Urinary Concentration (mg/100ml)	A	"	89.1	106.3	10.52	NS
	B	"	32.8	52.8	5.95	**
Serum Concentration (mg/100ml)	A	"	3.30	4.09	0.154	***
	B(b)	"	3.50	4.16	0.123	***

(a) Two missing values
(b) Eleven missing values

TABLE 52

THE EFFECT OF TWO DIETARY LEVELS OF PHOSPHORUS (0.4 and 0.75% of air-dry diet) ON SOME ASPECTS OF MAGNESIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			0.4% PHOSPHORUS	0.75% PHOSPHORUS		
Digestibility (%)	A(a)	32	46.1	43.3	2.07	NS
	B(b)	"	42.0	48.0	2.42	*
Urinary Excretion (mg/14 days)	A	"	4395	2865	365.8	***
	B	"	8237	6537	775.6	*
Retention (mg/14 days)	A(a)	"	5460	5520	830	NS
	B(b)	"	4380	6340	980	NS
Urinary Concentration (mg/100ml)	A	"	130.3	65.1	10.52	***
	B	"	56.4	29.3	5.95	***
Serum Concentration (mg/100ml)	A	"	3.73	3.66	0.154	NS
	B(b)	"	3.96	3.71	0.123	NS

(a) Two missing values
(b) Eleven missing values

increased in trial B by the higher level of dietary phosphorus.

(iv) Interactions

Digestibility

There was a significant interaction between calcium and magnesium in both metabolic trials (Table 53a). In trials A and B the higher dietary level of magnesium reduced digestibility of magnesium whereas the higher dietary level of calcium increased it in trial A and reduced it in trial B. However, in both trials the combined effect of the higher levels of calcium and magnesium was to increase magnesium digestibility (Table 53b).

Table 53b

The effect of the interaction between dietary calcium and magnesium on magnesium digestibility (%) measured in lambs during trials A and B.

			Magnesium (%)	
			0.2	0.4
Trial A	Calcium(%)	0.4	40.4	37.6
		0.8	46.1	54.8
Trial B	Calcium(%)	0.4	48.1	38.2
		0.8	44.3	49.3

Urinary Excretion

There was a significant interaction between magnesium and phosphorus in trial A (Table 54a). The high dietary level of magnesium increased urinary excretion of magnesium whilst the diet high in phosphorus reduced magnesium excretion. Combined in the diet at their upper levels these

TABLE 53a

THE EFFECT OF INTERACTION BETWEEN MINERALS ON DIGESTIBILITY (%) OF MAGNESIUM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A(a)	32	5.8	2.07	**
	B(b)	"	7.5	2.42	**
Calcium/Phosphorus	A(a)	"	-0.9	2.07	NS
	B(b)	"	1.7	2.42	NS
Magnesium/Phosphorus	A(a)	"	-0.2	2.07	NS
	B(b)	"	2.2	2.42	NS
Calcium/Magnesium/ Phosphorus	A(a)	"	-2.3	2.07	NS
	B(b)	"	2.4	2.42	NS

(a) Two missing values
(b) Eleven missing values

TABLE 54a

THE EXTENT OF INTERACTION BETWEEN MINERALS ON URINARY EXCRETION (mg/14 days) OF MAGNESIUM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A	32	159	365.8	NS
	B	"	161	775.6	NS
Calcium/Phosphorus	A	"	175	365.8	NS
	B	"	1330	775.6	NS
Magnesium/Phosphorus	A	"	773	365.8	*
	B	"	1314	775.6	NS
Calcium/Magnesium/ Phosphorus	A	"	280	365.8	NS
	B	"	952	775.6	NS

minerals served to reduce urinary magnesium excretion below that produced by the phosphorus alone (Table 54b).

Table 54b

The effect of the interaction between dietary magnesium and phosphorus on urinary excretion of magnesium (mg/14 days) measured in lambs during trial A.

		Phosphorus(%)	
		0.4	0.75
Magnesium(%)	0.2	3730	2933
	0.4	5059	2797

Retention

There were significant interactions between calcium and magnesium and between calcium, magnesium and phosphorus in trial B (Table 55a). Both calcium and magnesium at the higher dietary level increased the retention of magnesium and their combined effect was greater than the sum of their individual effects. These effects were repeated in the second order interaction at both dietary levels of phosphorus. At the high dietary level of phosphorus the individual effects of high dietary calcium and magnesium on magnesium retention were more marked and although the value obtained for the combined effect was similar to that obtained at the low level of dietary phosphorus it was not greater than the sum of the individual effects. (Table 55b).

TABLE 55a

THE EXTENT OF INTERACTION BETWEEN MINERALS ON RETENTION (mg/14 days) OF MAGNESIUM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A(a)	32	-1450	830	NS
	B(b)	"	-2270	980	*
Calcium/Phosphorus	A(a)	"	1030	830	NS
	B(b)	"	170	980	NS
Magnesium/Phosphorus	A(a)	"	950	830	NS
	B(b)	"	-330	980	NS
Calcium/Magnesium/ Phosphorus	A(a)	"	340	830	NS
	B(b)	"	2090	980	*

(a) Two missing values
(b) Eleven missing values

Table 55b

The effect of interactions between dietary magnesium and calcium and between magnesium, phosphorus and calcium on magnesium retention (mg/14 days) measured in lambs during trial B.

		Magnesium(%)	
		0.2	0.4
Calcium (%)	0.4	1120	4560
	0.8	3890	11870

	Magnesium(%)	0.2		0.4	
	Phosphorus(%)	0.4	0.75	0.4	0.75
Calcium(%)	0.4	1270	980	2280	6840
	0.8	2110	5670	11860	11880

Urinary Concentration

There was a significant interaction between calcium and phosphorus during both trials (Table 56a). Urinary magnesium concentration was increased in both trials by the high dietary level of calcium and reduced by high dietary phosphorus. When calcium and phosphorus were combined at the higher dietary levels urinary magnesium concentration was reduced to a value below that produced by phosphorus alone in trial A although in trial B the effect was not so marked. (Table 56b).

TABLE 56a

THE EXTENT OF INTERACTION BETWEEN MINERALS ON URINARY CONCENTRATION (mg/100ml) OF MAGNESIUM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A	32	-14.15	10.52	NS
	B	"	- 9.95	5.95	NS
Calcium/Phosphorus	A	"	37.00	10.52	***
	B	"	18.90	5.95	**
Magnesium/Phosphorus	A	"	17.35	10.52	NS
	B	"	6.25	5.95	NS
Calcium/Magnesium/ Phosphorus	A	"	8.78	10.52	NS
	B	"	3.93	5.95	NS

Table 56b

The effect of the interaction between dietary calcium and phosphorus on the urinary concentration of magnesium (mg/ 100 ml) of lambs measured during trials A and B.

			Phosphorus (%)	
			0.4	0.75
Trial A	Calcium (%)	0.4	99.1	70.8
		0.8	161.6	59.3
Trial B	Calcium (%)	0.4	28.8	20.6
		0.8	83.9	37.9

Serum Concentration

There were no significant interactions (Table 57).

3:2:3:1:4. Sodium

(i) Calcium Level (Table 58)

The higher dietary level of calcium significantly increased sodium retention and urinary concentration in both trials and urinary excretion and serum sodium concentration in trial B. Sodium digestibility was significantly reduced by the high dietary level of calcium in trial B.

(ii) Magnesium Level (Table 59)

The high dietary magnesium level increased the value of all the measurements made. Significant changes occurred in sodium retention in both trials, in digestibility (trial A only) and in urinary excretion (trial B only).

(iii) Phosphorus Level (Table 60)

Sodium retention was significantly reduced in trial B by the high dietary level of phosphorus.

TABLE 57

THE EXTENT OF INTERACTION BETWEEN MINERALS ON SERUM CONCENTRATION (mg/100ml) OF MAGNESIUM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A	32	-0.06	0.154	NS
	B(b)	"	-0.18	0.123	NS
Calcium/Phosphorus	A	"	-0.29	0.154	NS
	B(b)	"	-0.12	0.123	NS
Magnesium/Phosphorus	A	"	-0.01	0.154	NS
	B(b)	"	-0.04	0.123	NS
Calcium/Magnesium/ Phosphorus	A	"	-0.08	0.154	NS
	B(b)	"	-0.10	0.123	NS

(b) Eleven missing values

TABLE 58

THE EFFECT OF TWO DIETARY LEVELS OF CALCIUM (0.4 and 0.8% of air-dry diet) ON SOME ASPECTS OF SODIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			0.4% CALCIUM	0.8% CALCIUM		
Digestibility (%)	A(c)	32	69.3	70.0	2.19	NS
	B(d)	"	74.9	69.9	1.99	*
Urinary Excretion (mg/14 days)	A	"	193	285	63.6	NS
	B	"	257	504	91.6	**
Retention (mg/14 days)	A(a)	"	550	1230	90	***
	B(b)	"	800	2070	110	***
Urinary Concentration (mg/100ml)	A	"	4.3	7.5	1.25	*
	B	"	0.5	6.0	1.59	***
Serum Concentration (mg/100ml)	A	"	326.5	329.6	1.99	NS
	B(b)	"	320.6	329.5	2.48	***

(a) Two missing values
(b) Eleven missing values
(c) Four missing values
(d) Twelve missing values

TABLE 59

THE EFFECT OF TWO DIETARY LEVELS OF MAGNESIUM (0.2 and 0.4% of air-dry diet) ON SOME ASPECTS OF SODIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			0.2% MAGNESIUM	0.4% MAGNESIUM		
Digestibility (%)	A(c)	32	66.8	72.5	2.19	*
	B(d)	"	70.9	73.9	1.99	NS
Urinary Excretion (mg/14 days)	A	"	205	273	63.6	NS
	B	"	268	493	91.6	*
Retention (mg/14 days)	A(a)	"	700	1080	90	***
	B(b)	"	1040	1830	110	***
Urinary Concentration (mg/100 ml)	A	"	4.7	7.0	1.25	NS
	B	"	1.7	4.8	1.59	NS
Serum Concentration (mg/100ml)	A	"	326.7	329.4	1.99	NS
	B(b)	"	323.9	326.2	2.48	NS

(a) Two missing values
(b) Eleven missing values
(c) Four missing values
(d) Twelve missing values

TABLE 60

THE EFFECT OF TWO DIETARY LEVELS OF PHOSPHORUS (0.4 and 0.75% of air-dry diet) ON SOME ASPECTS OF SODIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			0.4% PHOSPHORUS	0.75% PHOSPHORUS		
Digestibility (%)	A(c)	32	69.1	70.2	2.19	NS
	B(d)	"	71.0	73.8	1.99	NS
Urinary Excretion (mg/14 days)	A	"	205	273	63.6	NS
	B	"	340	422	91.6	NS
Retention (mg/14 days)	A(a)	"	890	890	90	NS
	B(b)	"	1560	1310	110	*
Urinary Concentration (mg/100ml)	A	"	5.2	6.5	1.25	NS
	B	"	3.3	3.2	1.59	NS
Serum Concentration (mg/100ml)	A	"	328.2	328.0	1.99	NS
	B(b)	"	324.2	325.8	2.48	NS

(a) Two missing values
(b) Eleven missing values
(c) Four missing values
(d) Twelve missing values

(iv) Interactions

Digestibility

There were significant interactions between calcium and magnesium in trial B, between calcium and phosphorus and between magnesium and phosphorus during both trials (Table 61a). Both calcium and phosphorus at the high dietary level reduced sodium digestibility in each trial. Their combined effect in trial A was to increase digestibility and in trial B to slightly reduce digestibility of sodium.

Both magnesium and phosphorus at the higher dietary level increased sodium digestibility in both trials although their combined effect was not as great as that exerted by the diet containing the high level of magnesium.

Both magnesium and calcium at their high dietary levels reduced sodium digestibility although when combined there was little reduction in digestibility. (Table 61b).

Table 61b

The effect of the interactions between dietary calcium and phosphorus, between magnesium and phosphorus and between magnesium and calcium on sodium digestibility (%) measured in lambs during trials A and B.

			Phosphorus (%)	
			0.4	0.75
Trial A	Calcium(%)	0.4	71.9	66.7
		0.8	66.4	73.7
Trial B	Calcium(%)	0.4	75.9	73.8
		0.8	66.0	73.8
Trial A	Magnesium(%)	0.2	63.7	70.0
		0.4	74.6	70.5
Trial B	Magnesium(%)	0.2	66.8	74.9
		0.4	75.1	72.6

TABLE 61a

THE EXTENT OF INTERACTION BETWEEN MINERALS ON DIGESTIBILITY (%) OF SODIUM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A(c)	32	4.0	2.19	NS
	B(d)	"	6.5	1.99	**
Calcium/Phosphorus	A(c)	"	6.3	2.19	**
	B(d)	"	5.0	1.99	*
Magnesium/Phosphorus	A(c)	"	-5.2	2.19	*
	B(d)	"	-5.4	1.99	*
Calcium/Magnesium/ Phosphorus	A(c)	"	1.7	2.19	NS
	B(d)	"	3.6	1.99	NS

(c) Four missing values
(d) Twelve missing values

			Magnesium (%)	
			0.2	0.4
Trial B	Calcium(%)	0.4	76.6	73.1
		0.8	65.2	74.6

Urinary Excretion

There were significant interactions between magnesium and phosphorus and between calcium, magnesium and phosphorus during trial B (Table 62a).

Both phosphorus and magnesium at the high dietary level increased sodium excretion and when combined the effect was intermediate between their individual effects.

The high dietary level of calcium reduced sodium excretion at the low level of phosphorus whilst the high level of magnesium increased excretion. Calcium and magnesium combined at the high level of dietary inclusion produced a considerable increase in urinary sodium excretion at the low level of dietary phosphorus. At the upper level of phosphorus inclusion in the diet the higher levels of calcium and magnesium increased the excretion of sodium although their combined effect was less than either of their individual effects. (Table 62b).

TABLE 62a

THE EXTENT OF INTERACTION BETWEEN MINERALS ON URINARY EXCRETION (mg/14 days) OF SODIUM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A	32	-24	63.6	NS
	B	"	69	91.6	NS
Calcium/Phosphorus	A	"	-48	63.6	NS
	B	"	70	91.6	NS
Magnesium/Phosphorus	A	"	25	63.6	NS
	B	"	191	91.6	*
Calcium/Magnesium/ Phosphorus	A	"	6	63.6	NS
	B	"	272	91.6	***

Table 62b

The effect of interactions between dietary magnesium and phosphorus and between calcium, magnesium and phosphorus in the urinary excretion of sodium (mg/14 days) by lambs during trial B.

		Phosphorus (%)	
		0.4	0.75
Magnesium (%)	0.2	132	405
	0.4	548	439

	Magnesium (%)	0.2		0.4	
	Phosphorus(%)	0.4	0.75	0.4	0.75
Calcium (%)	0.4	143	215	219	452
	0.8	120	595	876	425

Retention

There were significant interactions between calcium and magnesium in both trials, between magnesium and phosphorus in both trials and between calcium, magnesium and phosphorus in trial B only (Table 63a). The high dietary level of calcium increased sodium retention whilst the high dietary level of magnesium had the opposite effect. In combination, high dietary magnesium and calcium produced a marked increase in sodium retention.

Both magnesium and phosphorus at high levels in the diet increased retention of sodium although when combined at the higher level these minerals produced an increased retention which was less than that produced by magnesium alone.

Both magnesium and calcium at higher levels in the diet reduced sodium retention by lambs fed low levels of phosphorus

TABLE 63a

THE EXTENT OF INTERACTION BETWEEN MINERALS ON RETENTION (mg/14 days) OF SODIUM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A(a)	32	-520	90	***
	B(b)	"	-1130	110	***
Calcium/Phosphorus	A(a)	"	-150	90	NS
	B(b)	"	-150	110	NS
Magnesium/Phosphorus	A(a)	"	290	90	**
	B(b)	"	630	110	***
Calcium/Magnesium/ Phosphorus	A(a)	"	140	90	NS
	B(b)	"	330	110	**

(a) Two missing values
(b) Eleven missing values

in the diet. When the higher levels of calcium and magnesium were combined at this dietary phosphorus level there was a marked increase in sodium retention. At the higher dietary phosphorus level retention of sodium was reduced and this was much more marked in the presence of the higher level of magnesium. The higher level of calcium combined with that of phosphorus had the opposite effect. The combination of high levels of the three minerals increased sodium retention but not as much as the combination of high magnesium and calcium. (Table 63b).

Table 63b

The effect of the interactions between dietary magnesium and calcium, between phosphorus and magnesium and between calcium, magnesium and phosphorus on sodium retention (mg/14 days) by lambs during trials A and B.

			Magnesium (%)	
			0.2	0.4
Trial A	Calcium (%)	0.4	627	488
		0.8	782	1688
Trial B	Calcium (%)	0.4	965	635
		0.8	1118	3039
Trial A	Phosphorus(%)	0.4	556	1237
		0.75	853	938
Trial B	Phosphorus(%)	0.4	855	2280
		0.75	1228	1394

Trial B	Magnesium(%)	0.2		0.4	
	Phosphorus(%)	0.4	0.75	0.4	0.75
Calcium(%)	0.4	1016	914	989	282
	0.8	694	1542	3571	2507

Urinary Concentration

There were no significant interactions (Table 64).

TABLE 64

THE EXTENT OF INTERACTION BETWEEN MINERALS ON URINARY CONCENTRATION (mg/100ml) OF SODIUM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A	32	-1.75	1.25	NS
	B	"	-2.96	1.59	NS
Calcium/Phosphorus	A	"	-0.76	1.25	NS
	B	"	0.11	1.59	NS
Magnesium/Phosphorus	A	"	0.02	1.25	NS
	B	"	1.96	1.59	NS
Calcium/Magnesium/ Phosphorus	A	"	-0.79	1.25	NS
	B	"	1.99	1.59	NS

Serum Concentration

The second order interaction in trial B was significant (Table 65a). Both calcium and magnesium at the higher dietary level increased serum sodium at the low level of dietary phosphorus and their combined effect produced a greater increase than either of the individual effects. At the higher level of phosphorus in the diet, serum sodium was increased. A further increase was obtained by adding calcium to achieve the higher dietary rate (0.8%). However the higher level of magnesium caused a reduction in serum sodium although in combination with the high dietary level of calcium serum sodium was increased. (Table 65b).

Table 65b

The effect of the interaction between dietary calcium, phosphorus and magnesium on serum sodium concentration (mg/100ml) in lambs during trial B.

	Magnesium (%)	0.2		0.4	
	Phosphorus (%)	0.4	0.75	0.4	0.75
Calcium (%)	0.4	318.4	324.9	323.9	315.2
	0.8	326.6	325.7	328.1	337.4

3:2:3:1:5. Potassium

(i) Calcium Level (Table 66)

The higher dietary level of calcium significantly increased urinary potassium concentration in both trials and retention and digestibility of potassium in trial A only.

(ii) Magnesium Level (Table 67)

Retention and digestibility of potassium were significantly increased in trial A and B respectively by the high

TABLE 65a

THE EXTENT OF INTERACTION BETWEEN MINERALS ON SERUM CONCENTRATION (mg/100ml) OF SODIUM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A	32	1.37	1.99	NS
	B(b)	"	-4.40	2.48	NS
Calcium/Phosphorus	A	"	-0.45	1.99	NS
	B(b)	"	-2.70	2.48	NS
Magnesium/Phosphorus	A	"	-2.66	1.99	NS
	B(b)	"	1.25	2.48	NS
Calcium/Magnesium/ Phosphorus	A	"	-2.11	1.99	NS
	B(b)	"	-6.35	2.48	*

(b) Eleven missing values

TABLE 66

THE EFFECT OF TWO DIETARY LEVELS OF CALCIUM (0.4 and 0.8% of air-dry diet) ON SOME ASPECTS OF POTASSIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			0.4% CALCIUM	0.8% CALCIUM		
Digestibility (%)	A(a)	32	71.0	80.4	3.61	*
	B(b)	"	81.6	83.5	2.12	NS
Urinary Excretion (mg/ 14 days)	A	"	14436	15939	1621.8	NS
	B	"	32507	32218	2154.1	NS
Retention (mg/ 14 days)	A(a)	"	8000	12460	1970	*
	B(b)	"	2400	5600	2440	NS
Urinary Concentration (mg/100ml)	A	"	348	501	43.9	***
	B	"	119	299	28.1	***
Serum Concentration (mg/100ml)	A	"	20.22	20.85	0.464	NS
	B(b)	"	20.40	20.37	0.569	NS

(a) Two missing values
(b) Eleven missing values

TABLE 67

THE EFFECT OF TWO DIETARY LEVELS OF MAGNESIUM (0.2 and 0.4% of air-dry diet) ON SOME ASPECTS OF POTASSIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			0.2% MAGNESIUM	0.4% MAGNESIUM		
Digestibility (%)	A(a)	32	70.4	81.0	3.61	**
	B(b)	"	80.9	84.1	2.12	NS
Urinary Excretion (mg/14 days)	A	"	14539	15836	1621.8	NS
	B	"	34241	30484	2154.1	NS
Retention (mg/14 days)	A(a)	"	9500	10950	1970	NS
	B(b)	"	-200	8200	2440	**
Urinary Concentration (mg/100ml)	A	"	413	435	43.9	NS
	B	"	209	209	28.1	NS
Serum Concentration (mg/100ml)	A	"	20.91	20.16	0.464	NS
	B(b)	"	21.06	19.71	0.569	*

(a) Two missing values
(b) Eleven missing values

dietary level of magnesium although serum concentration was significantly reduced in trial B.

(iii) Phosphorus Level (Table 68)

The high dietary level of phosphorus significantly reduced the digestibility, urinary excretion and concentration of potassium during both trials.

(iv) Interactions

Digestibility

There was a significant interaction between calcium and magnesium during both trials (Table 69a). Both calcium and magnesium at their higher levels increased potassium digestibility. Their combined effect in trial A was greater than either of their individual effects although in trial B it was less. (Table 69b).

Table 69b

The effect of the interaction between dietary calcium and magnesium on potassium digestibility (%) in lambs during trials A and B.

			Magnesium (%)	
			0.2	0.4
Trial A	Calcium(%)	0.4	61.6	80.5
		0.8	79.2	81.6
Trial B	Calcium(%)	0.4	76.9	86.3
		0.8	85.0	82.0

Urinary Excretion

There were significant interactions between calcium and magnesium in both trials, and in trial B between calcium and phosphorus, magnesium and phosphorus and between calcium, magnesium and phosphorus (Table 70a).

TABLE 68

THE EFFECT OF TWO DIETARY LEVELS OF PHOSPHORUS (0.4 and 0.75% of air-dry diet) ON SOME ASPECTS OF POTASSIUM METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

MEASUREMENTS	TRIAL	n	MEANS		SED	SIGNIFICANCE
			0.4% PHOSPHORUS	0.75% PHOSPHORUS		
Digestibility (%)	A(a)	32	84.8	66.7	3.61	***
	B(b)	"	88.1	77.0	2.12	***
Urinary Excretion (mg/14 days)	A	"	19055	11320	1621.8	***
	B	"	36661	28065	2154.1	***
Retention (mg/14 days)	A(a)	"	9830	10630	1970	NS
	B(b)	"	3700	4400	2440	NS
Urinary Concentration (mg/100ml)	A	"	577	272	43.9	***
	B	"	250	168	28.1	**
Serum Concentration (mg/100ml)	A	"	20.89	20.18	0.464	NS
	B(b)	"	20.70	20.07	0.569	NS

(a) Two missing values
(b) Eleven missing values

TABLE 69a

THE EXTENT OF INTERACTION BETWEEN MINERALS ON DIGESTIBILITY OF POTASSIUM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A(a)	32	-8.3	3.61	*
	B(b)	"	-6.1	2.12	**
Calcium/Phosphorus	A(a)	"	4.1	3.61	NS
	B(b)	"	0.4	2.12	NS
Magnesium/Phosphorus	A(a)	"	-3.6	3.61	NS
	B(b)	"	-1.3	2.12	NS
Calcium/Magnesium/ Phosphorus	A(a)	"	4.1	3.61	NS
	B(b)	"	3.6	2.12	NS

(a) Two missing values
(b) Eleven missing values

TABLE 70a

THE EXTENT OF INTERACTION BETWEEN MINERALS ON URINARY EXCRETION (mg/14 days) OF POTASSIUM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A	32	4029	1.62	*
	B	"	5721	2.15	**
Calcium/Phosphorus	A	"	1290	1.62	NS
	B	"	-7363	2.15	**
Magnesium/Phosphorus	A	"	1572	1.62	NS
	B	"	5501	2.15	*
Calcium/Magnesium/ Phosphorus	A	"	3086	1.62	NS
	B	"	6094	2.15	**

When fed separately, both the higher dietary levels of calcium and magnesium increased the urinary excretion of potassium but when they were combined the effect in trial A was less than the individual effects and in trial B excretion was less than when the minerals were fed at the lower level.

The upper level of dietary phosphorus reduced potassium excretion whereas magnesium increased urinary losses. The combined effect of the upper levels of these two minerals was to reduce excretion to a value less than that produced by the minerals at their lower levels.

Both calcium and phosphorus at their upper dietary levels reduced urinary potassium excretion although their combined effect was similar to that produced by calcium alone.

In the second order interaction calcium and phosphorus interacted in a similar manner to that seen in the first order interaction. At the upper level of magnesium the excretion of potassium was greater when the other two minerals were present at their low levels although when combined at their upper level they effectively halved the excretion of potassium. (Table 70b).

Table 70b

The effect of interactions between dietary magnesium and calcium, phosphorus and calcium, phosphorus and magnesium and between magnesium, phosphorus and calcium on the urinary excretion of potassium (mg/14 days) by lambs during trials A and B.

		Magnesium (%)	
		0.2	0.4
Trial A Calcium(%)	0.4	11773	17099
	0.8	17305	14573
Trial B Calcium (%)	0.4	31525	33489
	0.8	36957	27479
Trial B Phosphorus (%)	0.4	35789	37532
	0.75	32694	23435

		Phosphorus (%)	
		0.4	0.75
Trial B Calcium %	0.4	40487	24528
	0.8	32835	31602

Trial B Magnesium %		0.2		0.4	
Phosphorus %		0.4	0.75	0.4	0.75
Calcium %	0.4	39801	23250	41172	25806
	0.8	31776	42138	33893	21065

Retention

There were significant interactions between magnesium and phosphorus in trial A and calcium and phosphorus in trial B (Table 71a). Both magnesium and phosphorus at the higher level of dietary inclusion almost doubled potassium retention although when combined they only produced about a 30% increase in retention.

TABLE 71a

THE EXTENT OF INTERACTION BETWEEN MINERALS ON RETENTION (mg/14 days) OF POTASSIUM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A(a)	32	-2620	1970	NS
	B(b)	"	-3500	2440	NS
Calcium/Phosphorus	A(a)	"	1740	1970	NS
	B(b)	"	12000	2440	***
Magnesium/Phosphorus	A(a)	"	4800	1970	*
	B(b)	"	1300	2440	NS
Calcium/Magnesium/ Phosphorus	A(a)	"	-400	1970	NS
	B(b)	"	1450	2440	NS

(a) Two missing values
(b) Eleven missing values

Both calcium and phosphorus at their higher levels in the diet produced considerable increases in potassium retention although when combined their effects were nullified (Table 71b).

Table 71b

The effect of interactions between dietary phosphorus and magnesium in trial A and phosphorus and calcium in trial B on potassium retention (mg/14 days) by lambs.

			Phosphorus (%)	
			0.4	0.75
Trial A	Magnesium(%)	0.2	6710	12300
		0.4	12950	8950
Trial B	Calcium (%)	0.4	-3900	8800
		0.8	11200	-100

Urinary Concentration

There was a significant interaction between calcium and phosphorus in trial A (Table 72a). The upper level of calcium in the diet increased urinary potassium concentration whereas the upper level of phosphorus had the opposite effect and furthermore, when these minerals were combined the effect of calcium was lost and the value obtained very similar to that obtained with phosphorus alone (Table 72b).

Table 72b

The effect of the interaction between dietary calcium and phosphorus on urinary potassium concentration (mg/100ml) in lambs during trial A.

		Phosphorus (%)	
		0.4	0.75
Calcium(%)	0.4	434	262
	0.8	720	282

TABLE 72a

THE EXTENT OF INTERACTION BETWEEN MINERALS ON URINARY CONCENTRATION (mg/100ml) OF POTASSIUM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A	32	72.5	43.9	NS
	B	"	13.0	28.1	NS
Calcium/Phosphorus	A	"	133.0	43.9	***
	B	"	-3.0	28.1	NS
Magnesium/Phosphorus	A	"	41.5	43.9	NS
	B	"	14.5	28.1	NS
Calcium/Magnesium/ Phosphorus	A	"	26.8	43.9	NS
	B	"	16.8	28.1	NS

Serum Concentration

There was a significant interaction between calcium and magnesium in trial B (Table 73a). The upper dietary level of calcium increased serum potassium whereas the higher level of magnesium had little effect except when combined with that of calcium. In this case serum potassium was reduced to a value below that obtained when the minerals were present at low level (Table 73b).

Table 73b

The effect of the interaction between dietary calcium and magnesium on serum potassium concentration (mg/100ml) in lambs during trial B.

		Magnesium (%)	
		0.2	0.4
Calcium (%)	0.4	20.41	20.39
	0.8	21.72	19.03

3:2:3:2. Water Metabolism

3:2:3:2:1. Water Intake

The higher dietary level of calcium significantly reduced water intake in trial B (Table 74). There were no significant interactions (appendix 8:2:4).

3:2:3:2:2. Urine Output

The higher dietary level of calcium significantly reduced urine output in trial B (Table 75). There were no significant interactions (appendix 8:2:4.).

3:2:3:2:3. Water Retention

The higher dietary level of calcium significantly reduced the water retained by lambs during trial B (Table 76).

TABLE 73a

THE EXTENT OF INTERACTION BETWEEN MINERALS ON SERUM CONCENTRATION (mg/100ml) OF POTASSIUM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

INTERACTION	TRIAL	n	INTERACTION VALUE	SE	SIGNIFICANCE
Calcium/Magnesium	A	32	0.78	0.464	NS
	B(b)	"	1.34	0.569	*
Calcium/Phosphorus	A	"	-0.39	0.464	NS
	B(b)	"	0.49	0.569	NS
Magnesium/Phosphorus	A	"	-0.63	0.464	NS
	B(b)	"	-0.99	0.569	NS
Calcium/Magnesium/ Phosphorus	A	"	0.06	0.464	NS
	B(b)	"	-0.87	0.569	NS

(b) Eleven missing values

TABLE 74

THE EFFECT OF TWO DIETARY LEVELS OF CALCIUM, MAGNESIUM AND PHOSPHORUS ON WATER INTAKE (ml/week) BY WETHER LAMBS MEASURED DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A)[a] AND ELEVEN (TRIAL B)[b] WEEKS OF AGE

MINERAL	DIETARY LEVEL (% AIR DRY DIET)		TRIAL	N	MEANS		SED	SIGNIFICANCE
	LOW	HIGH			LOW	HIGH		
Calcium	0.4	0.8	A	32	6034	5562	832	NS
			B	"	34459	14314	3731	***
Magnesium	0.2	0.4	A	"	5597	6000	832	NS
			B	"	21563	27210	3731	NS
Phosphorus	0.4	0.75	A	"	5430	6167	832	NS
			B	"	21636	27137	3731	NS

(a) One missing value
(b) Eleven missing values

TABLE 75

THE EFFECT OF TWO DIETARY LEVELS OF CALCIUM, MAGNESIUM AND PHOSPHORUS ON URINE OUTPUT (ml/week) BY WETHER LAMBS MEASURED DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A)[a] AND ELEVEN (TRIAL B)[b] WEEKS OF AGE

MINERAL	DIETARY LEVEL (% AIR DRY DIET)		TRIAL	n	MEANS		SED	SIGNIFICANCE
	LOW	HIGH			LOW	HIGH		
Calcium	0.4	0.8	A	32	2940	2529	685	NS
			B	"	27247	9398	3696	***
Magnesium	0.2	0.4	A	"	2621	2848	685	NS
			B	"	16121	20524	3696	NS
Phosphorus	0.4	0.75	A	"	2318	3151	685	NS
			B	"	15610	21034	3696	NS

(a) Two missing value
(b) Eleven missing values

TABLE 76

THE EFFECT OF TWO DIETARY LEVELS OF CALCIUM, MAGNESIUM AND PHOSPHORUS ON WATER RETENTION (ml/24 hrs.) BY WETHER LAMBS MEASURED DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) [(a)] AND ELEVEN (TRIAL B) [(b)] WEEKS OF AGE

MINERAL	DIETARY LEVEL (% AIR DRY DIET)		TRIAL	n	MEANS		SED	SIGNIFICANCE
	LOW	HIGH			LOW	HIGH		
Calcium	0.4	0.8	A	32	352	353	24	NS
			B	"	876	612	71	***
Magnesium	0.2	0.4	A	"	341	363	24	NS
			B	"	664	824	71	*
Phosphorus	0.4	0.75	A	"	353	351	24	NS
			B	"	695	793	71	NS

(a) Two missing values
(b) Eleven missing values

There were no significant interactions (appendix 8:2:4).

3:2:3:3. Protein Bound Hexose Metabolism

3:2:3:3:1. Urinary Concentration

The higher dietary level of calcium significantly increased the urinary concentration of protein bound hexose in trial B whilst the upper level of phosphorus significantly reduced protein bound hexose concentration in trial A (Table 77a).

There was a significant interaction between calcium and phosphorus in trial A (appendix 8:2:5). The higher dietary level of calcium increased the concentration of hexose whereas the upper level of phosphorus had little effect. However in combination at their upper dietary levels these minerals reduced the urinary concentration of hexose below the value obtained when these minerals were present at their lower dietary levels. (Table 77b).

Table 77b

The effect of the interaction between dietary calcium and phosphorus on urinary concentration of protein bound hexose (mg/100ml) in lambs during trial A.

		Phosphorus (%)	
		0.4	0.75
Calcium (%)	0.4	46.5	46.2
	0.8	71.2	38.5

3:2:3:3:2. Urinary Excretion

The higher level of dietary calcium significantly reduced the excretion of protein bound hexose in trial B

TABLE 77a

THE EFFECT OF TWO DIETARY LEVELS OF CALCIUM, MAGNESIUM AND PHOSPHORUS ON URINARY CONCENTRATION OF PROTEIN-BOUND HEXOSE (mg/100ml) IN WETHER LAMBS MEASURED DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) (a) AND ELEVEN (TRIAL B) (b) WEEKS OF AGE

MINERAL	DIETARY LEVEL (% AIR DRY DIET)		TRIAL	n	MEANS		SED	SIGNIFICANCE
	LOW	HIGH			LOW	HIGH		
Calcium	0.4	0.8	A	32	46.4	54.8	4.69	NS
			B	"	14.5	28.1	2.77	***
Magnesium	0.2	0.4	A	"	52.1	49.1	4.69	NS
			B	"	19.8	22.9	2.77	NS
Phosphorus	0.4	0.75	A	"	58.9	42.3	4.69	***
			B	"	23.4	19.3	2.77	NS

(a) Two missing values
(b) Eleven missing values

(Table 78). There were no significant interactions (appendix 8:2:5).

3:2:3:3. Serum Concentration

There were no significant effects of mineral level on serum protein bound hexose (Table 79a). There was a significant interaction between phosphorus and calcium in trial A (appendix 8:2:5). Both calcium and phosphorus at their higher dietary levels reduced serum hexose although when combined at their upper levels they increased the concentration of serum hexose above the value obtained when the minerals were present at their lower levels. (Table 79b).

Table 79b

The effect of the interaction between dietary phosphorus and calcium on serum concentration of protein bound hexose (mg/100ml) in lambs during trial A.

		Phosphorus (%)	
		0.4	0.75
Calcium(%)	0.4	179.3	167.3
	0.8	175.8	183.6

3:2:3:4. Autopsy Findings

3:2:3:4:1. General

The number of animals destroyed during this experiment and the incidence of urolithiasis is shown in Table 80. The overall percentage of animals killed during the experiment was 20.3, the percentage diagnosed as calculi cases, 15.6 and the overall percentage incidence of urolithiasis, taking account of those cases diagnosed at autopsy, was 18.75.

TABLE 78

THE EFFECT OF TWO DIETARY LEVELS OF CALCIUM, MAGNESIUM AND PHOSPHORUS ON URINARY EXCRETION OF PROTEIN-BOUND HEXOSE (mg/24hr.) BY WETHER LAMBS MEASURED DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A)[a] AND ELEVEN (TRIAL B)[b] WEEKS OF AGE

MINERAL	DIETARY LEVEL (% AIR DRY DIET)		TRIAL	n	MEANS		SED	SIGNIFICANCE
	LOW	HIGH			LOW	HIGH		
Calcium	0.4	0.8	A	32	271	274	21	NS
			B	"	828	471	88	***
Magnesium	0.2	0.4	A	"	268	277	21	NS
			B	"	595	704	88	NS
Phosphorus	0.4	0.75	A	"	286	259	21	NS
			B	"	604	695	88	NS

(a) Two missing values
(b) Eleven missing values

TABLE 79a

THE EFFECT OF TWO DIETARY LEVELS OF CALCIUM, MAGNESIUM AND PHOSPHORUS ON SERUM CONCENTRATION OF PROTEIN-BOUND HEXOSE (mg/100ml) IN WETHER LAMBS MEASURED DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A)(a) AND ELEVEN (TRIAL B)(b) WEEKS OF AGE

MINERAL	DIETARY LEVEL (% AIR DRY DIET)		TRIAL	n	MEANS		SED	SIGNIFICANCE
	LOW	HIGH			LOW	HIGH		
Calcium	0.4	0.8	A	32	173	179	4.6	NS
			B	"	191	189	3.5	NS
Magnesium	0.2	0.4	A	"	174	178	4.6	NS
			B	"	192	188	3.5	NS
Phosphorus	0.4	0.75	A	"	177	175	4.6	NS
			B	"	193	188	3.5	NS

(a) One missing value
(b) Eleven missing values

TABLE 80

THE RELATIONSHIP BETWEEN TREATMENT AND THE NUMBER OF ANIMALS DESTROYED DURING EXPERIMENT II AND THE INCIDENCE OF CLINICAL UROLITHIASIS

TREATMENT*	NUMBER OF ANIMALS KILLED	REASON FOR DESTRUCTION		% CLINICAL CASES OF UROLITHIASIS
		UROLITHIASIS	OTHER	
1	2	2		25
2	1		1**	0
3	2	2		25
4	2	1	1***	12.5
5	1	1		12.5
6	1	1		12.5
7	1		1***	0
8	3	3		37.5
TOTALS	13	10	3	15.6

* Treatment as outlined in 3:2:2:3. ** Coccidiosis. *** Pneumonia.

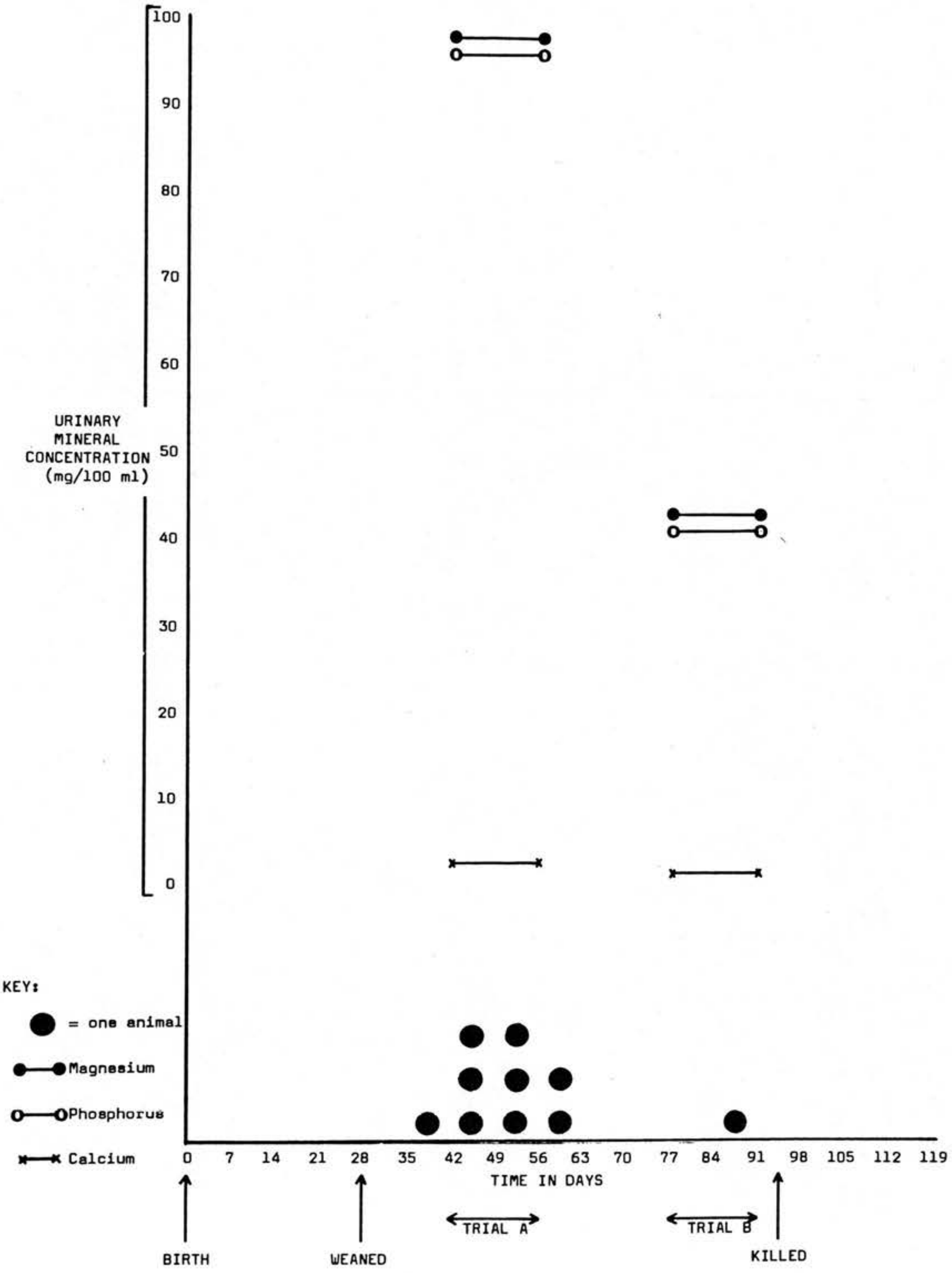
Animals killed for reasons other than urolithiasis were destroyed in period one of the experiment. The animal diagnosed as having coccidiosis was killed between metabolic trials. Faecal samples were taken from the remaining lambs and screened for the presence of coccidia. Fortunately there was no evidence of oocysts in the samples. It was assumed that the one animal was an isolated case and no treatment was prescribed. The two animals that developed pneumonia did not respond to antibiotic therapy and were subsequently killed between the metabolic trials (autopsy report, appendix 8:4:2).

During period two of the first experiment which was carried out during the summertime animals had been observed to show signs of heat stress. It had been realised at this time that the ventilation of the animal house was probably inadequate. It was suggested that poor ventilation might have contributed to the occurrence of these cases of pneumonia. In an effort to prevent the recurrence of this problem,air change was assisted by the use of two electric fans. However, it was apparent later on in this experiment that ventilation was still inadequate as animals were observed to pant. Humidity and temperature recordings were made at this time (appendix 8:6) and it was confirmed that temperatures and humidity were high in the building.

Although incidence of urolithiasis was not restricted to any particular treatment, most of the clinical cases became apparent within 4 weeks of weaning (Figure 1). A summary of the autopsy findings in animals killed during the experiment is shown in appendix 8:4:3. Characteristically there was

FIGURE 1

THE NUMBER OF ANIMALS KILLED PER WEEK IN EXPERIMENT II BECAUSE OF UROLITHIASIS IN RELATION TO MEAN URINARY CALCIUM, MAGNESIUM AND PHOSPHORUS CONCENTRATIONS (ALL TREATMENTS) MEASURED DURING METABOLIC TRIAL A AND B



urethral blockage which could lead to bladder or urethral rupture (Plate 2).

3:2:3:4:2. Renal Tissue

Histochemistry

(i) Cases without calculi

Calcium salt deposition occurred frequently in the medullary area and PAS positive material was observed in the interstitial tissue and also in some of the collecting tubules.

(ii) Cases with calculi

In one case, calculous material was identified in the renal pelvis, collecting tubules, some descending tubules and also in the lymphatics in the pelvic fat tissue. Acidic mucopolysaccharide was identified in the collecting tubules (Plate 3). In some cases there was evidence of accumulations of PAS positive material beneath the pelvic epithelium. In three cases, areas of the pelvic epithelium were seen bulging into the lumen. Occasionally the epithelium was ruptured (Plate 4). The material contained in these areas was von Kossa positive and occasionally stained PAS positive. Von Kossa positive areas were associated with the pelvic epithelium (Plate 5).

Mineral Analysis (Table 81)

The medulla had a greater ash and calcium content than the kidney cortex. Both phosphorus and potassium were higher in the cortex whilst the magnesium content was sometimes higher in the medulla. There was little difference in renal mineral content between grass-fed lambs and those

Plate 2

Lamb urinary tract showing urethral rupture.

(Lamb Number 12, Treatment 1, Period 3)

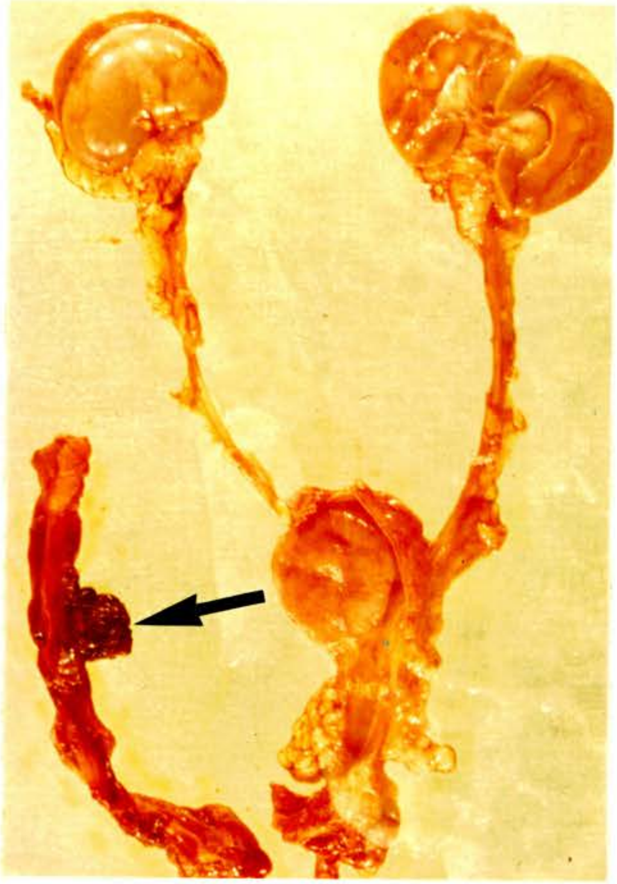


Plate 3

Lamb kidney showing distended collecting tubule containing mucoid secretion (Alcian green x 300).

/Lamb Number 8, Treatment 6, Period 1/

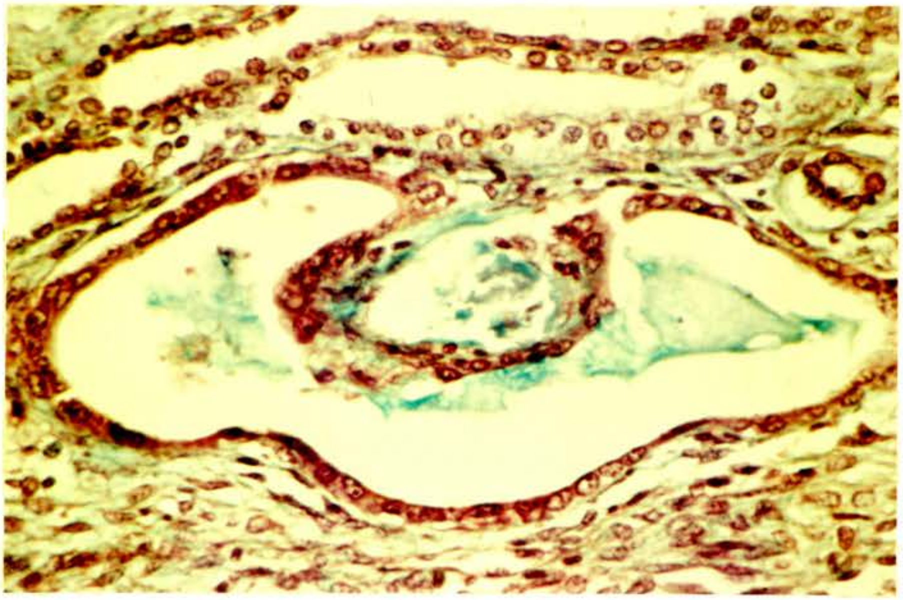


Plate 4

Lamb kidney pelvis showing rupture of pelvic wall (H. and E. x 80).

/Lamb Number 8, Treatment 6, Period 1/

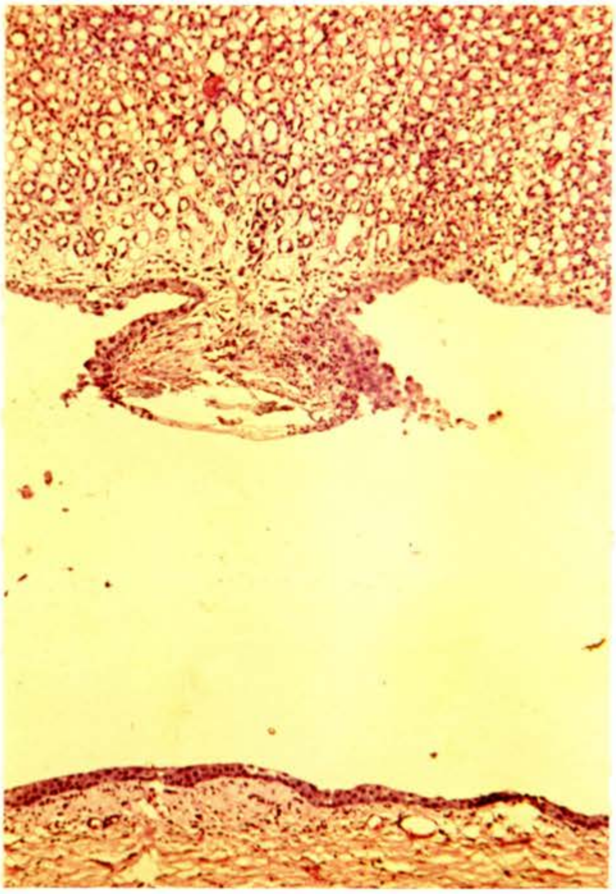


Plate 5

Lamb kidney pelvis showing calcium deposits adjacent to area of rupture (Von Kossa x 120).

/Lamb number 7, Treatment 8, Period 3/

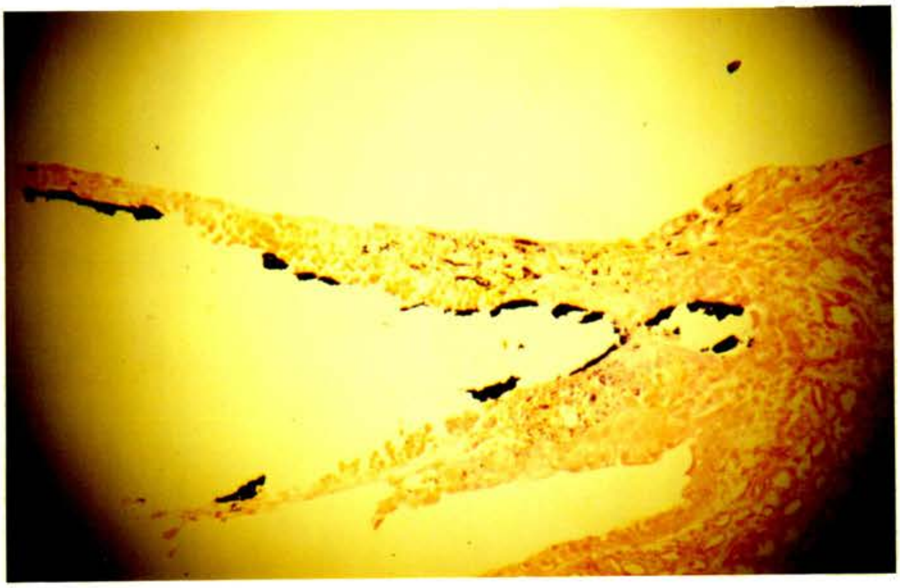


TABLE 81

MINERAL ANALYSIS OF RENAL TISSUE TAKEN FROM EXPERIMENTAL LAMBS AT THE END OF PERIOD FOUR COMPARED WITH RENAL TISSUE TAKEN FROM GRASS-FED LAMBS (TABULATED VALUES REPRESENT THE MEAN OF TWO ANIMALS)

* TREATMENT	g/100g DM		g/100g ASH									
	ASH		CALCIUM		PHOSPHORUS		MAGNESIUM		SODIUM		POTASSIUM	
	CORTEX	MEDULLA	CORTEX	MEDULLA	CORTEX	MEDULLA	CORTEX	MEDULLA	CORTEX	MEDULLA	CORTEX	MEDULLA
Grass Fed	5.42	7.41	0.64	2.37	24.79	17.07	1.96	1.70	13.82	37.55	27.64	16.60
1	5.96	6.69	0.67	1.11	22.54	16.36	2.04	1.58	13.56	27.67	26.91	19.83
2	5.52	6.98	0.86	7.55	25.15	20.31	2.11	2.33	14.69	31.98	29.44	20.74
3 **	6.58	7.25	0.41	1.52	20.17	18.08	1.74	2.07	15.70	26.06	24.96	20.53
4	5.53	10.29	1.46	7.47	25.59	16.04	1.95	1.79	15.48	26.99	26.31	10.67
5	5.30	8.66	0.69	2.08	24.43	15.08	2.06	1.57	15.88	37.43	27.01	14.32
6	5.99	8.37	0.65	3.12	23.35	17.06	1.87	1.99	13.05	28.25	25.20	14.88
7	5.64	7.62	0.78	5.98	23.99	20.55	1.98	2.33	13.48	30.32	27.26	17.61
8 **	6.30	8.45	1.69	4.25	22.50	17.86	1.98	2.29	16.43	22.61	23.45	14.90

** Includes data from one animal which was killed because it developed urolithiasis.

* Treatment diets as outlined under 3:2:2:3.

fed the experimental diets. Medullary calcium levels were extremely high on diets 2, 4, 7 and 8 which apart from 4 contained 0.75% phosphorus.

3:2:3:4:3. Calculous Material

The material obtained from urolithiasis cases always had the same appearance (see Plate 6) and was frequently located in the bladder (see Plate 7). It ranged in colour from grey to white and the shapes were spheroidal and tetrahedral. Calculus size seemed related to the numbers present. Where there was a lot of calculous material present there were only a few large calculi. Large calculi were between 3-4mm in diameter but the majority were less than 1mm in diameter. All of the material collected was easily crumbled. One of the greatest drawbacks to the examination of this material was the limited amount available. Very little calculous material was required to cause urethral blockage. A calculus of only 2mm in diameter was found on more than one occasion to be responsible for the blockage and subsequent urethral rupture.

Chemical Analysis (Table 82).

Hydroxyproline could not be detected in calculous material. To ensure that the hydrolysis was not destroying hydroxyproline a known weight of hydroxyproline was added to some calculous material before hydrolysis. Estimations of hydroxyproline after hydrolysis showed 100% recovery.

Nitrogen could only be detected in trace amounts and there was insufficient sample to carry out quantitative estimation.

Plate 6

Typical sample of calculous material removed from lamb urinary bladder (2.9 x magnification)

/Lamb number 16, Treatment 4, Period 3/.

Plate 7

Lamb urinary bladder showing location of calculous material.

/Lamb number 7, Treatment 8, Period 3/.

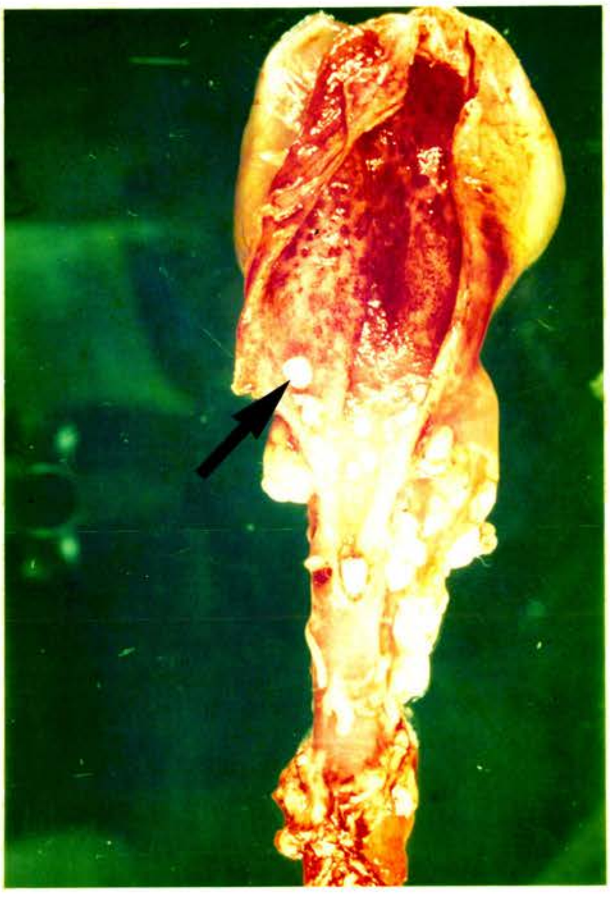


TABLE 82

CHEMICAL ANALYSIS OF CALCULOUS MATERIAL RECOVERED FROM THE URINARY TRACTS OF LAMBS WHICH DEVELOPED CALCULI DURING EXPERIMENT II

PERIOD	TREATMENT	g/100g OF DRY MATTER			g/100g OF ASH		
		ORGANIC MATTER	ASH	PROTEIN BOUND HEXOSE	CALCIUM	PHOSPHORUS	MAGNESIUM
1	6	37.29	62.71	1.47	0.67	21.41	41.44
1	1	IS	IS	IS	IS	IS	IS
2	1	IS	IS	IS	IS	IS	IS
2	5	43.51	56.49	1.01	3.17	24.80	34.80
3	3	43.43	56.57	0.65	1.89	24.26	34.12
3	8	36.13	63.87	0.32	1.08	22.54	27.37
3	8	35.42	64.58	0.29	2.90	21.81	28.69
3	4 *	40.27	59.73	IS	2.73	19.22	31.49
3	5 *	36.36	63.64	0.66	3.65	20.60	27.81
3	4	60.75	39.25	IS	6.98	20.87	34.17
4	8	44.44	55.56	0.60	2.05	21.76	33.82
4	3	58.09	41.91	IS	7.78	25.58	42.16
MEAN VALUES		43.58	56.42	0.71	3.29	22.29	33.59

* Found at Autopsy IS - Insufficient Sample

Protein bound hexose was present in samples that were analysed although only in small amounts. Apart from two samples, the organic matter content was about 40% and the ash content 55 to 60%. The calcium content was low in all cases and the phosphorus and magnesium contents were fairly consistent at around 22% and 33% respectively of the ash.

Physical Analysis

X-ray crystallography was attempted but unfortunately the calculous material obtained in this study was amorphous and not crystalline. The diffraction pattern obtained with amorphous ovine samples follows that shown in Plate 8 which may be compared with the discrete peaks obtained with crystalline calculous material taken from a dog (Plate 9).

There was only sufficient material remaining after chemical analysis to allow x-ray fluorescence to be carried out on two samples. The results indicated that there was 19% magnesium, 17.5% inorganic phosphorus and 0.9% calcium in dry calculous material. Assuming a mean ash content of 54% these results became 35.19g magnesium, 32.41g inorganic phosphorus and 1.67g calcium per 100g ash. Apart from the higher estimate for phosphorus the results agree quite well with those obtained by chemical means confirming that these calculi were very low in calcium and high in magnesium and phosphorus.

3:2:3:4:4. Bone (Table 83)

Radiography of the bones taken from animals receiving the 0.75% phosphorus diets failed to reveal any abnormality.

Plate 8.

A continuous chart record of the x-ray diffraction pattern of amorphous ovine phosphatic calculous material.

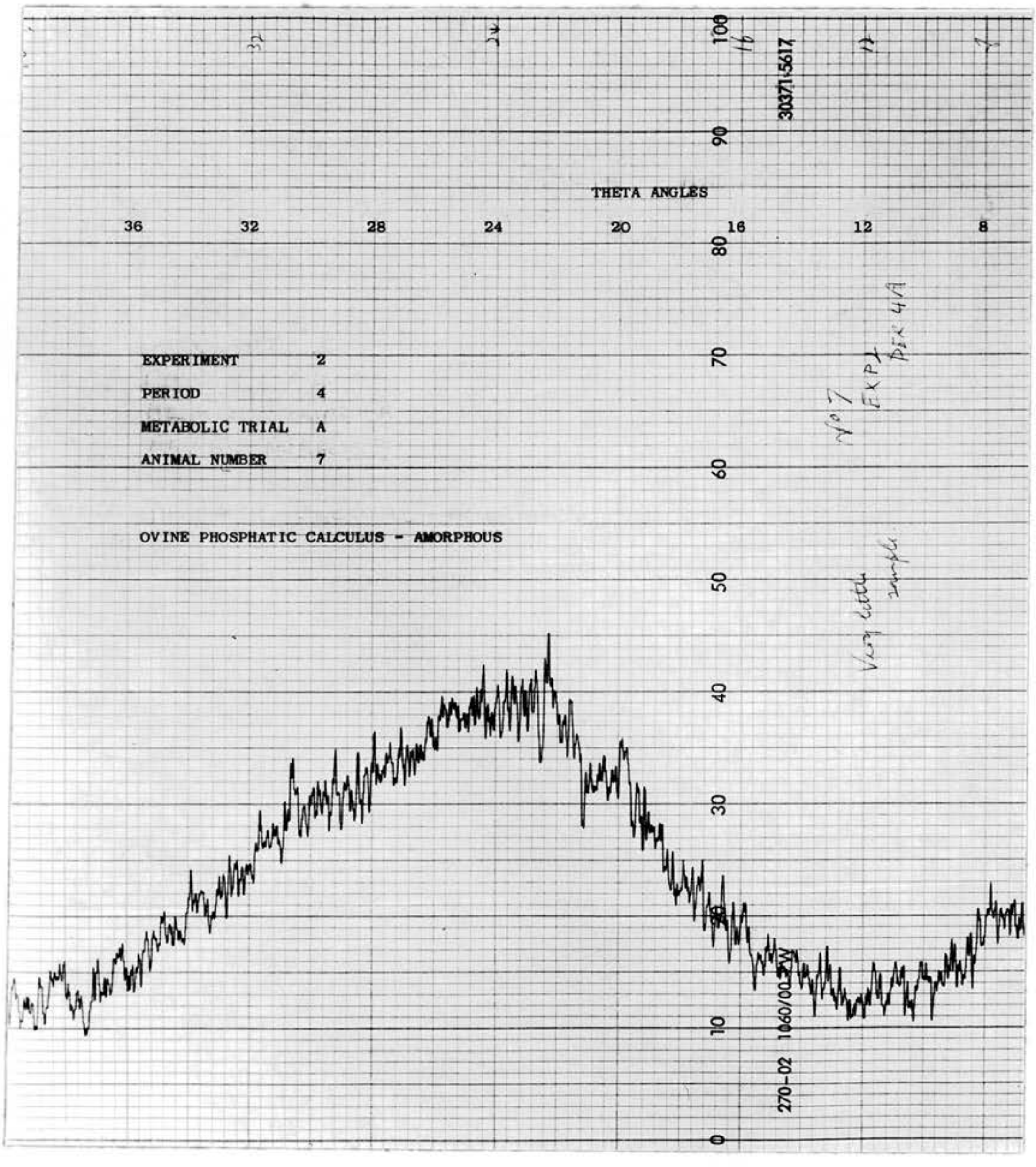


Plate 9

A continuous chart record of the x-ray diffraction pattern of crystalline canine phosphatic calculous material.

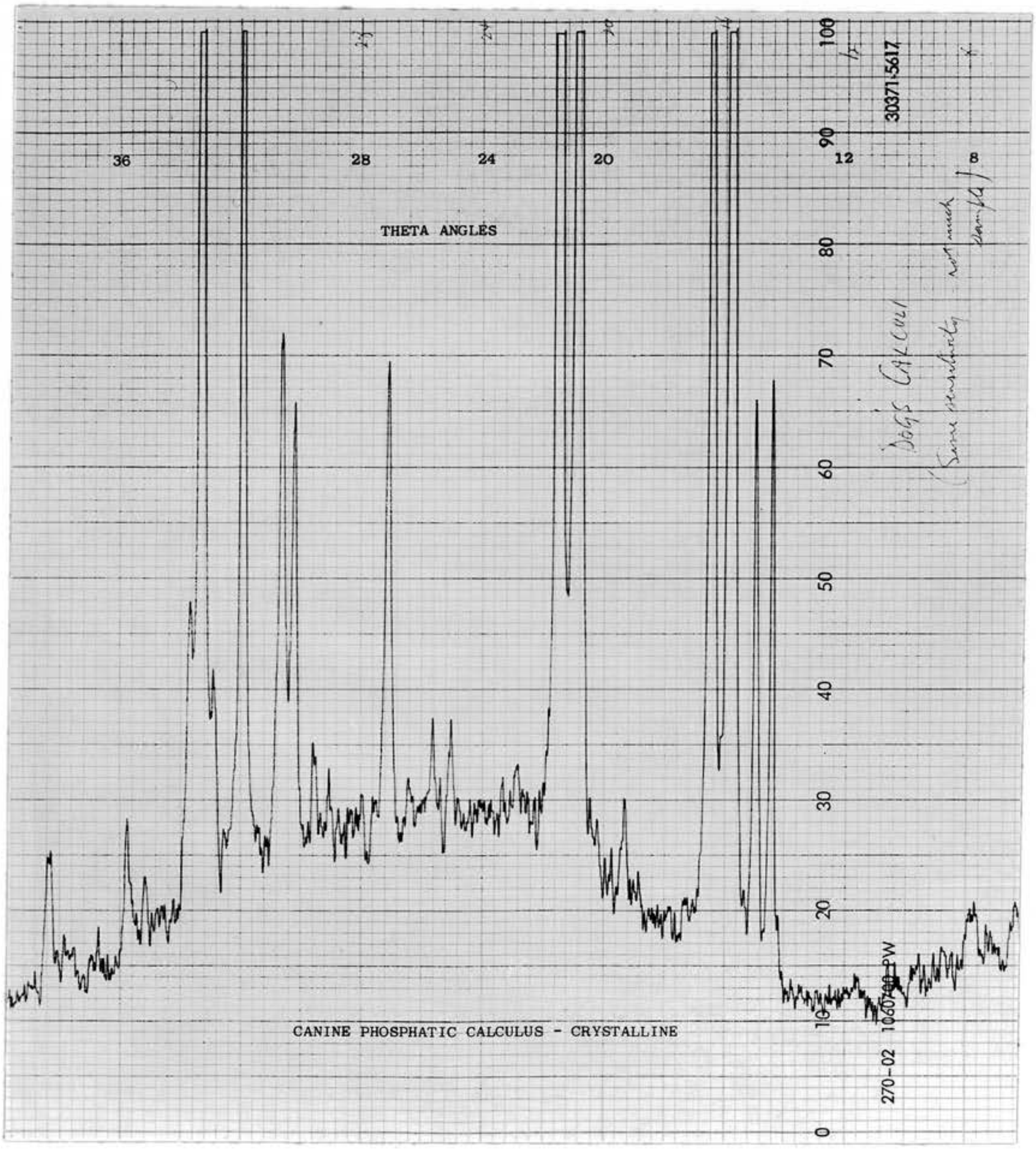


TABLE 83

MINERAL ANALYSIS OF BONE TAKEN FROM EXPERIMENTAL LAMBS AT THE END OF PERIOD THREE

TREATMENT	HUMERUS				FEMUR			
	g/100g dry fat free bone	g/100g Ash			g/100g dry fat free bone	g/100g Ash		
DIET	Ash	Calcium	Magnesium	Phosphorus	Ash	Calcium	Magnesium	Phosphorus
CONTROL	39.64	36.02	1.06	24.09	43.89	35.52	1.18	25.38
	44.66	35.15	1.00	23.44	45.55	34.29	1.05	24.35
MEAN	42.15	35.58	1.03	23.76	44.72	34.90	1.11	24.86
HIGH PHOSPHORUS	43.60	36.65	1.08	25.37	42.38	35.35	1.13	25.11
	42.75	37.89	1.05	23.69	46.28	36.17	1.01	22.82
MEAN	43.17	37.27	1.06	24.53	44.33	35.76	1.07	23.96
HIGH PHOSPHORUS AND MAGNESIUM	39.14	36.94	1.20	25.47	42.30	34.49	1.11	24.92
	45.34	35.55	1.15	24.04	45.77	35.61	1.16	25.13
MEAN	42.24	36.24	1.17	24.75	44.03	35.05	1.13	25.02
HIGH PHOSPHORUS AND CALCIUM	40.86	36.10	1.05	25.26	45.79	35.90	0.98	24.39
	46.98	34.16	1.19	25.12	44.08	34.96	1.13	24.21
MEAN	43.92	35.17	1.05	25.19	44.93	35.43	1.05	24.30

Furthermore, chemical analysis of these bones failed to demonstrate any differences between treatment on bone composition.

3:2:4. Discussion

3:2:4:1. Mineral Metabolism

Calcium

Although serum calcium levels were reduced by additional dietary magnesium and phosphorus, urine concentrations remained relatively unaffected. Losses of calcium via the urine - expressed as a proportion of total intake - were very small and mostly independant of treatment (figures 2 and 3). Gill, et al., (1959) have indicated that additional calcium in the diet of rats increased urinary calcium excretion; the opposite effect was demonstrated in this study. Bushman, et al., (1967) demonstrated an increase in the urinary calcium concentration in response to extra dietary calcium which was confirmed in this experiment. Furthermore, the finding by Bushman, et al., (1968) that calcium retention was increased by supplementing the diet with calcium was also confirmed in this experiment.

Phosphorus

In contrast to the small loss of calcium via the urine this route is an important one for phosphorus excretion. In this study, changes in dietary mineral content produced marked changes in urinary phosphorus excretion (figures 4 and 5). Thus, additional dietary phosphorus increased phosphorus digestibility; this was reflected in the relative decline in faecal losses and increased retention and urinary excretion. The increase in urinary phosphorus excretion by lambs following phosphorus supplementation of their diet has been observed by others in sheep (Bushman,

Figure 2. DAILY CALCIUM EXCRETION AND RETENTION EXPRESSED AS A PERCENTAGE OF INTAKE IN EXPERIMENT II TRIAL A.

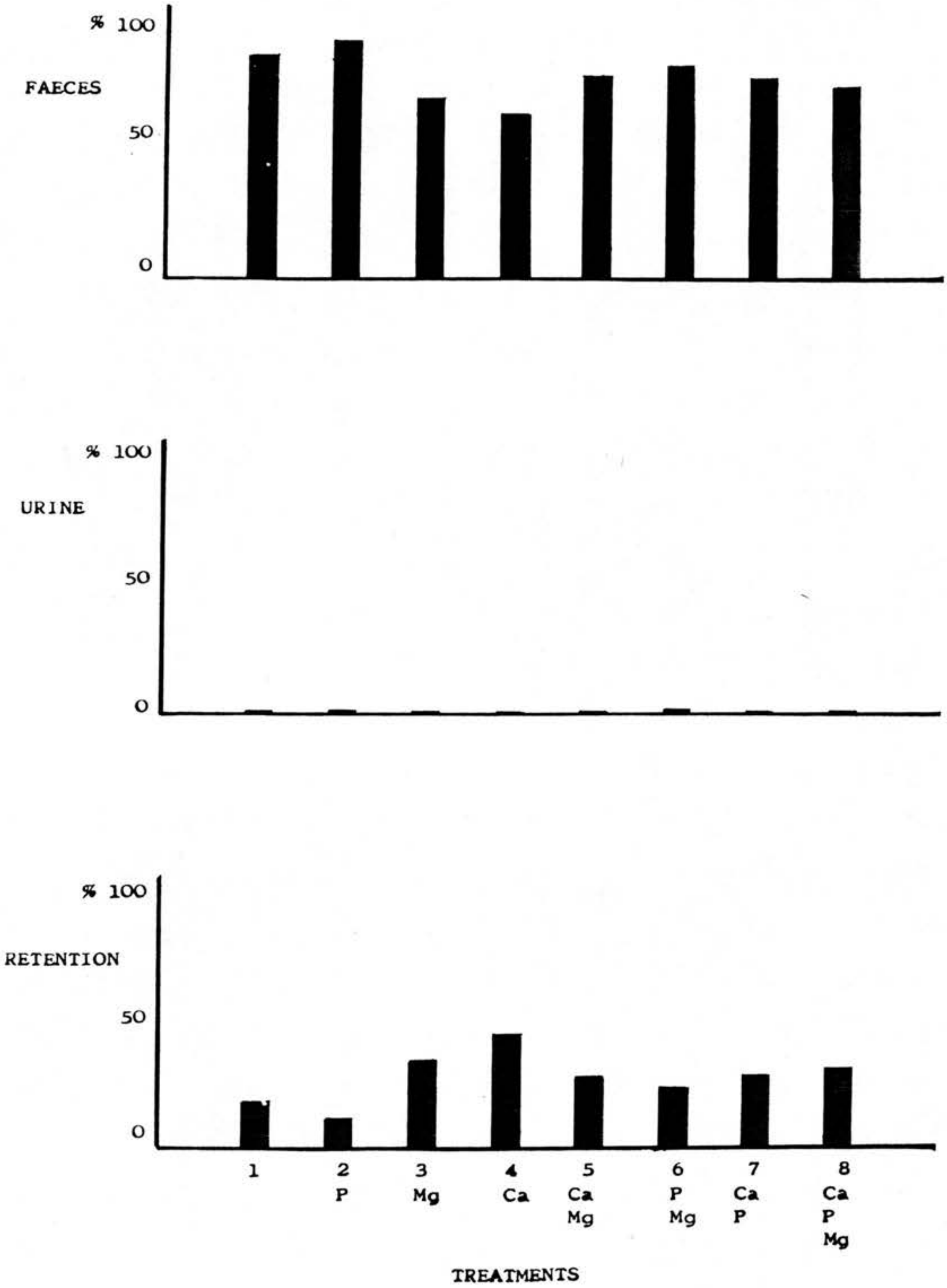


Figure 3. DAILY CALCIUM EXCRETION AND RETENTION EXPRESSED AS A PERCENTAGE OF INTAKE IN EXPERIMENT II TRIAL B.

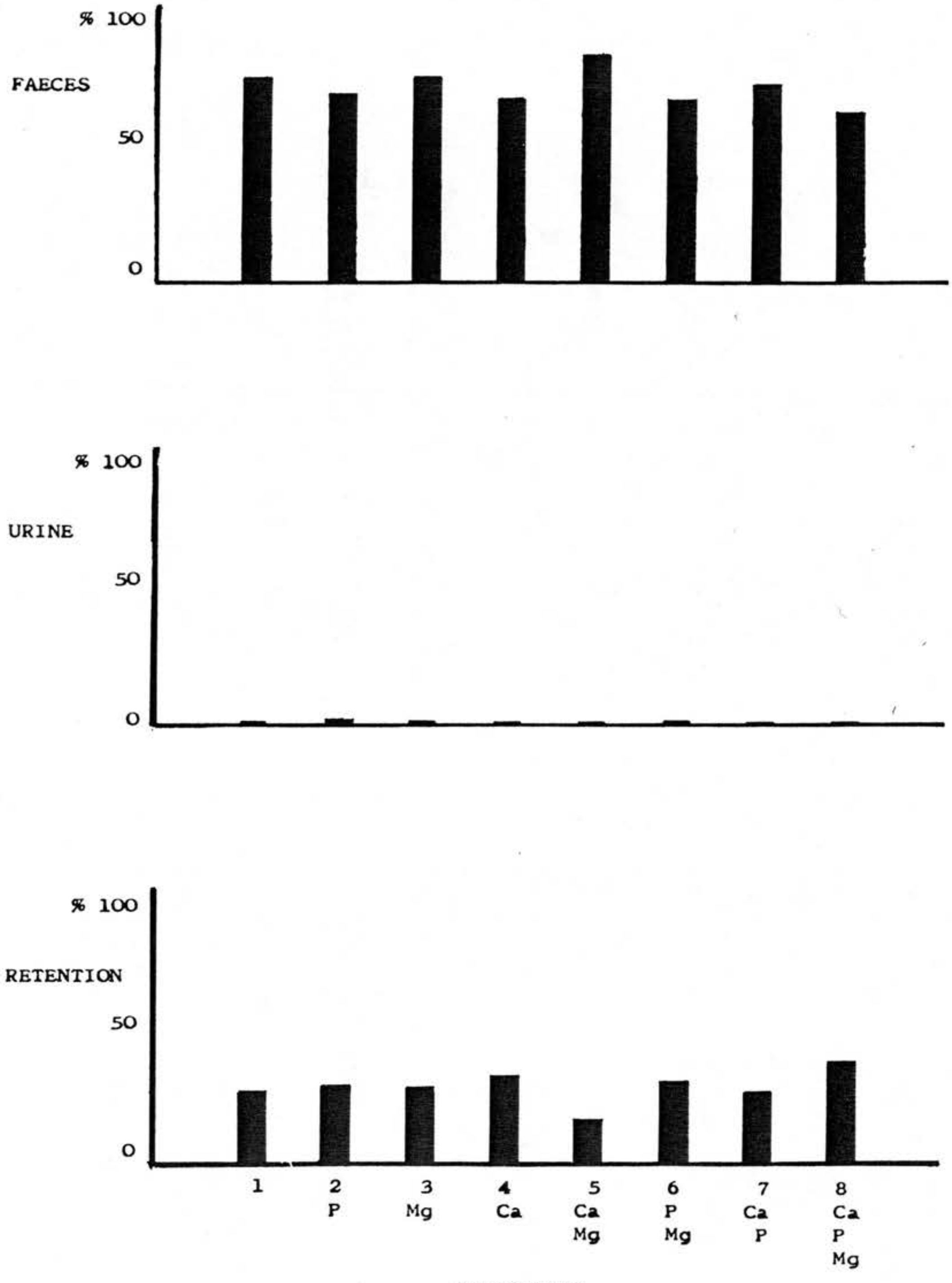


Figure 4. DAILY PHOSPHORUS EXCRETION AND RETENTION EXPRESSED AS A PERCENTAGE OF INTAKE IN EXPERIMENT II TRIAL A.

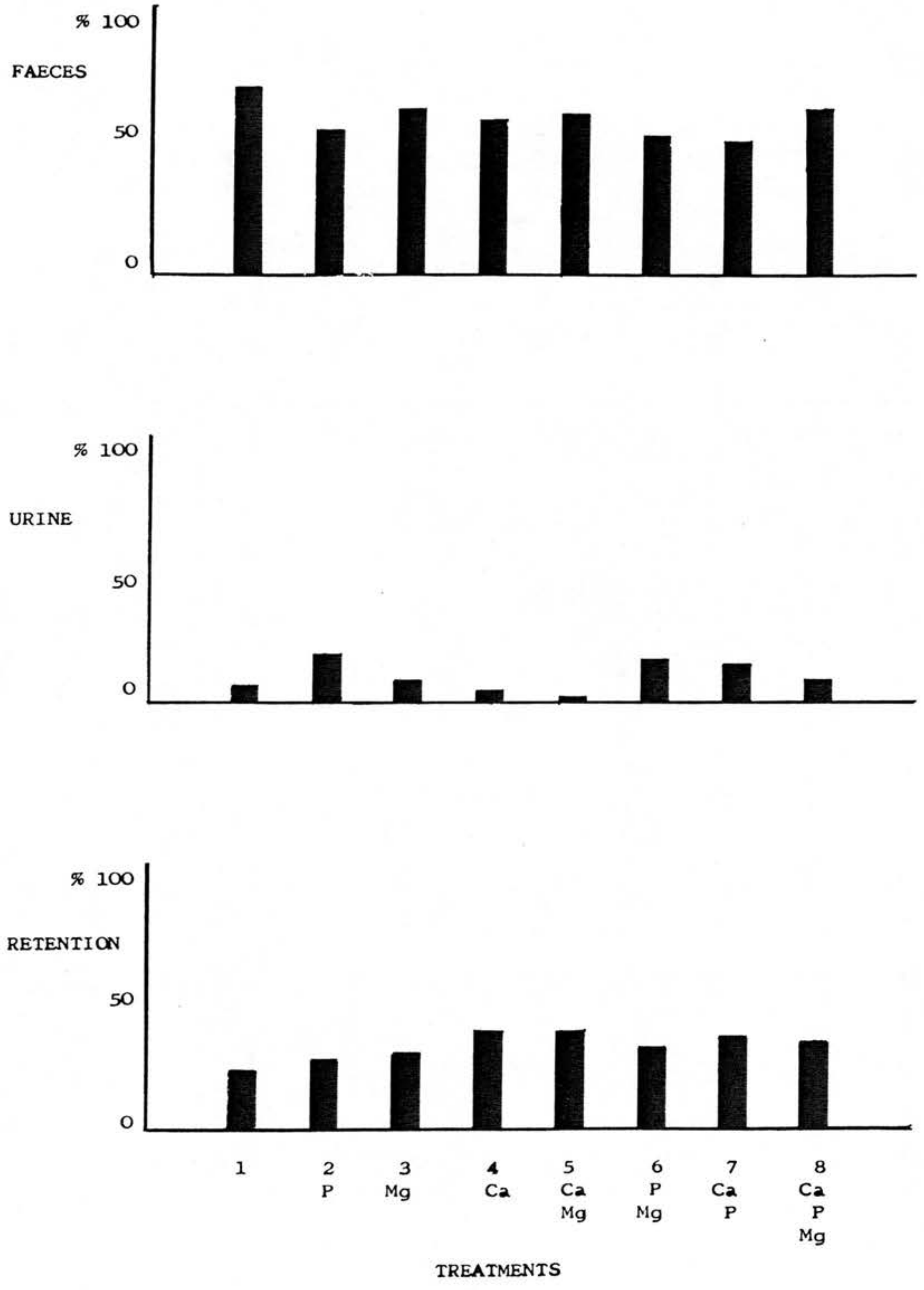
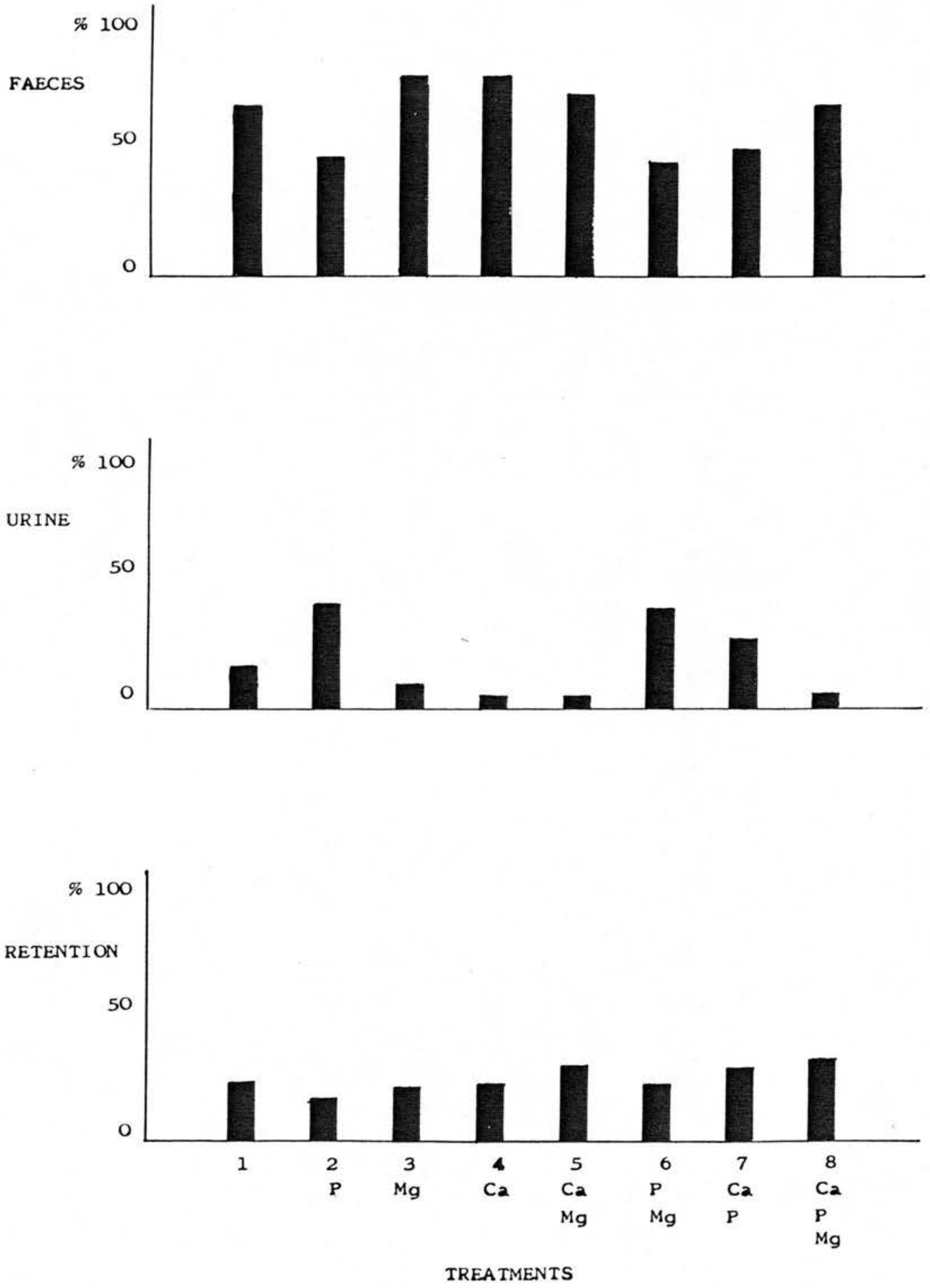


Figure 5. DAILY PHOSPHORUS EXCRETION AND RETENTION EXPRESSED AS A PERCENTAGE OF INTAKE IN EXPERIMENT II TRIAL B.



et al., 1965), calves (Kaushal, Dutt and Chakrabarti, 1972), rats (Gill and Vermeulen, 1964/5) and in man (Edwards, Russell and Hodgkinson, 1965). The reduction in urinary phosphorus excretion by lambs following calcium supplementation of their diet has been demonstrated in sheep (Bushman, et al., 1965) and rats (Ranganathan, 1931; Gill, et al., 1959). Furthermore, the ability of supplementary dietary magnesium to bring about a reduction in the amount of phosphorus excreted by lambs in their urine has been recognised by others (Bushman, et al., 1965; Bushman, 1968).

There are two likely mechanisms whereby calcium and magnesium supplements reduce urinary phosphorus excretion; firstly by reducing phosphorus absorption in the gut and secondly by increasing renal tubular reabsorption of phosphate. It is noteworthy, that ruminants excrete phosphorus principally by secretion into the lumen of the gut whereas monogastrics excrete it via the kidney. Scott (1972) showed that tubular reabsorption of phosphorus was reduced when phosphorus absorption from the gut increased in sheep and calves fed concentrate diets. Thus, one would expect that a reduction in phosphorus absorption from the gut would lead to a greater tubular reabsorption of phosphorus with a net reduction in urinary phosphorus excretion. The information relating to the control of phosphate handling by the renal tubule is incomplete and it has been pointed out recently that the renal tubule can adapt its capacity to transport phosphate in response to dietary phosphate in rats even in the absence of parathyroid hormone (Tröhler, Bonjour and

Fleisch, 1976); this indicates an as yet unknown regulatory factor involved in the urinary excretion of phosphate.

Supplementary dietary calcium was more effective than magnesium in reducing the amount of phosphorus excreted by lambs in their urine and there appeared to be a synergistic action when they were combined at their upper levels. This combination of minerals may have enhanced tubular reabsorption of phosphate. The effect of the interaction between calcium and magnesium on phosphorus metabolism has been recorded by Bushman (1967) in store lambs. He showed that a combination of a high level of magnesium (0.38%) and a low level of calcium (0.37%) was more effective in reducing urinary phosphorus excretion than a high level of calcium (1.27%) alone; however, a combination of a high dietary level of magnesium (0.38%) and a high level of calcium (1.27%) failed to exert an effect on urinary phosphorus excretion. The apparent discrepancy between Bushman's results and those obtained in the current study is probably due to the different dietary mineral levels used in the two experiments. The highest level of calcium used in this study was 0.8% in combination with 0.75% phosphorus and 0.4% magnesium. Examination of Bushman's data shows that a similar mineral combination, 0.77% calcium, 0.55% phosphorus and 0.38% magnesium, produced a much greater reduction in urine phosphorus excretion than a diet containing 0.77% calcium, 0.55% phosphorus and 0.18% magnesium. This substantiates the view that calcium and magnesium act synergistically with respect to reducing urinary phosphorus excretion.

Magnesium

An examination of the magnesium excretion data (figures 6 and 7) shows that urine is an important route for the excretion of magnesium. Furthermore, changes in dietary mineral intake were reflected in marked changes in urinary magnesium excretion. The large proportion of magnesium found in the calculi formed by lambs during this experiment was probably a reflection of the large quantities of magnesium lost via the urine.

Addition of calcium to the diet increased magnesium digestibility; this was reflected in an increased retention of magnesium and also an augmented urinary excretion. The latter effect was also demonstrated by Bushman (1968).

Supplementary dietary magnesium increased magnesium retention; furthermore, serum and urine concentrations of magnesium were also enhanced.

Addition of phosphorus to the diet of lambs reduced the concentration of magnesium in their urine, an effect noted by others (Edwards, et al., 1965). This effect was so marked that it overcame the effect of additional calcium and in trial B lambs fed high dietary levels of phosphorus (0.75%) hardly retained any magnesium (figure 7).

Sodium and Potassium

The precise role of sodium and potassium in relation to calculus formation in lambs is unclear. Lamprecht, Darroch and Crookshank (1969a) used multiple regression analysis to show that potassium exerts a curvilinear effect upon urolith formation. They found that increasing dietary potassium

Figure 6. DAILY MAGNESIUM EXCRETION AND RETENTION EXPRESSED AS A PERCENTAGE OF INTAKE IN EXPERIMENT II TRIAL A.

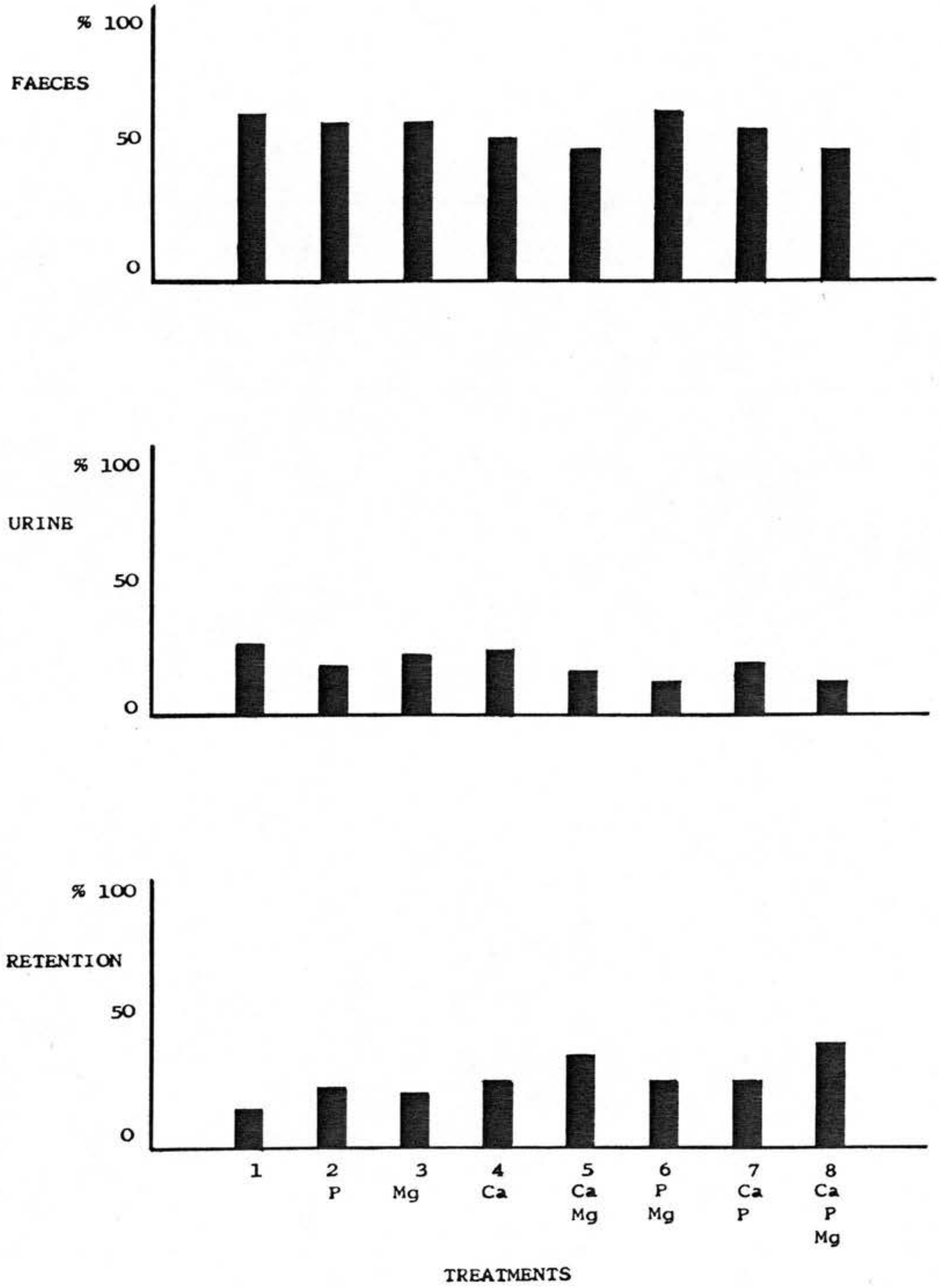
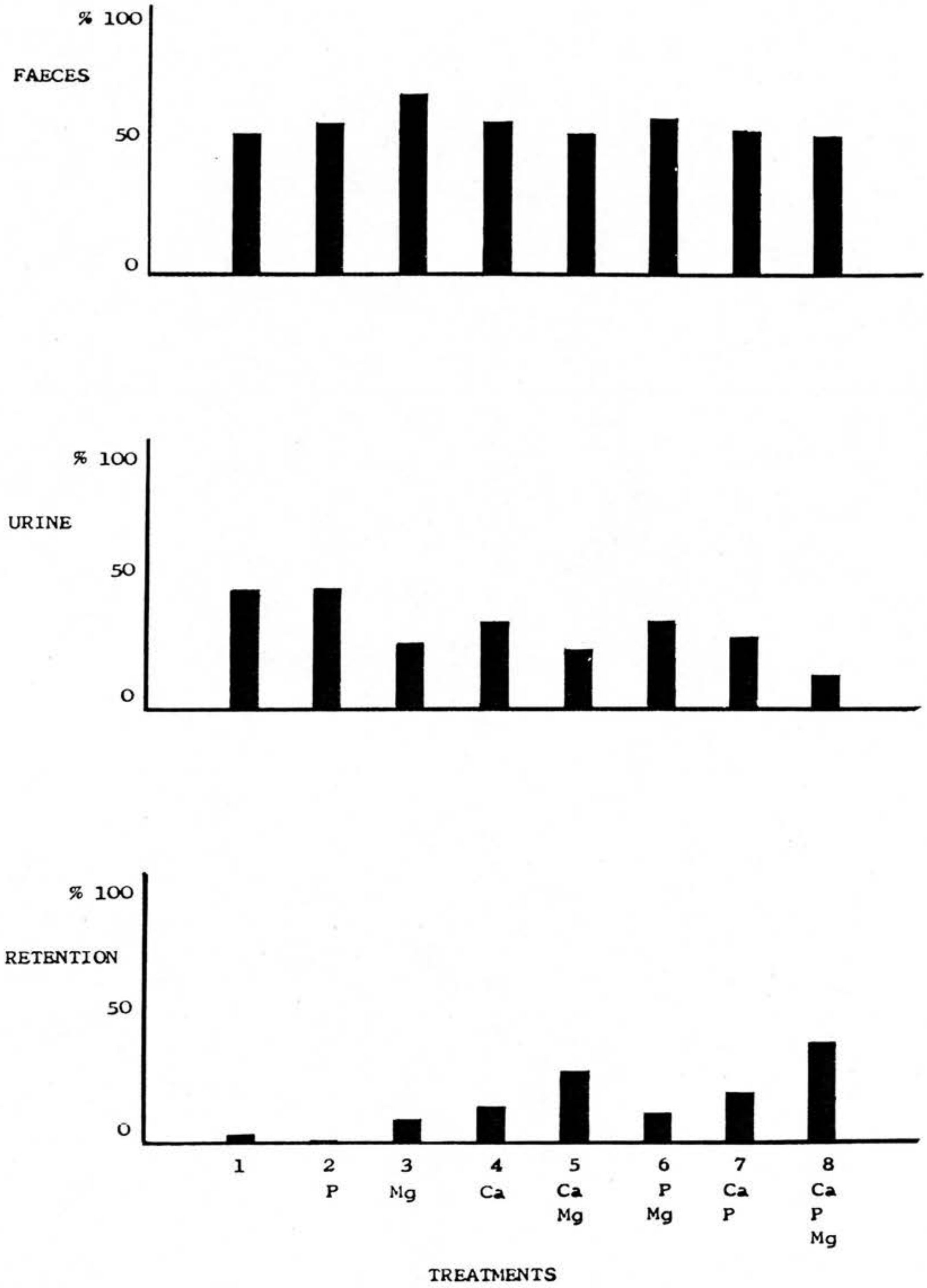


Figure 7. DAILY MAGNESIUM EXCRETION AND RETENTION EXPRESSED AS A PERCENTAGE OF INTAKE IN EXPERIMENT II TRIAL B.



was initially associated with an increased incidence of urolithiasis however, further increments of dietary potassium were associated with a reduction in calculi cases in other animals. In the current study, increasing the level of phosphorus in the diet to 0.75% had little effect on sodium excretion (figures 8 and 9) and reduced the excretion of potassium (figures 10 and 11). In contrast, increasing the dietary level of calcium or magnesium, increased the proportion of potassium excreted by lambs via their urine. Furthermore, there was an increased urinary sodium excretion. The effect of additional dietary calcium on urinary excretion of sodium and potassium has also been recorded by Yano, Sakurai and Kawashima (1975) in lambs.

Throughout this experiment, the sodium: calcium ratios ranged from 1:1 to 3:1 in contrast to the higher ratios of about 15:1 which were obtained in Experiment I. This is interesting in view of the greater number of calculi cases which occurred in this experiment. Additional calcium increased the urinary excretion of sodium; a result which may in part explain the beneficial effect of calcium supplements in reducing the incidence of urolithiasis. However, although the total excretion of sodium was increased, there was no significant change in the urinary sodium: calcium ratio.

Talos, Pop, Bugariu, Moraru and Roth (1970) have shown that serum sodium and potassium concentrations, measured in lambs with urolithiasis, remain in the normal range. Variations in dietary calcium, phosphorus and magnesium did not change serum sodium and potassium concentrations outwith the

Figure 8. DAILY SODIUM EXCRETION AND RETENTION EXPRESSED AS A PERCENTAGE OF INTAKE IN EXPERIMENT II TRIAL A.

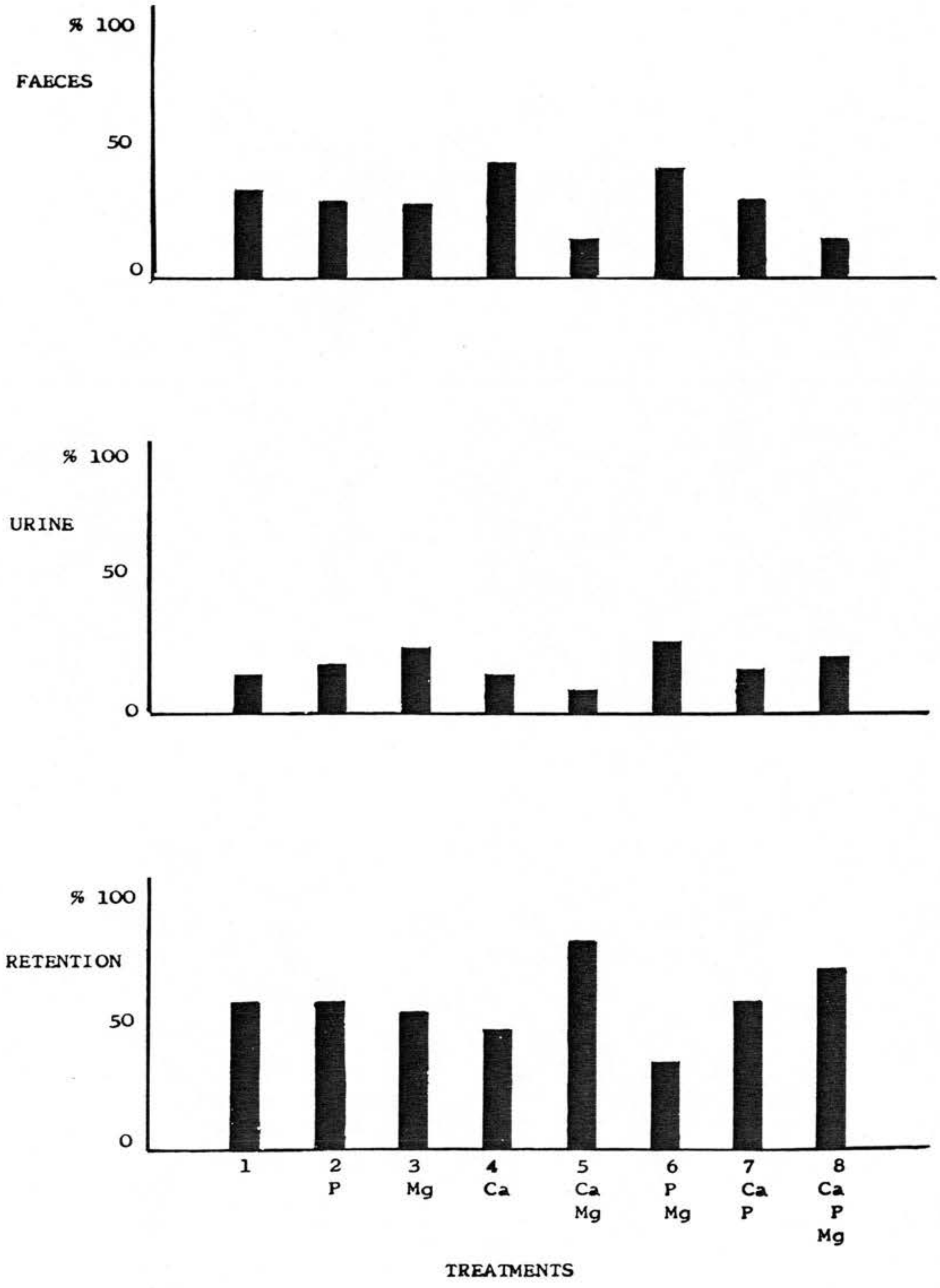


Figure 9. DAILY SODIUM EXCRETION AND RETENTION EXPRESSED AS A PERCENTAGE OF INTAKE IN EXPERIMENT II TRIAL B.

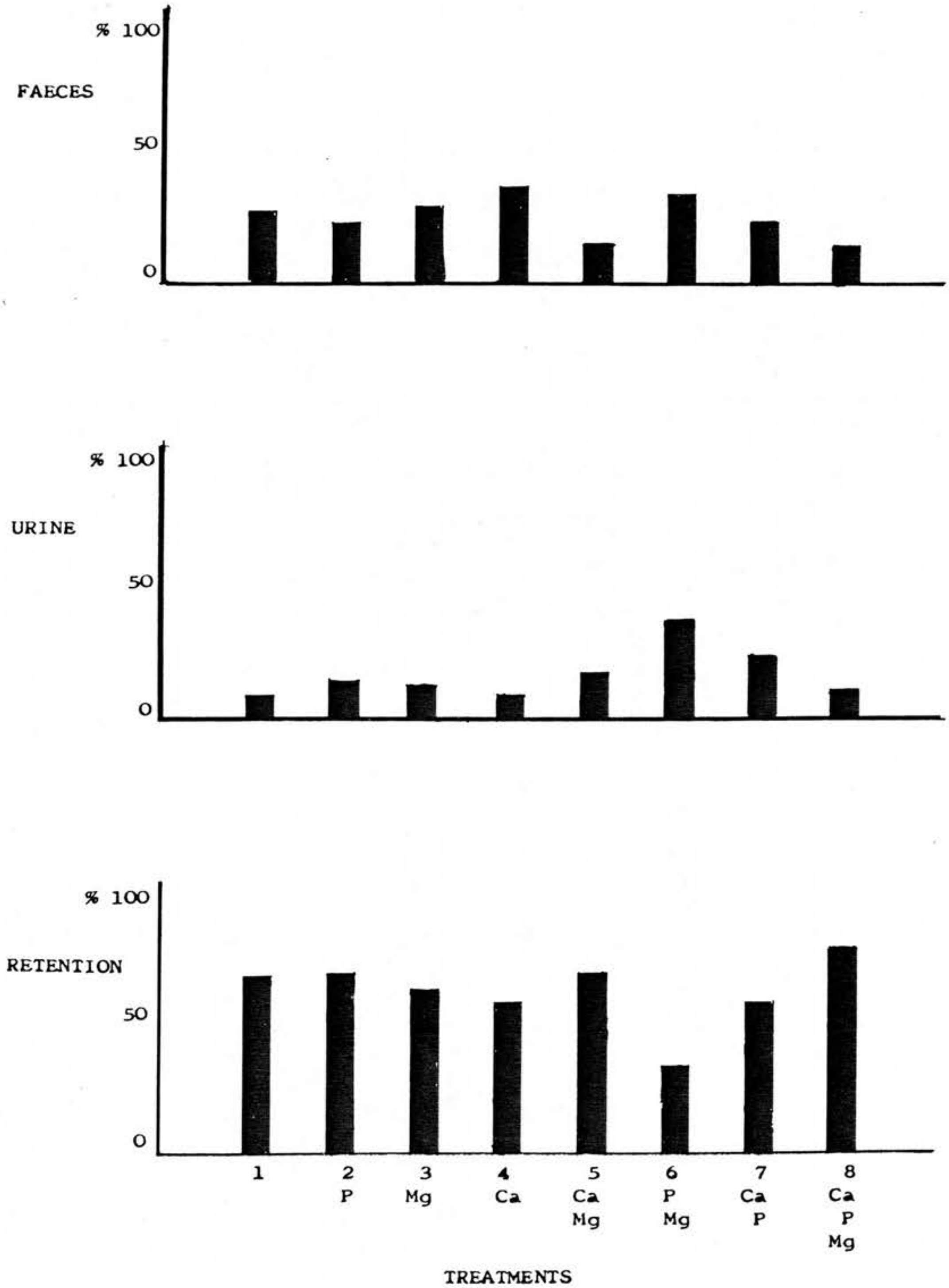


Figure 10. DAILY POTASSIUM EXCRETION AND RETENTION EXPRESSED AS A PERCENTAGE OF INTAKE IN EXPERIMENT II TRIAL A.

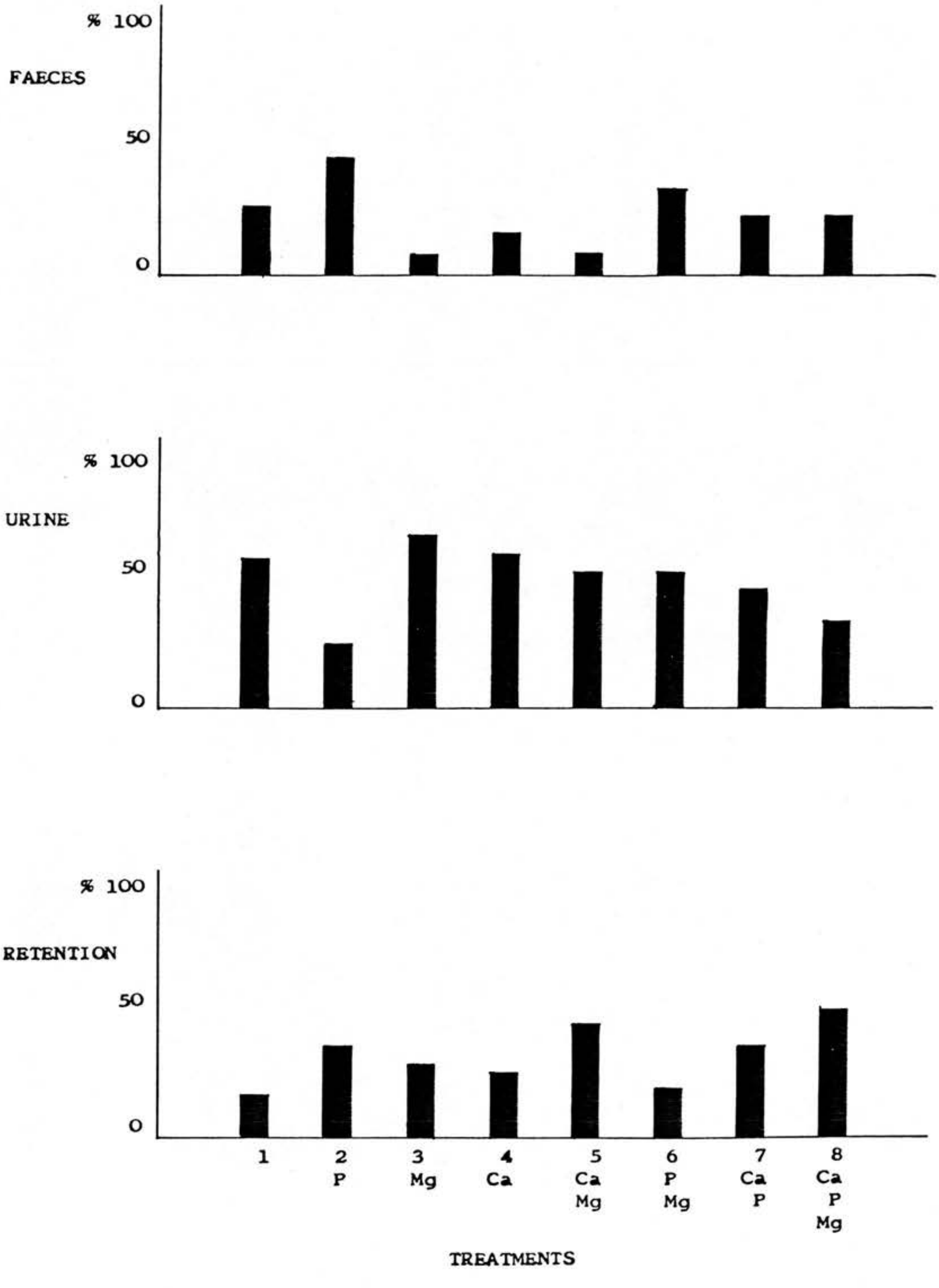
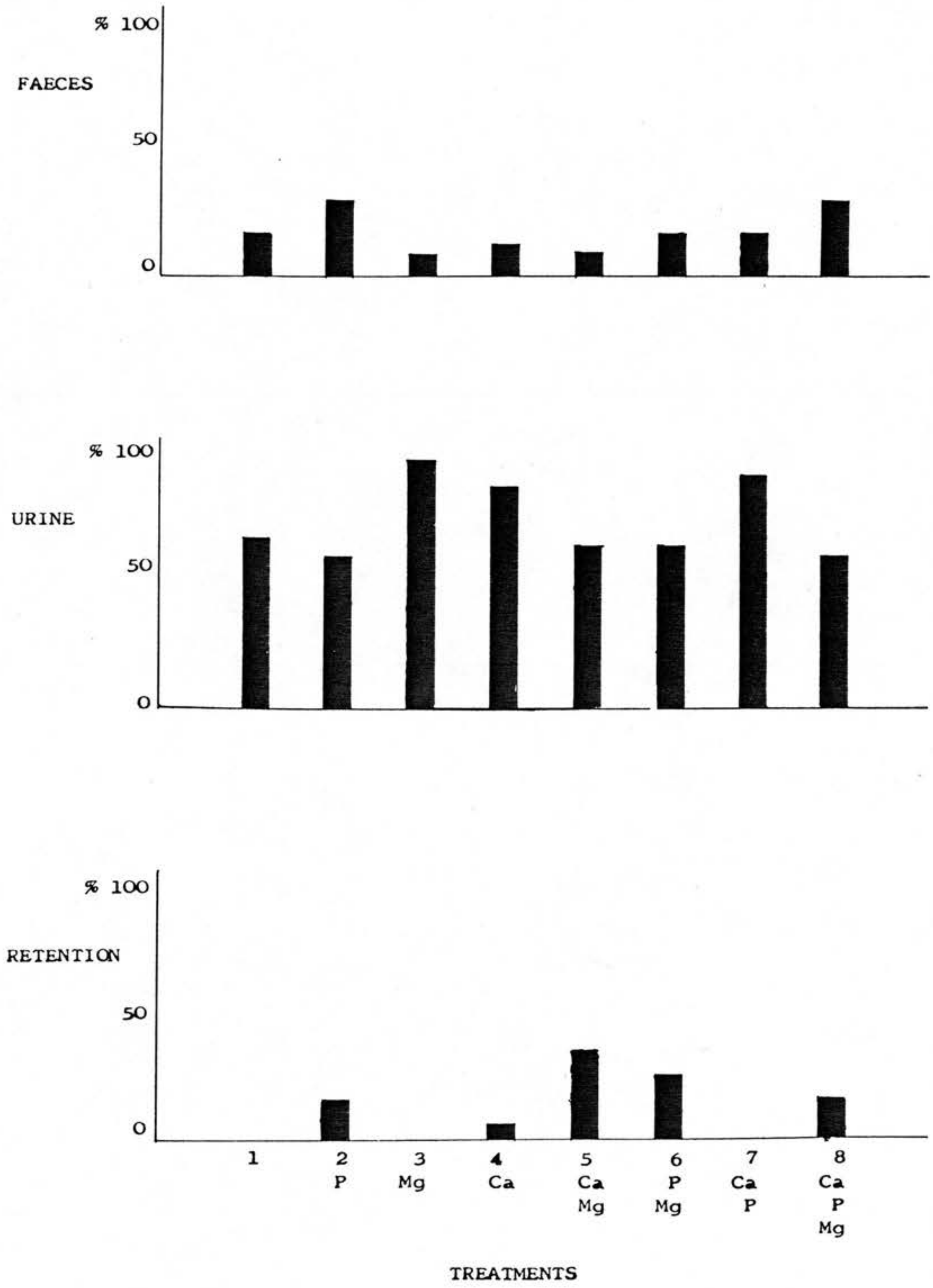


Figure 11. DAILY POTASSIUM EXCRETION AND RETENTION EXPRESSED AS A PERCENTAGE OF INTAKE IN EXPERIMENT II TRIAL B.



normal range during the current study.

3:2:4:2. Effect of time postweaning

There were distinct differences between metabolic trials A and B in most of the parameters measured: mineral digestibility, retention, urinary mineral excretion and concentration. Comparison of the data in this experiment shows that, during trial A, the urinary mineral concentration was approximately twice that measured in trial B (figure 1, p.182); it is established that urinary concentration of minerals is important in the formation of urinary calculi (Emerick et al., 1959; Nordin and Robertson, 1966; Pak, 1969). This concentration of urinary minerals during trial A is relevant in view of the fact that most clinical cases of urolithiasis occurred during the first few weeks of this experiment. This indicates that the immediate postweaning stage is of greater significance than first supposed although there is no reference in the literature relating this transitional period to the formation of calculi.

This time effect could be due to a number of different factors which are related to the physiological rather than the chronological age of the lamb. Symonds and Manston (1974) have shown that tubular reabsorption of phosphorus in the bovine kidney is between 95 and 99%, accounting for the low urinary phosphorus levels normally excreted by ruminants whereas the kidneys of most monogastrics have a far less efficient phosphorus reabsorptive capacity. One might thus surmise, that the relatively high urinary phosphorus concentration (95.6mg/100ml) during trial A (2 to 4 weeks postweaning) indicates that the lambs were excreting phosphorus in the manner characteristic of monogastrics whereas the relatively low phosphorus concentration (40.9mg/100ml) during trial B (7 to 9 weeks postweaning) was more like that of ruminants. Thus, the transition from a monogastric to a ruminant digestive process may be of considerable significance in the overall aetiology of urolithiasis in lambs. Furthermore, this could affect the digestibility of minerals as digestive processes, gut pH, digestive enzymes, and also food breakdown products will change during this period of time. Another factor which could be operating during this transitional period is that of hormonal change associated with sexual development and attainment of physical maturity. Some of the hormones that might be affected at this time are those associated with stress, ADH, growth hormone and the mineralocorticoids. Hormonal secretions will undoubtedly fluctuate during this period of time as various physiological mechanisms become established.

3:2:4:3. Water Metabolism

Low water intake and urine volume have been considered contributory factors to the formation of silica calculi in sheep (Forman, Sauer, Laughland and Davidson, 1958). Other workers (Udall, 1959; Schipper and Graves, 1968) have noted the benefit of enhanced water intake on reducing the incidence of phosphatic urolithiasis. This study has shown that the addition of calcium to the diet, a practice known to be beneficial in reducing calculus formation in lambs by reducing phosphorus digestibility and urinary phosphorus excretion, markedly reduced water intake and urine output by lambs. This latter effect is probably a reflection of the fact that the principle route of calcium excretion is via the faeces. In contrast, the addition of magnesium or phosphorus to the diet increased water intake and urine output. Bushman's (1967) balance trial data shows that lambs fed a diet containing 2% calcium carbonate voided the least urine (1122ml/24 hr.); even less than was produced (1277ml/24 hr.) by lambs fed the basal, unsupplemented diet. The most effective control of urolithiasis amongst lambs in Bushman's experiments was obtained by including 1% ammonium chloride in the diet. The volume of urine voided by these lambs was on average 1295ml/24 hr.; a figure little different from that obtained with the control lambs. Thus the results obtained by Bushman (1967) and those obtained in the current study indicate that water intake and urine output <u>per se</u> are not of primary importance in the aetiology of phosphatic calculus formation between 2 and 9 weeks postweaning.

Similar conclusions have been reached by other workers (Swingle and Marsh, 1953; Whiting, Connell and Forman, 1958) who were unable to relate water restriction to the occurrence of silica urolithiasis in cattle. However, Bailey (1969) demonstrated that the formation of siliceous urinary calculi was reduced in cattle given additional drinking water, although not prevented; Munakata, Suda and Ikeda (1974) observed that limiting the supply of drinking water to calves exacerbated the problem of urolithiasis.

3:2:4:4. Protein bound hexose metabolism

Udall (1959b) showed that the concentration of mucoprotein was inversely proportional to the levels of minerals in the diet but that the ash content of the diet did not affect the total amount excreted daily. Hoar, et al., (1969) found that the concentration of non-dialysable organic constituents of the urine was lowest in groups of lambs with the highest incidence of urolithiasis. Hoar (1970) showed that a calculus-provoking diet produced no increase in these urinary constituents. These findings are in agreement with those of the current study.

3:2:4:5. Autopsy findings

Renal Deposits

Mucopolysaccharide was observed in kidney tubules and in association with the pelvic epithelium, a finding similar to that made by Cornelius and Moulton (1960). Furthermore, calcium deposits and accumulations of PAS postive material were found in the medullary area of the kidneys taken from lambs at autopsy. Sahoo and Rao (1972) found similar deposits in the kidneys taken from sheep and goats. Schneeberger and Morrison (1965) fed rats a magnesium-deficient diet and induced the formation of calcium and PAS positive deposits in their renal medullary tubules. Davis, et al., (1969) fed a calcium-rich diet to lambs and found both tubular and intestitial deposits in renal tissue taken from these animals between 4 to 6 months of age. These animals did not form clinically apparent calculi and these authors suggested that medullary precipitates were a normal finding in domestic sheep. Renal tissue taken from human cadavers has been shown to contain calcium deposits/microcalculi although the cases were apparently 'normal' (Vermooten, 1942; Anderson and McDonald, 1946). The results of the current study support the view that renal deposits may be present in apparently normal subjects without subsequent progression to a clinical condition.

Renal Tissue

Phosphorus: calcium ratios were never less than 3:1 and in several cases were more than 10:1 in renal tissue taken from lambs in the current study. Gill and Vermeulen (1964/5) found that the phosphorus content of normal rat kidney was much higher than the calcium content and furthermore, was increased by dietary supplements of phosphorus. Renal cortical phosphorus content in lambs was not increased by phosphorus supplements in this study; however there was an increase in medullary phosphorus content. It is likely that renal mineral deposits would be increased in animals fed a calculus-provoking diet as kidneys taken from lambs with urolithiasis show histological evidence of mineral accumulations.

Barlet, Thériez and Molénat (1973) found that the renal phosphorus content of lambs developing calculi was about twice that of lambs which did not form calculi. Although the average calcium and magnesium values obtained in the current study were similar to the values obtained by Barlet, et al., (1973) the phosphorus levels were twice as much. McCance and Widdowson (1946) analysed normal ovine kidneys and obtained values similar to those reported in this thesis which indicates that the values obtained by Barlet, et al., (1973) were extremely low, even in cases where calculi developed. Although there are indications that renal phosphorus content is responsive to dietary supplements of phosphorus the results of the current study, although limited to an analysis of renal tissue taken from only 16 lambs, do not support a causal relationship between renal phosphorus content and calculus formation.

Calculous Material

Weaver (1966) considered that calculi recovered from sheep in Scotland were primarily composed of phosphates in association with amorphous organic material; the commonest form was magnesium ammonium phosphate. In the current study, lambs formed amorphous calculi composed largely of magnesium and phosphorus with a small amount of calcium and traces of ammonia nitrogen. Although it was impossible to confirm the precise chemical nature of these stones by x-ray crystallography, it was considered that these calculi were predominantly magnesium phosphate associated with a small amount of calcium phosphate. Calculi of a similar chemical form have been recovered from lambs fed concentrate diets (Packett and Hauschild, 1964; Packett and Coburn, 1965).

A survey (Prien, 1963) involving the crystallographic analysis of urinary calculi taken from man showed that magnesium ammonium phosphate hexahydrate was a calculus constituent in six to 14% of the calculi cases examined each year over a period of 23 years. These extensive crystallographic studies (Prien and Frondel, 1947; Prien, 1949; Prien, 1955; Prien and Prien, 1968) revealed that calculi were nearly always formed in alkaline urine and were associated with infection; phosphatic calculi were not invariably associated with infection. Apart from one case of cystitis, there was no evidence of urinary tract infection in animals which formed calculi in the current study.

Bone Material

Radiological examination and chemical analysis of bones taken from lambs at the end of the third period of the current experiment revealed no differences between those fed the control diet and those fed phosphorus supplements. Although, it has been shown that feeding different dietary mineral combinations to rats altered the bone ash of their femurs (Bushman, 1967). A search of the literature has failed to reveal any data on the composition of young lamb bones; however, Stewart (1934/5) published some data relating to the bones of 30kg lambs which indicates that the ash content of the bones of lambs in the current experiment were low. This might be explained by the fact that whole bones were analysed in this study and the epiphyses were probably not fully mineralised. It seems that although some of the treatment diets were grossly imbalanced with respect to minerals, they had no measurable effect on bone composition. Furthermore, the absence of hydroxyproline in the calculous material that was recovered suggests that bone was not being resorbed and contributing material to the formation of calculi.

3:2:4:6. Occurrence of calculi cases

Lambs fed treatment diets two and seven did not form calculi. This was surprising in view of the fact that treatment two contained a mineral combination (0.4% calcium, 0.75% phosphorus and 0.2% magnesium) which has been shown (Emerick and Embry, 1963; Bushman, et al., 1965a) to induce calculus formation in wether lambs. Weaver (1963) had a similar experience, in that he used a diet, shown by American workers to induce calculus formation, which failed to produce clinical symptoms of urolithiasis when fed to Blackface ram lambs. However, Weaver was able to explain this discrepancy on the basis of a changed calcium: phosphorus ratio in the diet; this explanation does not pertain to the current study, except perhaps in lambs fed the control diet who developed calculi.

Another surprising occurrence in this study was the formation of calculi in lambs fed a diet containing a mineral combination (0.8% calcium, 0.4% phosphorus and 0.2% magnesium) which has been shown not to produce calculi (Bushman, 1967). This random occurrence of calculi, apparently unrelated to treatment, suggests that minerals per se were not responsible for the initiation of calculus formation in lambs during the current experiment. It appears that the aetiology of calculus formation in these young lambs is different from that described in relation to the occurrence of urolithiasis amongst wethers kept in feedlots.

A feature of the calculi cases in this current study was that the majority occurred within a short time of weaning (figure 1, p.182). This is a situation previously described in calves (Black, 1976) and ascribed to a sudden change in fluid intake and urine output. One change in circumstances, common to all the experimental lambs in the current study, was the changeover from a liquid-based diet to a dry diet at weaning. Thus, there is probably a change in fluid economy and mineral control at this time, albeit of short duration. Vermeulen, Lyon, Ellis and Borden (1967) considered that peaks in urinary concentration, even if temporary, were causal; they considered that a brief drastic episode, such as this, could initiate the stone-forming process. Consequently, there may have been sufficient change in fluid economy at weaning to 'trigger' the stone-forming process in these young lambs. Thus, a further experiment was conducted to quantify any changes in fluid economy that take place at weaning (Experiment III).

3:3. EXPERIMENT III

3:3:1. Introduction

In Experiment II, most cases of urolithiasis were clinically apparent within 4 weeks of weaning. The most obvious change taking place at this time is the transference from a wholly liquid diet to a dry diet. This change might be expected to affect the lamb's total water and mineral intake, which in turn would modify urine volume and urinary mineral concentration.

With this in mind, an experiment was designed to investigate the precise nature of these effects at weaning. Furthermore, an attempt was made to assess the significance of the means of supplying water in relation to total water intake postweaning.

3:3:2. Materials and Methods

3:3:2:1. Plan of Investigation

Two groups of lambs, one containing three individuals and the other two, were to be weaned at 6 weeks of age from a milk substitute diet to a mostly concentrate diet. The age of weaning was decided on the basis that future experiments might involve surgical procedures and to allow sufficient recovery time; the youngest age at which operations could be readily carried out was thus 3 to 4 weeks. Furthermore, it was considered that the rumen should be allowed time to develop and acquire its 'adult position' within the body cavity.

In an attempt to (i) reduce the experimental errors associated with small numbers of lambs, (ii) correct for within-day and between-day variations of input and output of water and (iii) allow sufficiently long a period for attainment of a state of equilibrium after weaning, water intake and urine output were measured daily for 14 days before and after weaning. Lambs were individually fed milk substitute via a teat connected to a tube immersed in the lamb's allowance of substitute contained in a bucket. Postweaning, one group of lambs were to be provided with additional water using this means of supply. If lambs were reluctant to drink water from a bucket this could be of importance in practical rearing situations. The significance of water supply to lambs has been ignored and this was an opportunity to determine the effect of mode of supply.

3:3:2:2. Animals

Five 3 day old Suffolk-cross lambs (Suffolk ♂ x Half-bred ♀) were purchased for this experiment; three were females and two were males.

3:3:2:3. Diet

The lambs received their mother's milk up to the age of 3 days. At this age they were changed abruptly onto a milk substitute diet (appendix 8:1:1) which was individually fed via a teat. The lambs were kept together as a group although they were fed individually. Initially, they were fed four times a day; this was reduced to twice a day at 3 weeks of age. They were offered 850ml daily until 4 weeks of age when the allowance was increased to 1140ml per day. Abrupt weaning took place at 6 weeks of age. From 3 days of age to termination of the experiment at 8 weeks of age, good hay, creep feed (appendix 8:1:6) and fresh water were available ad lib..

3:3:2:4. Accommodation

Up to the age of 3 weeks the animals were kept together in a loose box containing straw bedding. At this age they were removed to a metabolic crate where they were held individually until the end of the experiment.

3:3:2:5. Experimental Procedure

When placed in the metabolic crates the animals were divided into two groups, group I and group II. Both groups were allowed 1 week to adapt to their new environment before water intake and urine output were measured. These measurements were made for the 14 days before the animals were weaned at 6 weeks of age. At weaning, the two ewe lambs and one ram lamb in group I continued to receive ad lib. fresh water, hay and creep feed. The remaining ewe and ram lamb in group II were offered additional water twice daily in place of their liquid milk substitute feeds using the same teat/tube/bucket system. Intake and output measurements were continued for 14 days postweaning when the experiment was terminated when the lambs were 8 weeks old.

The contribution of dietary and metabolic water was ignored in the calculations. It is unlikely that the contribution of dietary water would exceed 10% of total water intake in lambs fed a dry diet. Furthermore, the object of this experiment was to determine gross differences in intake and output.

3:3:3. Results

There was a considerable fall in water intake at weaning and this was reflected in the volume of urine excreted (appendix 8:5:1). This phenomenon was observed in both groups of lambs although the changes observed in group II animals were not so dramatic.

Expressing the mean water intake and mean urine output postweaning as a percentage of preweaning values (Table 84) serves to emphasise the magnitude of the change which takes place at weaning.

Table 84

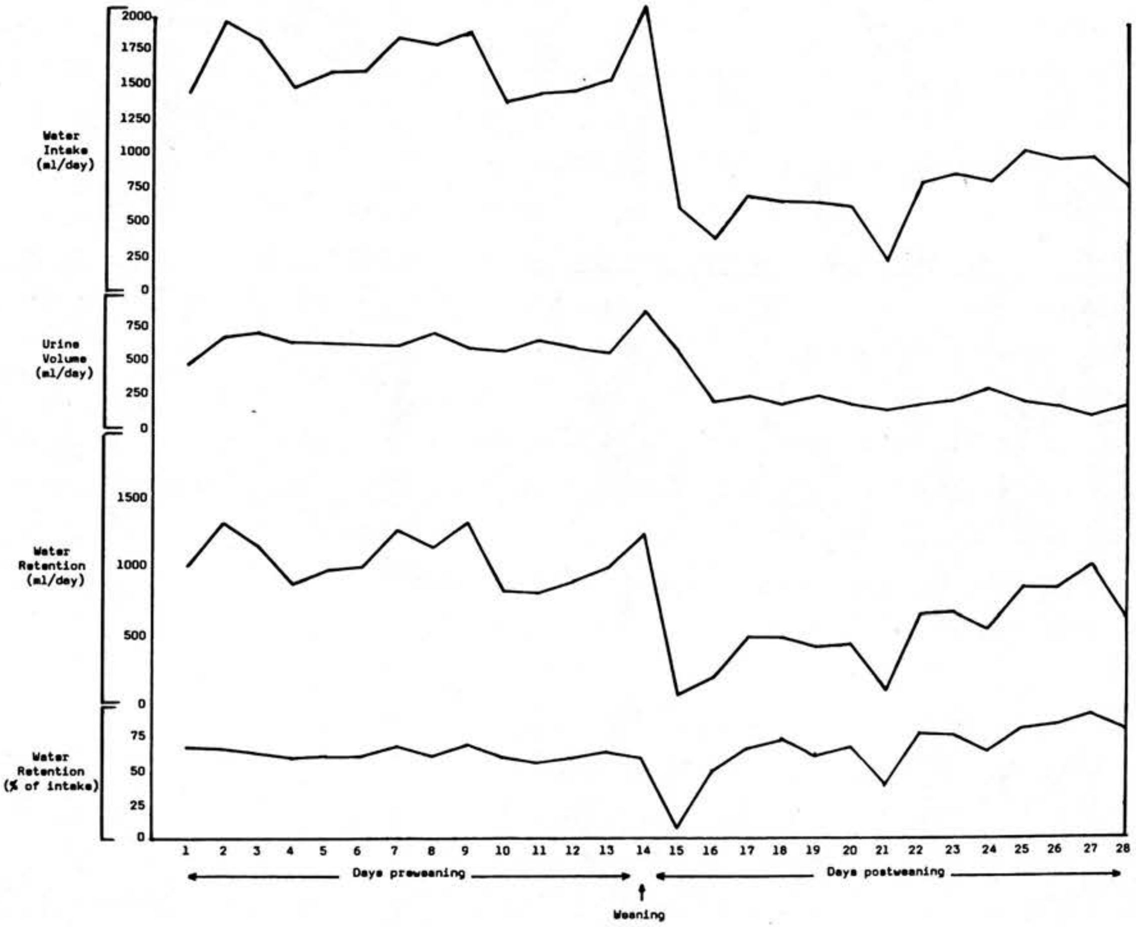
Overall daily means (ml/day) of water intake and urine output preweaning and postweaning.

Group	n	Measurement	Preweaning	Postweaning	$\frac{\text{Postweaning}}{\text{Preweaning}} \times 100$ %
1	3	Water intake	1676	744	44.4
		Urine output	629	221	35.1
II	2	Water intake	1963	1063	54.2
		Urine output	851	369	43.4

The results obtained with the group I animals are plotted graphically in figure 12. Both water retention and retention expressed as a percentage of intake show a marked fall at weaning. These results further show that in preweaned lambs, the volume of water retained by the animals is a function of water consumed. However, in weaned lambs, the volume of water retained is not a function of water consumed but of kidney function.

FIGURE 12

MEAN WATER INTAKE (ML/DAY), URINE OUTPUT (ML/DAY), WATER RETENTION (ML/DAY), AND WATER RETENTION EXPRESSED AS A PERCENTAGE OF INTAKE OF THREE LAMBS 14 DAYS BEFORE AND 14 DAYS AFTER WEANING AT 6 WEEKS OF AGE



3:3:4. Discussion

The reduction in urine output postweaning will effectively mean that the concentration of urinary constituents will be doubled if nutrient intake postweaning is the same as that preweaning. This concentrating effect could be particularly important in relation to urinary mineral content as various workers (Emerick and Embry, 1963; Packett and Hauschild, 1964) have shown that high urinary phosphorus values are associated with calculus formation.

A comparison of ewe's milk, milk substitute and creep feed shows that they have a similar mineral composition (Table 85). Probable dry matter intakes preweaning would be 300g milk substitute powder and 100g of creep feed. Postweaning lambs would consume about 400g creep feed and no milk substitute. Thus, using the data in Table 85, it is possible to assess probable mineral intakes pre- and postweaning (Table 86). These calculations indicate that mineral intake is slightly greater postweaning. This would exacerbate any situation where there was already a concentrating effect taking place in the kidney. Furthermore, it would seem that water intake is partially related to the mode of supply. The lambs (Group I) which were solely bucket-fed consumed the least water and therefore were likely to excrete a more concentrated urine than those in group II who received additional water via a teat.

Table 85

Comparison of the mineral content (g/kg DM) of ewe's milk, ewe milk substitute and lamb creep feed.

	Calcium	Phosphorus	Magnesium	Source
Ewe's milk	9.74	7.69	0.91	ARC (1965)
Milk substitute	6.36	5.32	0.88	(calculated values)
Creep Feed	9.20	6.90	1.70	(Laboratory analysis)

Table 86

Probable mineral intakes (g/day) of lambs pre- and postweaning.

	Preweaning			Postweaning
	Milk Substitute Powder (300g)	Creep Feed (100g)	Total	Creep Feed (400g)
Calcium	1.86	0.80	2.66	3.20
Phosphorus	1.56	0.60	2.16	2.40
Magnesium	0.26	0.15	0.41	0.59

It has been proposed (Vermeulen, et al., 1967; Vermeulen and Lyon, 1968) that transient phenomena are responsible for the idiopathic syndromes of urolithiasis seen in man. These authors refer to the importance of peaks in urinary concentration, although temporary, which may 'trigger' the stone-forming process. The relationship, observed in preweaned lambs in the current study, between water retention and water consumption indicates that the renal control of water balance is weak. In contrast, in the weaned lamb, renal control is important. Water retained by lambs immediately postweaning was dramatically reduced; this quantity only returned to the preweaning level 10 to 14 days after weaning. It is likely, therefore, that lambs are under considerable physiological stress during this 10 day postweaning period. Furthermore, it may be predicted that the inability of the lamb to maintain the water content of its 'milieu interior' during this period may well have elicited the secretion of ADH by the pituitary with a subsequent concentration of the urine. This could act as a potent stimulus to calculus formation. Koch and Turner (1961) have indicated that in ovines, the high potassium genotype begins to be expressed at 8 to 9 weeks of age and the animal assumes its adult characteristics. It is possible that the lambs regulatory mechanisms are not fully developed until it reaches this age.

3:4. EXPERIMENT IV

3:4:1. Introduction

It was established (3:3) that water intake and urine output by lambs were dramatically reduced at weaning. A hypothesis was formulated suggesting that urinary constituents would be concentrated postweaning and that this might lead to calculus formation. In Experiment II most cases of urolithiasis occurred shortly after weaning. Black (1976) postulated that reduced water intake after weaning, due to the cessation of milk substitute feeding, was probably responsible for calculus formation in Zebu calves 3 to 6 weeks postweaning. Holstein calves did not form calculi, possibly because they maintained a higher water intake postweaning compared to Zebu calves.

A small pilot experiment was carried out to investigate the relation between the observed changes in water economy at weaning and the microscopic structure of lamb kidney.

3:4:2. Materials and Methods

3:4:2:1. Plan of Investigation

Two groups of two lambs each were to be weaned at 6 weeks of age from a milk substitute diet to a mostly concentrate diet. One group was to receive additional water via an infant feeding tube postweaning. The purpose of this was to maintain the preweaning water intake postweaning. The other group would be allowed to undergo the 'normal' postweaning reduction in water intake. Fourteen days after weaning, both groups of lambs would be killed and the kidneys examined both macroscopically and microscopically for evidence of calculus formation.

3:4:2:2. Animals

Four 3 day old Cheviot-cross ram lambs (Cheviot ♀ x Border Leicester ♂) were purchased for this experiment.

3:4:2:3. Diets

The diets used in this experiment and the feeding management was as described in 3:3:2:3.

3:4:2:4. Accommodation

This was the same as that described in 3:3:2:4.

3:4:2:5. Experimental Procedure

At 3 weeks of age each lamb was fitted with a 40cm long infant feeding tube,[*] FG6. The tubes were passed via the nares and oesophagus into the stomach. In order to confirm the correct location of the tube a syringe was attached to the free end and some of the stomach contents aspirated. This procedure was not always successful and an alternative method was employed. Surgical stainless steelwire,[**] 24 SWG, was introduced into the feeding tube. A 35cm length was used to avoid the possibility of damaging any internal organs. The animal was x-rayed to confirm correct positioning of the feeding tube and then the wire was withdrawn. Local anaesthetic[***] was injected into the nasal area and the hub of the feeding tube sutured to the skin adjacent to the nares using 2/0 nylon sutures.

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[*] Vygon Ltd., Unit 3, Eskdale Road, Uxbridge, Middlesex.

[**] Ethicon Ltd., Bankhead Avenue, Edinburgh.

[***] Xylocaine, Astra Chemicals Ltd., Animal Health Division, Watford, Hertfordshire.

Lambs were placed in metabolic crates and allowed 1 week to adapt to their new environment. Water intake and urine output were measured from the beginning of their second week in the metabolic crates. It was intended that when the lambs were weaned at 6 weeks of age, two of the four animals would be connected to water reservoirs via blood administration sets**** attached to their indwelling feeding tubes. Thus, it would have been possible to maintain the preweaning level of water intake postweaning until termination of the experiment when the lambs were 8 weeks old.

3:4:3. Results

The lambs persistently regurgitated the feeding tubes during the second week in the metabolic crates and the experiment was abandoned.

3:4:4. Discussion

Infant feeding tubes were unsatisfactory as a means of supplying additional water to lambs. The tubes were probably regurgitated because the lambs were consuming small amounts of hay as well as concentrate and were thus starting to ruminate. Furthermore, the tubes were designed for use with infants and were very soft and pliable so that they could easily coil and move in the gut. Regurgitation of feeding tubes has been encountered in carnivora (Darke, 1978) and in this case was overcome by inserting the tube via a pharyngostomy, a procedure described by Lane (1977).

**** Avon Blood Administration Set A15, Avon Medicals Ltd., Birmingham, B30 3DR.

3:5. EXPERIMENT V

3:5:1. Introduction

For the reasons outlined in 3:4:1. an attempt was made to augment water intake postweaning. After the failure of the oesophageal cannula (3:4) it was decided to administer water directly into the rumen via a fistula. To enhance the possibility of obtaining some renal changes, a low calcium, high phosphorus diet was fed.

3:5:2. Materials and Methods

3:5:2:1. Plan of Investigation

Essentially, this was the same as outlined in 3:4:2:1. except that at 3 weeks of age, a fistula was to be created in the left flank of each lamb so that water could be directly administered into the rumen. Two of the lambs were to be killed at 6 weeks of age in case there were any renal changes preweaning.

3:5:2:2. Animals

Six 3 day old Suffolk-cross ram lambs (Suffolk ♂ x Halfbred ♀) were purchased for this experiment.

3:5:2:3. Diets

The diet and feeding management of the lambs was the same as that described in 3:3:2:3. except at 4 weeks of age the daily milk substitute allowance was increased to 2000ml. The creep feed (appendix 8:1:6) was supplemented with 23kg of FSL No.4* (appendix 8:1:3:4) per tonne so that the final diet contained 0.58, 1.00 and 0.14% of calcium, phosphorus and magnesium respectively on an air dry basis.

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* Feed Service (Livestock) Ltd., Hartham, Corsham, Wiltshire, SW13 0QB.

3:5:2:4. Accommodation

This was the same as described in 3:3:2:4. except that two of the lambs remained in the loose box until they were killed at 6 weeks of age.

3:5:2:5. Experimental Procedure

3:5:2:5:1. Surgical

Surgery was performed on four of the lambs at 3 weeks of age. Anaesthesia was induced and maintained with a halothane/nitrous oxide/oxygen mixture. A ruminal fistula was created in the left upper flank of each lamb, 2 to 3 cm behind the costal margin. At this site, a 2 to 4 cm long laparotomy incision was made into which the rumen was elevated and anchored to the abdominal musculature using chromic cat gut (3.5mm). An intravenous cannula* 120mm long, FG13 was inserted for its full length into the reticulo-rumen and the butterflies at the proximal end of the cannula were sutured to the skin (plates 10, 11 and 12).

3:5:2:5:2. Postoperative

Following cannula insertion, the four lambs were removed to a metabolic crate and allowed 1 week to acclimatise. At the beginning of the second week in the crate, two of the lambs were attached via their respective cannulae to blood administration sets** which in turn were connected to reservoirs (plate 13) which were suspended above the metabolic crate. Throughout the 2 weeks preweaning, water intake and urine output of the four lambs was recorded. Dry-matter intake was measured for 1 week preweaning. At

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* Vygon Ltd., Unit 3, Eskdale Road, Uxbridge, Middlesex.

** Avon A15, Avon Medicals Ltd., Birmingham, B30 3DR.

Plate 10.

Lamb - left flank showing indwelling cannula.

Plate 11

Lamb - forestomachs and abomasum showing point of insertion of cannula.

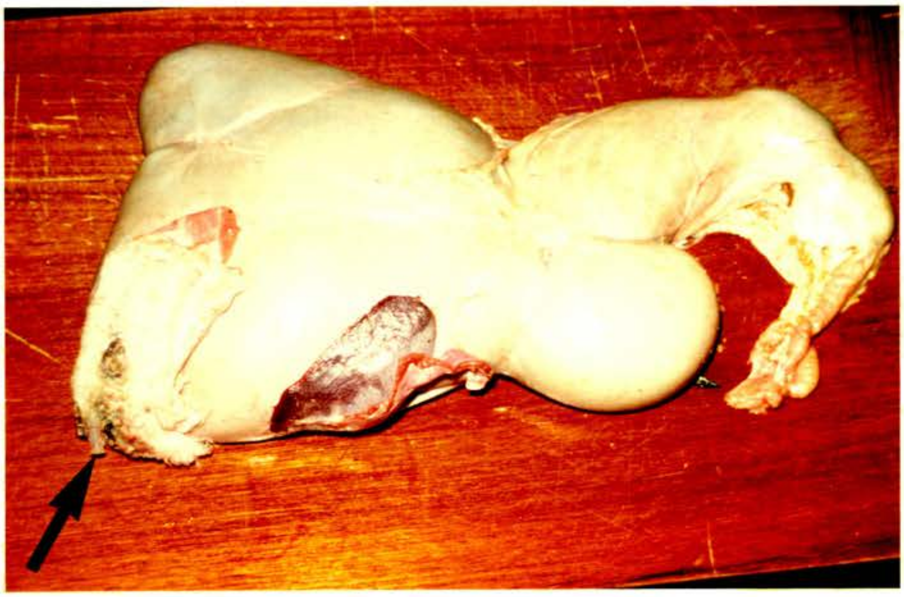
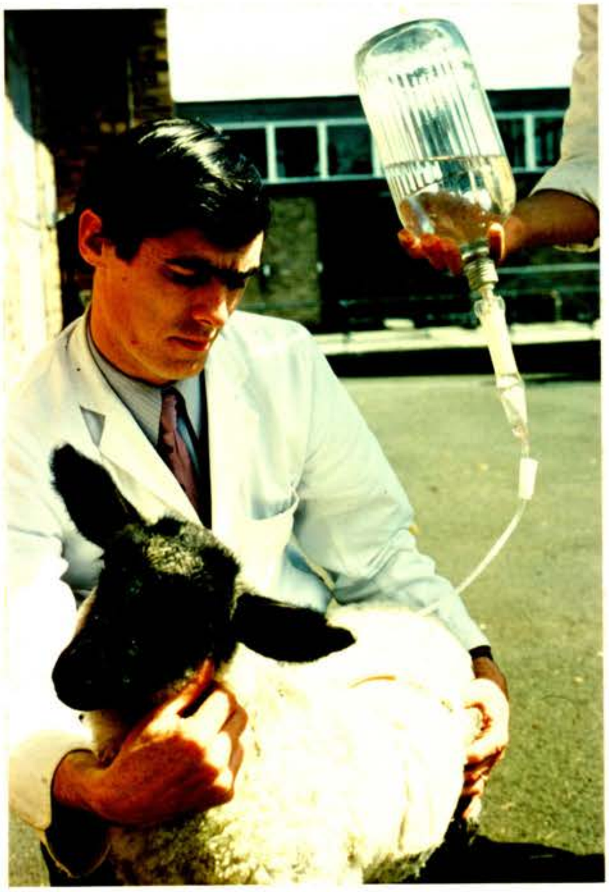


Plate 12

Lamb rumen opened to show position of cannula.

Plate 13.

Apparatus for introducing deionised water directly into rumen.



weaning, the reservoirs were filled with deionised water and 2000ml of this water was dripped into each of the two lambs throughout a 24 hr period for the next 14 days. During this period of time, water and drymatter intake and urine output of each of the four lambs was measured daily. At the end of the 14 day postweaning period, the four lambs were killed and autopsied as described in 3:1:2:7:5. as were the two lambs that were killed at 6 weeks of age.

3:5:2:6. Analytical Methods

Renal tissue was examined as described in 3:1:2:8:4.

3:5:3. Results

3:5:3:1. Water Metabolism

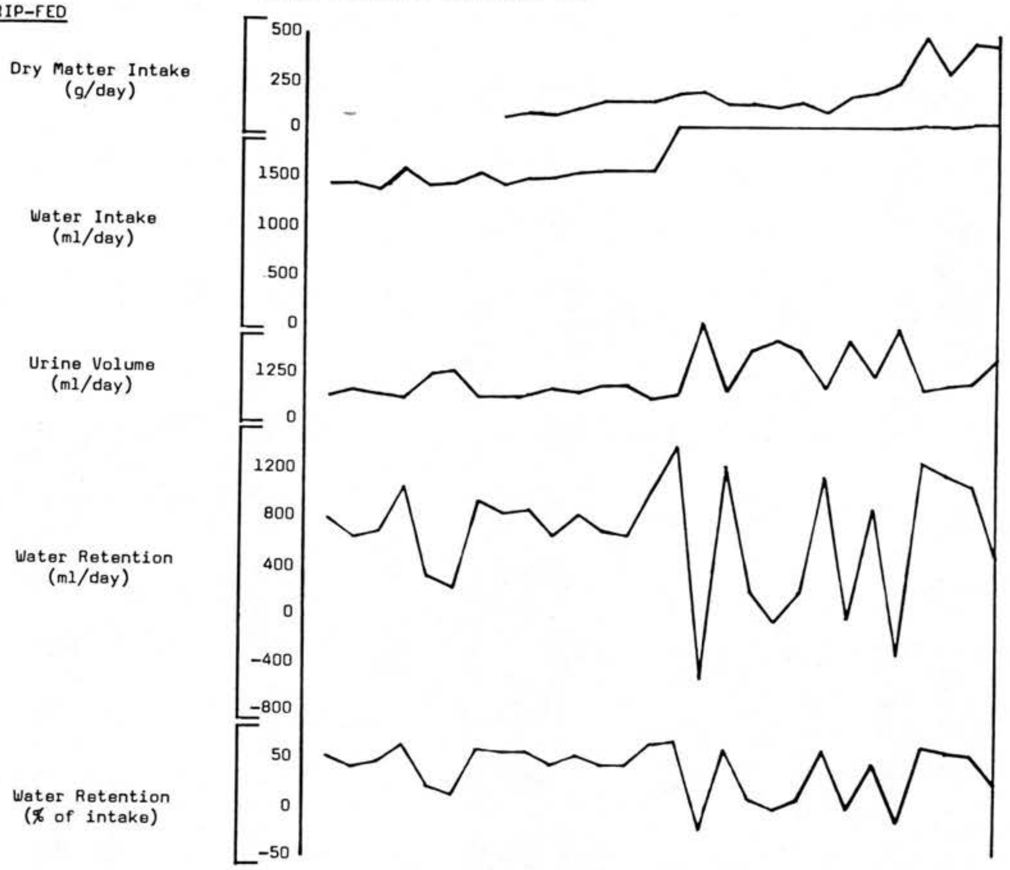
The mean daily values (of two lambs) for the two groups of lambs are recorded in appendix 8:5:2. and shown graphically in figure 13. The mean water intake and urine output postweaning of the two lambs not receiving water via a drip was much less than those connected to the dripset. The former showed the drop in water intake (29.7%) and in urine output (47.9%) postweaning that was characterised in Experiment III.

The water intake, urine volume and consequently water retention were lower in the 'non-drip' group preweaning. The overall volume of water retained by this group was no different pre- and postweaning although the volume retained during the first 10 days postweaning was much less than that retained by the 'drip' group. These animals may have

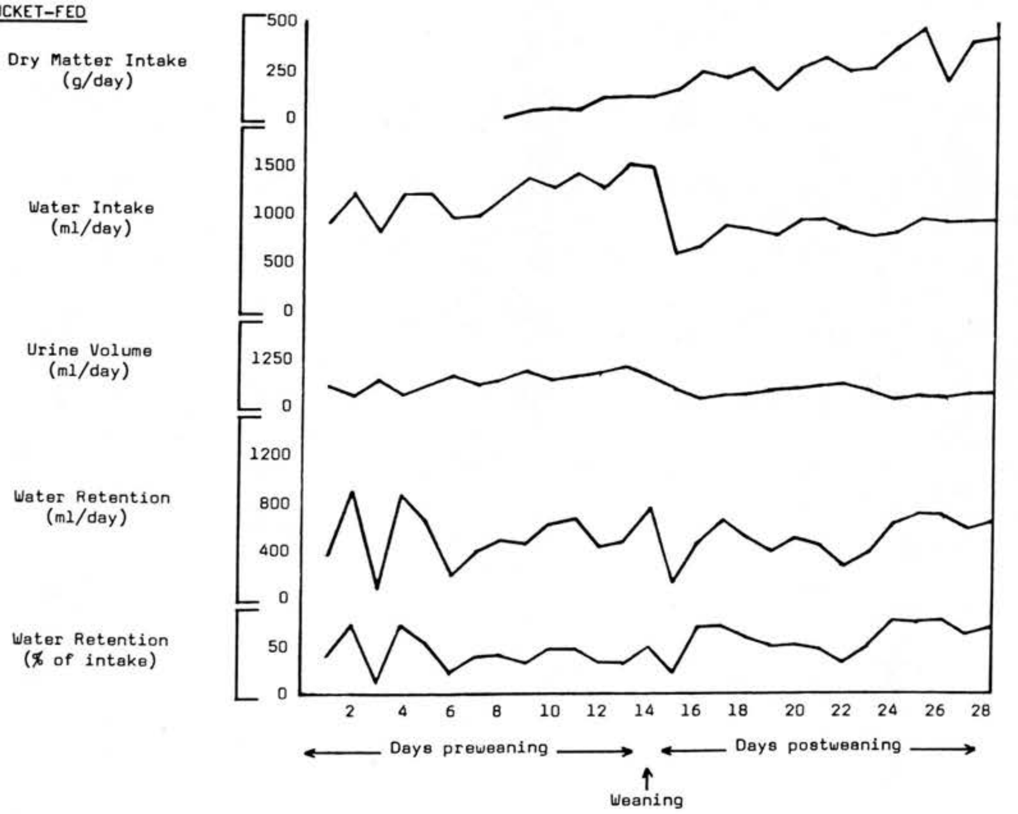
FIGURE 13

MEAN DRY MATTER INTAKE (G/DAY), WATER INTAKE (ML/DAY), URINE OUTPUT (ML/DAY), WATER RETENTION (ML/DAY), AND WATER RETENTION EXPRESSED AS A PERCENTAGE OF INTAKE OF TWO LAMBS DRIP-FED WATER (a) AND OF TWO LAMBS BUCKET-FED WATER (b), 14 DAYS BEFORE AND 14 DAYS AFTER WEANING AT 6 WEEKS OF AGE

(a) DRIP-FED



(b) BUCKET-FED



been experiencing some degree of non-clinical dehydration which may have involved an increased secretion of ADH and furthermore, they may have excreted greater than normal concentrations of minerals during this 10 day period which would increase the likelihood of stone formation.

During the postweaning period, the'drip'group exhibited several negative water retentions.

3:5:3:2. Autopsy

No abnormality could be detected in the viscera taken from the two lambs killed at 6 weeks of age.

There was no calculous material in the urinary tracts of the lambs killed at 8 weeks of age. Furthermore, microscopic examination of the kidneys taken from these four lambs revealed no changes which could be associated with calculus formation. The kidneys taken from the two lambs connected to the dripsets (numbers 2 and 4) showed a degree of hypertrophy (plate 14).

3:5:4. Discussion

Although the 'normal' postweaning reduction in water intake and urine output occurred in two of the lambs, this could not be associated with any evidence of calculus formation in the kidneys of these animals.

The negative water retentions of the 'drip' lambs may reflect a total inhibition of ADH release as a result of over-infusion (the volume of water infused was about 500ml greater than that normally consumed preweaning); such inhibition may have caused the episodic fluctuations in water

Plate 14.

Sectioned kidneys from four lambs. One and three untreated controls, two and four intraruminal infusion.

retention. It is noteworthy however, that the mean retention over the 14 day postweaning period was within the realms of normality. The over-infusion (over-hydration) was reflected by the hypertrophied kidneys of the 'drip-fed' lambs.

The small group numbers involved in this experiment could account for the failure to show renal changes associated with the formation of calculi. A high incidence of clinical urolithiasis of 10% in a flock would require that at least 10 animals be used to give an expectation of one clinical case; although it is a fair assumption that for every clinical case there should be several subclinical cases as demonstrated by Weaver (1963); he failed to produce clinical symptoms in lambs using a high phosphorus diet, although autopsy revealed renal calculi in 25% of the lambs used in the trial. If the current experiment had been continued for a further 2 weeks, some changes might have been evident; clinical cases of urolithiasis occurred up to 4 weeks postweaning in Experiment II.

The findings of this experiment support those of Experiment III that the immediate postweaning period is one of considerable physiological stress and therefore potentially of great significance in the aetiology of calculus formation in early weaned lambs.

4. General Discussion

General Discussion

The initial objective of undertaking this study was to examine the relationship between various dietary parameters and urinary calculus formation in early weaned lambs. The results obtained from Experiment I indicated that although the levels (10, 14 and 18% crude protein) and sources (urea, and soya/fishmeal) of dietary nitrogen used did not result in urolithiasis there was evidence of enhanced urinary phosphorus excretion by lambs fed protein supplemented diets. In addition, the pelleting of diets caused an increase in the digestibility of minerals and subsequent urinary excretion and was therefore more likely to cause calculi; supplementation of the diet with urea would seem to have the opposite effect. The results of Experiment II showed that dietary mineral combinations, shown by others (e.g. Bushman, 1968) to induce calculus formation in store lambs, did not induce the formation of urinary calculi in early weaned lambs. Thus, it seems that the aetiology of calculus formation in early weaned lambs is different from that established for store lambs. The formation of calculi in the early postweaning period suggests that a link exists between change in diet at weaning and the susceptibility of early weaned lambs to urolithiasis. For example, the results of Experiments III and V showed a marked reduction in water intake by lambs when they were changed from a liquid milk substitute diet to an air dry concentrate diet. Unfortunately, these small-scale exploratory experiments failed to establish a clear association between a reduction in water consumption and calculus formation, in that no renal deposits were observed in the kidneys of the lambs used in these experiments.

An aetiology which appears to conform to the evidence obtained in these experiments is one first postulated by Randall in various publications in the period between 1930 and 1950 (Randall, 1936; Randall, 1937a and b; Randall, 1940; Randall, 1944). In this theory of calculus formation, Randall suggested that subsurface deposits of calcium in the wall of the renal papilla represent the initiating lesion. Subsequently, the epithelial covering is removed exposing the calcium plaque to the urine, and allowing growth. Calcium deposits (von Kossa positive) such as Randall described were observed underlying the pelvic epithelium of kidneys taken from calculi cases in Experiment II.

Randall (1940) further proposed that intratubular deposits could occur, which might lead to formation of a calculus where the collecting tubule enters the pelvic area. Rosenow (1940) and Posey (1942) both confirmed the presence of these calcium deposits during routine human autopsy. They suggested that the papillary lesion preceded clinical stone disease and that subsequent stone growth depended on factors such as urine concentration, infection etc. Based on the evidence obtained from their extensive crystallographic studies, Prien and Prien (1968) also supported Randall's plaque theory.

Complementary to Randall's theory of calculus formation is the concept of embryogenesis put forward recently by another group of workers. It was suggested (Vermeulen, et al., 1967; Vermeulen and Lyon, 1968) that minute 'embryos' appear inside the ducts in the papilla. The small size of the duct lumen retards passage of these 'embryos' and they have the opportunity to grow before being discharged into the pelvis. These authors introduced the idea of 'triggering' which relies on some brief drastic episode to induce papillary embryogenesis and, although conditions may return to normal, stone growth continues.

Various workers have indicated that mineral deposits are a normal feature of the renal pelvic area. Vermooten (1942) could not account for the generalised distribution of calcification observed in human kidneys examined at postmortem. He suggested that when calcium deposits were present under the pelvic epithelium they might ulcerate through this layer of cells. Anderson and McDonald (1946) found microscopic calculi in the kidneys taken from both normal and abnormal human cases. They concluded that this form of calculus occurred in the kidneys of all people more than 10 years old. Kunkel, Whitaker, Packett and Crookshank (1961) conducted a histopathological study of kidneys taken from store lambs and found 76% showed signs of incipient urolithiasis. Microcalculi were located in the collecting tubules and renal pelvis and these authors suggested the explanation of this occurrence lay not simply in nutritional terms. Furthermore, calcium deposits and calculi have been observed beneath the epithelium, lining the renal pelvis of rat kidney (Coburn and Packett, 1962; Wexler, 1963a and b). Examination of the kidneys taken from calculi cases in Experiment II showed subepithelial calcium deposits ulcerating through or bursting-out of the pelvic epithelium into the lumen (Plate 4, p.186). One of the photomicrographs produced by Anderson and McDonald (1946) demonstrates an identical phenomenon.

One of the attractions of a 'triggering' concept is that it explains away idiopathic conditions where an investigation fails to yield meaningful data. This was precisely the situation in Experiment II where although calculi occurred, there was no apparent explanation for their formation. The histopathological evidence accumulated during this experiment supports Randall's view of the initiating lesion. The question arises however, what causes these renal deposits? The most obvious factor is the change in fluid economy at weaning. Both Experiments III and V showed that there is a dramatic fall in urine output immediately postweaning. Furthermore, the results of Experiment II showed that urinary mineral concentrations in trial A were twice those of trial B. Thus, the effect of the fall in urine output postweaning is exacerbated by the large quantities of minerals excreted in the urine. It is apparent that lambs are under considerable physiological stress for at least the first 10 days postweaning and this could act as the 'trigger' for the formation of renal deposits. Unfortunately, the kidneys taken from lambs at the end of Experiment V did not contain any renal deposits. However, Vermeulen's concept of embryogenesis merely states that the"embryos have the opportunity to grow" and it might well be that many are discharged into the pelvis and are subsequently excreted leaving no trace of their existence in the kidney. Indeed, calculi do not invariably form in lambs fed so-called 'calculus-inducing diets'.

The results recorded in Experiment I do not support the hypothesis that a reduction in water intake at weaning is the 'trigger' for calculus formation. The two cases of urolithiasis which did occur in this experiment were evident much later than those in Experiment II. Furthermore, histopathological examination of the kidneys taken from the remaining lambs in Experiment I did not reveal any significant calcium /mucopolysaccharide deposits. In view of the fact that lambs used in each experiment were of the same type and managed in a similar fashion, it is hard to explain why, if there is a 'weaning effect', it did not manifest itself in Experiment I. Nevertheless, there were differences in mineral excretions between trials and the urinary calcium concentration in trial A was about ten times that seen in lambs during trial A of Experiment II.

An attempt was made, using the data from Experiment II, to identify which other factor/factors might be involved in the formation of calculi by comparing lambs which formed calculi with those that did not. However, between period differences precluded amassing the data from all animals that developed calculi. Since the comparisons were limited to within periods, period three was selected because most calculi cases (six) occurred during this period. The 't' test was used to compare water intakes, urine volumes, urine and serum minerals of the calculus-forming group with the remaining animals. However, these comparisons were not valid as the variances of the two samples were unequal. Notwithstanding this, it was apparent that both serum magnesium and urinary phosphorus levels were elevated in the calculus-forming animals. These findings support those of Eveleth and Millen (1939) and Kunkel, et al., (1961) who demonstrated that a significant correlation exists between serum magnesium and the quantal incidence of calculi in store lambs. Furthermore, others (Bushman, et al., 1965b; Barlet, et al., 1973) have demonstrated a phosphaturia in store lambs that developed calculi. However, elevation of serum magnesium and urinary phosphorus can be achieved simply by dietary manipulation and these phenomena do not clarify the mechanism of calculus formation.

Comparison of the various measurements made during Experiments I and II reveals no evidence to support the theory that high dietary magnesium levels are conducive to the formation of calculi. The levels of magnesium incorporated in the experimental diets were between five to ten times ARC (1965) recommended levels and there was no apparent association between calculus formation and dietary magnesium level, apart from the fact that the calculi were composed of magnesium phosphate.

The incidence of calculi in the control lambs in Experiment II was possibly due to the narrowness of the calcium: phosphorus ratio (1:1). It has been demonstrated (Hoar, 1970) that calculi form in store lambs fed a diet containing a calcium: phosphorus ratio less than 1.5:1. Certainly, the ARC (1965) stipulate a calcium: phosphorus ratio of 1.8:1 widening to 2.1:1.

Scott and Dobson (1965) have shown that urine does not normally appear to be a major pathway for phosphorus excretion in sheep. They quantified daily excretion of phosphate by sheep fed a pelleted grass diet and found it averaged only about 160mg; this amounted to about 8% of dietary intake. Furthermore, Scott (1969) has shown that urinary phosphorus excretion by sheep was less than 100mg daily. In both Experiments I and II, the urine was an important route of phosphorus excretion in early weaned lambs, although its relative importance decreased with time postweaning. This might be because the transfer to a concentrate diet induces a transient metabolic acidosis which could involve phosphate acting as a buffer to maintain acid-base balance. As the animal matures, it becomes physiologically adapted to its new diet and changes from a monogastric to a ruminant type of digestion. Values obtained for urinary phosphorus excretion during Experiments II were as much as ten times greater than those of Scott (1969) and furthermore, urine losses accounted for as much as 20% of dietary intake. The work of Young, Lofgreen and Luick (1966) showed that ruminants do not rely on the kidney as a way of excreting phosphate, unlike the monogastric, and that major variations in phosphate balance are controlled by intestinal secretion. Thus, it may be concluded that these lambs were behaving as monogastrics in their handling of phosphate. The reticulo-rumen of these lambs was found to be thin-walled and generally poorly developed when examined at postmortem. It is conceivable that the feeding of all-concentrate diets to early weaned lambs delays their physiological development into ruminants and that such a delay may be a causal factor in the development of embryonic calculi in their kidneys.

Practical implications of experimental findings

1. The indications are that lambs reared artificially on milk substitute drink less water when changed from a teat system to a bucket-only system. Consideration should be given to encouraging lambs to drink milk substitute from a bucket rather than via a teat.
2. The changeover at weaning should be a gradual process, ensuring that water is supplied well before weaning actually takes place. Furthermore, the supply of milk substitute should be reduced gradually so that an abrupt weaning is avoided.
3. Consideration should be given to weaning lambs later than 28 days of age so that they are better adapted to dry diets, can consume more food and their reticulo-rumen is better developed.

4. Avoid using concentrate diets which are rich in minerals - phosphates in particular - and ensure an adequacy of calcium. The calcium: phosphorus ratio should be at least 1.5:1. Consider including salt in the early weaning concentrate to encourage water intake.
5. Conventional protein supplements might be replaced by urea in concentrate diets although there may be a penalty in terms of reduced animal production.
6. Avoid using pelleted diets, particularly when the diet is rich in minerals as pelleting will invariably improve their digestibility and increase the likelihood of raised urinary mineral levels and thus calculus formation.

5. Topics requiring further research work.

5:1. Time scale of calculus development postweaning.

In Experiment II, histopathological examination of the kidneys of two 4 week old lambs at weaning revealed no apparent lesions. The first case of urolithiasis was apparent between 1 to 2 weeks postweaning although most cases occurred between 2 to 5 weeks postweaning. However, it is not known how much time passes between the initiating lesion and the occurrence of clinically apparent urolithiasis and this is an area which requires further research.

5:2. Water economy around the time of weaning.

The work initiated in Experiments III and V should be repeated with larger groups of lambs to obtain more precise measurements of the changes that take place at weaning. In addition, to measuring fluid balance, osmolality and urine mineral levels should be investigated to determine any changes in mineral excretion which take place that might contribute to calculus formation. Electron microscopic studies of renal tissue taken from these lambs might provide information on the precise mode of calculus formation and on the effect of water restriction on renal ultrastructure. Weaning lambs at different ages would show if all lambs take about 10 days postweaning to recover control of their water balance and if this is a function of age. The precise relation between water retention and water intake remains to be established to see if there is a threshold level of water intake. Lastly, to see if there is a juvenile control of water metabolism which is superseded by adult mechanisms.

5:3. Infection

Lambs undergo considerable stress at weaning and infection cannot be excluded as an aetiological agent. It is quite conceivable that lambs suffer subclinical infections at weaning. Keyser (1945) has suggested that the nephritic lesions which precede renal stone may be produced by bacterial toxins and Vermooten (1942) considered that these toxins could injure the lining epithelium of the collecting tubules and thus play a part in plaque formation. Certainly, some workers (e.g. Packett, *et al.*, 1958) have demonstrated that the inclusion of antibiotics in the diets for lambs reduced the incidence of urolithiasis. It is possible that these antibiotics suppressed a subclinical infection although with the levels employed this was unlikely. Nevertheless, it is another topic which requires further investigation.

5:4. Vitamin A

Over the years, vitamin A deficiency has been held to be the cause of urolithiasis in rats (Van Leersum, 1928a and b), guinea pigs (Steiner, Zuger and Kramer, 1939) and calves (Vasudevan and Dutt, 1969). However, no critical experiments have been carried out using adult sheep or lambs. Richards (1972) has demonstrated marked changes in renal function in calves as they become retinol depleted but before there was clinical evidence of a deficiency. Associated with the change in renal function, there was a phosphaturia. About 10% of these calves developed calculi and several had tubular deposits in their kidneys (Richards, 1978). Furthermore, Webb, *et al.*, (1968) indicated that vitamin A-deficient wethers showed a marked polyuria and phosphaturia. The latter effect has been established as being conducive to calculus formation. In view of the fact that lambs are born with low liver reserves of retinol and if housed intensively before weaning onto concentrate diets, they will be totally dependent on dietary additions of retinol to meet their requirements. Thus, the role of vitamin A in urolithiasis amongst lambs requires investigation.

5:5. Genetic Aspects

Evans (1957) reported considerable variation in the water metabolism of Blackface sheep and was able to show that part of this variation was attributable to the potassium (K) type of the red blood cells. He demonstrated that the mean water intake and urine output for 19 low K sheep was 1845 and 552 ml/24 hrs. whereas the means for 17 high K sheep were 2459 and 1073 ml/24 hrs. respectively. In further work (Evans, Harris and Warren, 1958), he examined the frequency of the high blood K type in different breeds of British sheep. Generally speaking, the hill breeds showed a much higher frequency than the lowland breeds. The biological significance of this feature requires investigation in relation to the incidence of urolithiasis.

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8. APPENDIX

8.1 Food Composition Tables

8.1:1 Composition of milk substitute powder (% air dry)

Fat	30.00
Mean solid not fat	66.25
Moisture	3.50
Fibre	0.25
	100.00

8.1:2 Composition of the creep feed used in Experiments I and II (% air dry)

Barley	40.00
Flaked Maize	20.00
Locust Bean Meal	15.00
Linseed Chips	10.00
Dried grass	5.00
Molasses	5.00
Fishmeal	3.00
Vitamins/Minerals	2.00
	100.00

8.1:3 Composition of mineral/vitamin supplements

8.1.3.1 Coopers Beta 212 (g/Kg as fed)

Calcium	187
Phosphorus	40
Magnesium	32
Vitamin A	0.078
Vitamin D_3	0.002

8:1:3:2. Coopers Beta No. 102 TE. (g/Kg as fed)

Vitamin A	.88
Vitamin D_3	.022

+ trace minerals

8:1:3:3. Ibex (g/Kg as fed)

Calcium	149
Phosphorus	238

8:1:3:4. FSL No. 4 (g/Kg as fed)

Calcium	175
Phosphorus	185

8.1:4. Mean Composition of Experiment I treatment diets (g/kg DM)

	Treatments							
Constituents	1	2	3	4	5	6	7	8
Crude Protein	115.3	116.8	206.8	207.8	167.3	160.4	195.1	194.1
Calcium	8.2	10.1	9.6	9.9	10.2	11.3	8.4	10.5
Phosphorus	7.1	7.5	7.8	7.8	7.8	7.7	6.5	7.3
Magnesium	2.1	2.2	2.1	2.4	2.1	2.6	1.9	2.1
Sodium	2.3	2.6	3.1	2.9	2.9	2.7	2.4	2.4
Potassium	4.6	3.3	6.5	6.2	5.6	4.4	4.4	4.5

8.1:5. Mean Composition of Experiment II treatment diets (g/kg DM)

	Treatments							
Constituents	1	2	3	4	5	6	7	8
Crude Protein	164.3	161.8	164.7	166.4	168.2	162.0	159.1	165.5
Calcium	5.4	5.5	4.4	10.0	10.0	5.1	10.9	9.9
Phosphorus	4.6	9.6	4.5	4.3	4.8	9.0	9.8	9.3
Magnesium	2.7	2.9	5.1	2.7	5.5	4.0	2.9	4.9
Sodium	0.7	0.65	0.7	0.7	0.7	0.6	0.7	0.6
Potassium	6.1	6.9	6.8	6.4	7.4	6.6	7.2	7.0

8.1:6. Composition of creep feed used in Experiments III, IV and V (% air dry)

Rolled oats	50
Decorticated ground nut cake	10
Linseed meal	10
Flaked maize	10
Bran	10
Locust bean meal	10
	100

10 kgs trace mineral/vitamin mixture added/tonne finished feed.

8:2. Statistical Tables

TABLE 8:2:1.

THE EFFECT OF UREA SUPPLEMENTATION ON SOME ASPECTS OF METABOLISM MEASURED IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENT	TRIAL	TREATMENT MEANS			COMPARISONS			
					T2 vs. T6		T6 vs. T8	
		BD(T2)	LUD(T6)	HUD(T8)	SE	SIG	SE	SIG
CALCIUM METABOLISM								
Digestibility	A	28.4	34.1	31.1	7.12	NS	8.99	NS
(%)	B	26.5	31.1	31.2	8.33	NS	7.66	NS
Urinary Excretion	A	712	385	751	150.27	*	206.71	NS
(mg/14 days)	B	1568	606	1154	340.92	*	389.92	NS
Retention	A	18324	27455	17927	5.86	NS	6.55	NS
(mg/14 days)	B	27050	28447	26452	8.60	NS	7.80	NS
Urinary Concentration	A	27.6	15.1	22.9	6.81	NS	5.81	NS
(mg/100ml)	B	29.7	12.7	18.1	6.63	*	6.82	NS
Serum Concentration	A	11.7	11.6	11.6	0.38	NS	0.36	NS
(mg/100ml)	B	12.0	12.3	12.2	0.50	NS	0.46	NS
PHOSPHORUS METABOLISM								
Digestibility	A	50.3	45.7	52.9	6.72	NS	6.62	NS
(%)	B	53.5	52.6	46.5	7.26	NS	7.95	NS
Urinary Excretion	A	6038	3286	4733	880.33	**	1325.80	NS
(mg/14 days)	B	12208	6338	8069	2018.98	*	2632.09	NS
Retention	A	17777	18517	14959	3320	NS	3330	NS
(mg/14 days)	B	28348	30260	21935	4.29	NS	5.34	NS
Urinary Concentration	A	236.5	134.5	164.9	63.08	NS	60.16	NS
(mg/100ml)	B	235.8	145.5	130.7	54.02	NS	53.79	NS
Serum Concentration	A	9.0	8.7	9.0	0.57	NS	0.49	NS
(mg/100ml)	B	10.1	9.8	9.6	0.56	NS	0.47	NS

MAGNESIUM METABOLISM (continued over)

MEASUREMENT	TRIAL	TREATMENT MEANS			COMPARISONS			
					T2 vs. T6		T6 vs. T8	
		BD(T2)	LUD(T6)	HUD(T8)	SE	SIG	SE	SIG
MAGNESIUM METABOLISM								
Digestibility	A	51.5	50.3	50.3	3.97	NS	4.04	NS
(%)	B	51.6	53.5	49.6	4.76	NS	4.82	NS
Urinary Excretion	A	4050	3407	2916	690.4	NS	1215.0	NS
(mg/14 days)	B	6151	3972	4479	963.4	*	1040.2	NS
Retention	A	3535	4921	3177	1.01	NS	0.9	NS
(mg/14 days)	B	5905	7803	4766	1.48	NS	1.32	*
Urinary Concentration	A	146.0	132.1	84.9	31.42	NS	32.22	NS
(mg/100ml)	B	112.2	79.4	70.2	23.40	NS	21.59	NS
Serum Concentration	A	2.8	2.8	2.6	0.12	NS	0.09	NS
(mg/100ml)	B	2.9	2.8	2.8	0.14	NS	0.09	NS
SODIUM METABOLISM								
Digestibility	A	88.2	88.6	90.5	2.48	NS	2.44	NS
(%)	B	90.2	90.5	90.0	2.27	NS	1.68	NS
Urinary Excretion	A	10303	11283	8886	1681.7	NS	1546.63	NS
(mg/14 days)	B	19436	18213	19315	3826.21	NS	3841.49	NS
Retention	A	3978	2712	2709	1.20	*	1.10	NS
(mg/14 days)	B	4327	5254	2027	2.93	NS	2.23	NS
Urinary Concentration	A	386.8	434.8	288.5	86.02	NS	62.00	*
(mg/100ml)	B	363.3	314.0	292.7	73.61	NS	59.51	NS
Serum Concentration	A	356.6	358.2	358.1	3.66	NS	4.03	NS
(mg/100ml)	B	356.8	356.3	356.8	4.14	NS	3.17	NS

POTASSIUM METABOLISM (continued over)

MEASUREMENT	TRIAL	TREATMENT MEANS			COMPARISONS			
					T2 vs. T6		T6 vs. T8	
		BD(T2)	LUD(T6)	HUD(T8)	SE	SIG	SE	SIG
POTASSIUM METABOLISM								
Digestibility	A	82.0	86.5	87.4	2.69	NS	2.45	NS
(%)	B	86.9	90.5	89.4	1.78	NS	1.78	NS
Urinary Excretion	A	9164	10355	7541	1831.82	NS	1898.46	NS
(mg/14 days)	B	15345	16291	18363	4620.36	NS	5609.99	NS
Retention	A	16538	13813	17353	5.47	NS	5.02	NS
(mg/14 days)	B	24824	26040	23037	6.53	NS	8.01	NS
Urinary Concentration	A	349.9	403.4	254.5	94.18	NS	80.65	NS
(mg/100ml)	B	280.8	264.3	267.2	49.99	NS	48.47	NS
Serum Concentration	A	21.8	21.7	21.5	0.74	NS	0.80	NS
(mg/100ml)	B	20.6	20.4	20.3	0.53	NS	0.54	NS
WATER METABOLISM								
Water Retention	A	435.4	414.8	374.2	62.57	NS	59.50	NS
(ml/24hr)	B	801.9	838.0	760.8	133.08	NS	99.78	NS
Urine Volume	A	226.3	220.2	241.5	49.91	NS	46.41	NS
(ml/24hr)	B	475.7	450.8	491.2	123.02	NS	88.32	NS
PROTEIN-BOUND HEXOSE METABOLISM								
Urinary Concentration	A	40.9	42.6	37.5	6.01	NS	6.12	NS
(mg/100ml)	B	31.8	31.6	28.8	4.46	NS	3.40	NS
Urinary Excretion	A	159.8	159.6	158.4	20.96	NS	19.89	NS
(mg/24hr)	B	247.4	254.7	257.1	42.03	NS	34.82	NS
Serum Concentration	A	0.147	0.145	0.142	0.08	NS	0.08	NS
(g/100ml)	B	0.149	0.153	0.150	0.08	NS	0.08	NS

8:2:2.

THE EFFECT OF PROTEIN SUPPLEMENTATION ON SOME ASPECTS OF METABOLISM MEASURED IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

MEASUREMENT	TRIAL	TREATMENT MEANS			COMPARISONS			
		BD(T2)	LPD(T5)	HPD (T4)	T2 vs T5		T5 vs T4	
					SE	SIG	SE	SIG
CALCIUM METABOLISM								
Digestibility	A	28.4	28.1	34.7	7.89	NS	5.87	NS
(%)	B	26.5	30.7	35.1	5.56	NS	4.89	NS
Urinary Excretion	A	712	571	760	138.35	NS	448.78	NS
(mg/14 days)	B	1568	996	955	391.03	NS	409.91	NS
Retention	A	18324	18875	24871	5640	NS	3990	NS
(mg/14 days)	B	27050	30537	33542	7930	NS	5970	NS
Urinary Concentration	A	27.5	17.4	14.8	6.88	NS	8.55	NS
(mg/100ml)	B	29.7	14.2	11.3	6.61	*	5.17	NS
Serum Concentration	A	11.7	12.0	11.6	0.40	NS	0.38	NS
(mg/100ml)	B	12.0	12.4	12.0	0.48	NS	0.38	NS
PHOSPHORUS METABOLISM								
Digestibility	A	50.3	54.8	47.3	9.06	NS	8.66	NS
(%)	B	53.5	59.5	56.8	8.01	NS	8.01	NS
Urinary Excretion	A	6038	6836	6086	1754.39	NS	2248.09	NS
(mg/14 days)	B	12208	14332	13635	2018.98	*	5199.47	NS
Retention	A	17777	21061	19343	4210	NS	3900	NS
(mg/14 days)	B	28348	34082	34675	6090	NS	6940	NS
Urinary Concentration	A	236.5	209.9	140.0	56.36	NS	50.46	NS
(mg/100ml)	B	235.8	201.5	168.4	54.02	NS	51.60	NS
Serum Concentration	A	9.0	9.0	8.9	0.54	NS	0.37	NS
(mg/100ml)	B	10.1	9.9	10.1	0.45	NS	0.42	NS

MAGNESIUM METABOLISM (continued over)

MEASUREMENT	TRIAL	TREATMENT MEANS			COMPARISONS			
					T2 vs T5		T5 vs T4	
		BD(T2)	LPD(T5)	HPD(T4)	SE	SIG	SE	SIG
MAGNESIUM METABOLISM								
Digestibility (%)	A	51.5	48.7	54.9	4.11	NS	2.93	NS
	B	51.6	52.0	55.8	2.26	NS	2.30	NS
Urinary Excretion (mg/14 days)	A	4050	3765	3749	650.47	NS	682.91	NS
	B	6151	5118	4553	818.9	NS	1101.5	NS
Retention (mg/14 days)	A	3535	2988	5865	1000	NS	820	**
	B	5905	6345	9425	1680	NS	1990	NS
Urinary Concentration (mg/100ml)	A	146.0	117.1	92.3	19.96	NS	13.32	NS
	B	112.2	73.5	58.3	16.06	*	16.37	NS
Serum Concentration (mg/100ml)	A	2.8	2.7	2.8	0.13	NS	0.11	NS
	B	2.9	2.9	2.9	0.15	NS	0.19	NS
SODIUM METABOLISM								
Digestibility (%)	A	88.2	91.7	89.8	1.31	*	2.01	NS
	B	90.2	92.5	89.4	2.01	NS	2.29	NS
Urinary Excretion (mg/14 days)	A	10303	12397	12481	1596.1	NS	1899.8	NS
	B	19436	24504	26340	3501.7	NS	11791.9	NS
Retention (mg/14 days)	A	3978	4203	5592	1.21	NS	2.07	NS
	B	4327	2419	158	2.43	NS	1.37	*
Urinary Concentration (mg/100ml)	A	386.8	411.0	331.3	71.89	NS	60.49	NS
	B	363.3	346.9	315.6	56.04	NS	56.82	NS
Serum Concentration (mg/100ml)	A	356.6	353.0	358.6	3.48	NS	4.15	NS
	B	356.8	354.7	355.8	4.13	NS	3.34	NS
POTASSIUM METABOLISM								
Digestibility (%)	A	82.0	89.0	88.7	2.37	*	2.78	NS
	B	86.9	90.3	90.9	3.30	NS	2.04	NS
Urinary Excretion (mg/14 days)	A	9164	14147	16651	2000.2	*	5876.1	NS
	B	15345	20655	30109	3151.4	NS	3787.3	*

Retention/....

MEASUREMENT	TRIAL	TREATMENT MEANS			COMPARISONS			
					T2 vs T5		T5 vs T4	
		BD(T2)	LPD(T5)	HPD(T4)	SE	SIG	SE	SIG
Retention (mg/14 days)	A	16538	20586	23628	6.61	NS	7.19	NS
	B	24824	33093	29531	6.45	NS	7.23	NS
Urinary Concentration (mg/100ml)	A	349.9	463.3	451.7	85.06	NS	99.26	NS
	B	280.8	306.6	378.1	59.52	NS	84.78	NS
Serum Concentrations (mg/100ml)	A	21.8	22.4	22.2	1.03	NS	1.27	NS
	B	20.6	21.4	20.7	0.64	NS	0.64	NS
WATER METABOLISM								
Water Retention (ml/24hr)	A	435.4	522.9	538.8	82.01	NS	70.49	NS
	B	801.9	879.8	902.0	173.55	NS	165.30	NS
Urine Volume (ml/24hr)	A	226.3	246.5	329.9	48.15	NS	48.91	NS
	B	475.7	534.3	684.1	116.94	NS	107.75	NS
PROTEIN-BOUND HEXOSE METABOLISM								
Urinary Concentration (mg/100ml)	A	40.9	43.8	36.1	6.45	NS	6.18	NS
	B	31.8	29.7	28.0	4.12	NS	3.47	NS
Urinary Excretion (mg/24hr)	A	159.8	185.6	212.0	16.66	NS	17.63	NS
	B	247.4	291.4	338.7	44.34	NS	44.84	NS
Serum Concentration (g/100ml)	A	0.147	0.147	0.141	0.08	NS	0.08	NS
	B	0.149	0.154	0.154	0.08	NS	0.08	NS

TABLE 8:2:3.

THE EFFECT OF INTERACTION BETWEEN PHYSICAL FORM OF THE DIET AND LINEAR ADDITIONS OF PROTEIN OR UREA ON SOME ASPECTS OF PROTEIN BOUND HEXOSE METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE.

MEASUREMENTS	TRIAL	n	INTERACTIONS		SE		SIG	
			PROTEIN/ PELLETING	UREA/ PELLETING	(1)	(2)	(1)	(2)
Urinary Concentration (mg/100ml)	A	16	2.610	3.485	3.390	3.401	NS	NS
	B	"	1.635	-1.400	2.500	2.647	"	"
Urinary Excretion (mg/24 hr)	A	"	5.265	13.94	12.572	14.430	"	"
	B	"	-5.285	4.755	22.282	27.479	"	"
Serum Concentration (g/100ml)	A	"	0.0005	-0.0035	0.0038	0.0039	"	"
	B	"	0.0060	0.0000	0.0048	0.0049	"	"

TABLE 8:2:4

THE EFFECT OF INTERACTION BETWEEN DIFFERENT LEVELS OF CALCIUM (0.4 and 0.8%), MAGNESIUM (0.2 and 0.4%) AND PHOSPHORUS (0.4 and 0.75%) ON SOME ASPECTS OF WATER METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

		TRIAL A						TRIAL B					
		WATER INTAKE [a] (ml/week)		URINE OUTPUT [a] (ml/week)		WATER RETENTION [c] (ml/24 hrs)		WATER INTAKE [b] (ml/week)		URINE OUTPUT [b] (ml/week)		WATER RETENTION [b] (ml/24 hrs)	
INTERACTION	N	Interaction value	SE	Interaction value	SE	Interaction value	SE	Interaction value	SE	Interaction value	SE	Interaction value	SE
CALCIUM / MAGNESIUM	32	1111	832	820	685	43	24	4961	3731	4990	3696	42	71
CALCIUM / PHOSPHORUS	32	–744	832	882	685	13	24	1832	3731	1913	3696	58	71
MAGNESIUM / PHOSPHORUS	32	176	832	156	685	6	24	2444	3731	2082	3696	68	71
CALCIUM / MAGNESIUM / PHOSPHORUS	32	1091	832	678	685	48	24	3142	3731	2593	3696	30	71

a 1 Missing Value

b 11 Missing Values

c 2 Missing Values

TABLE 8:2:5

THE EFFECT OF INTERACTION BETWEEN LEVELS OF CALCIUM (0.4 and 0.8%), MAGNESIUM (0.2 and 0.4%) AND PHOSPHORUS (0.4 and 0.75%) ON SOME ASPECTS OF PROTEIN BOUND HEXOSE METABOLISM IN WETHER LAMBS DURING TWO 14 DAY METABOLIC TRIALS BEGUN AT SIX (TRIAL A) AND ELEVEN (TRIAL B) WEEKS OF AGE

		TRIAL A						TRIAL B[c]					
		URINE CONCENTRATION[a] (mg/100ml)		URINE EXCRETION[a] (mg/24 hrs)		SERUM CONCENTRATION[b] (mg/100ml)		URINE CONCENTRATION (mg/100ml)		URINE EXCRETION (mg/24 hrs)		SERUM CONCENTRATION (mg/100ml)	
INTERACTION	N	Interaction value	SE	Interaction value	SE	Interaction value	SE	Interaction value	SE	Interaction value	SE	Interaction value	SE
CALCIUM/ MAGNESIUM	32	−1.7	4.69	15	21	−2.2	4.6	−0.9	2.77	86	88	4.4	3.5
CALCIUM/ PHOSPHORUS	32	16.2**	4.69	−11	21	−9.9*	4.6	2.6	2.77	34	88	−0.8	3.5
MAGNESIUM/ PHOSPHORUS	32	0.7	4.69	3	21	−4.9	4.6	0.3	2.77	71	88	−6.3	3.5
CALCIUM/ MAGNESIUM/ PHOSPHORUS	32	4.7	4.69	24	21	−0.9	4.6	1.8	2.77	96	88	−0.8	3.5

[a] 2 Missing values
[b] I Missing value
[c] II Missing values

* $p < 0.05$
** $p < 0.01$

8:3. Analysis of Variance

Urine Volume data from Experiment II Trial A:

Periods	Animals	Treatments 1	2	3	4	5	6	7	8	Period Totals
1	1	126.2	383.9	464.6	272.3	132.3	1508.5	380.0	265.4	6163.3
	2	207.7	323.1	446.9	150.8	459.2	408.3	270.8	363.3	
2	3	228.6	411.9	216.4	151.1	196.8	217.7	189.3	345.7	4296.3
	4	367.8	189.3	241.1	217.5	197.0*	154.7	329.3	642.1	
3	5	496.0	247.5	435.0	257.9	136.7	534.3	2629.6	602.5	8864.5
	6	483.1	343.9	1065.4	295.7	558.6	285.0	207.3	286.0	
4	7	331.5	303.3	199.2	203.1	189.2	1032.9	291.5	214.0*	5636.7
	8	827.1	235.0	667.5	234.6	193.2	105.0	395.7	213.9	
Treatment Totals		3068.0	2437.9	3736.1	1783.0	2063.0	4246.4	4693.5	2932.9	

Correction factor $= \frac{(\Sigma x)^2}{n} = \frac{24960.8^2}{64}$ $n = 64$

The main effects of the three factors (calcium, magnesium and phosphorus) and their interaction may be calculated as follows:

	Source of Variation	1	2 +P	3 +Mg	4 +Ca	5 +Ca/Mg	6 +Mg/P	7 +Ca/P	8 +Ca/Mg/P	Factorial Effect Totals
		3068.0	2437.9	3736.1	1783.0	2063.0	4246.4	4693.5	2932.9	
Main Effects	Calcium	1	1	1	-1	-1	1	-1	-1	2016.0
	Magnesium	1	1	-1	1	-1	-1	1	-1	-996.0
	Phosphorus	1	-1	1	1	1	-1	-1	-1	-3660.6
Interactions	Calcium/Magnesium	1	1	-1	-1	1	-1	-1	1	-3957.2
	Calcium/Phosphorus	1	-1	1	-1	-1	-1	1	1	3900.2
	Magnesium/Phosphorus	1	-1	-1	1	-1	1	-1	1	- 900.2
	Calcium/Magnesium/ Phosphorus	1	-1	-1	-1	1	1	1	-1	3181.0

* Calculated missing values

The sum of squares of the main effects are obtained by squaring the factorial effect totals and dividing by n

e.g. sum of squares of the main effect of calcium $= \frac{2016^2}{64}$

Similarly, the sum of squares of the interactions are obtained by squaring the factorial effect totals and dividing by n

It is possible to begin constructing the analysis of variance table as follows:-

Source of Variation	df	Sum of Squares
Ca	1	$2016^2 \div 64$
Mg	1	$996^2 \div 64$
P	1	
Ca/Mg	1	etc.
Ca/P	1	
Mg/P	1	
Ca/Mg/P	1	

The period sum of squares is calculated by summing the squares of the period totals dividing by n (in this case 16) and then subtracting the correction factor i.e.

$$\frac{6163.3^2 + 4296.3^2 + 8864.5^2 + 5636.7^2}{16} - \frac{24960.8^2}{64}$$

The remaining source of variation which has to be quantified is the interaction between treatment and period. This necessitates firstly summing the data within periods i.e.

Urine volume data from Experiment II Trial A: /....

Periods	Treatments 1	2	3	4	5	6	7	8	Totals
1	333.9	707.0	911.5	423.1	591.5	1916.8	650.8	628.7	6163.3
2	596.4	601.2	457.5	368.6	393.8	372.4	518.6	987.8	4296.3
3	979.1	591.4	1500.4	553.6	695.3	819.3	2836.9	888.5	8864.5
4	1158.6	538.3	866.7	437.7	382.4	1137.9	687.2	427.9	5636.7
Totals	3068.0	2437.9	3736.1	1783.0	2063.0	4246.4	4693.5	2932.9	

The total period x treatment interaction sum of squares is obtained by:

$$\left[\frac{(333.9^2 + 707.0^2 + \dots + 427.9^2)}{2}\right] - \left[\left(\frac{(6163.3^2 + \cdots + 5636.7^2)}{16} - \frac{24960.8^2}{64}\right)\right] -$$

$$\left[\frac{(3068.0^2 + 2437.9^2 + \cdots + 2932.9^2)}{8} - \frac{24960.8^2}{64}\right] - \frac{24960.8^2}{64}$$

The period x treatment interaction sum of squares can be sub-divided into seven components each with three degrees of freedom:-

Calcium/Period
Magnesium/Period
Phosphorus/Period
Calcium/Magnesium/Period
Calcium/Phosphorus/Period
Magnesium/Phosphorus/Period
Calcium/Magnesium/Phosphorus/Period

To arrive at the sum of squares of each component it is necessary to identify the different sets of data. Reference to the table used to determine factorial effect totals shows that, for magnesium treatments 1, 2, 4 and 7 were compared with 3, 5, 6 and 8.

Thus,

333.9 + 707.0 + 423.1 + 650.8 may be compared with 911.5 + 591.5 + 1916.8 + 628.7 in period 1. This procedure is continued for periods 2, 3 and 4, enabling construction of the following table:-

Period	Total without Mg	Total with Mg	Total
1	2114.8	4048.5	6163.3
2	2084.8	2211.5	4296.3
3	4961.0	3903.5	8864.5
4	2821.8	2814.9	5636.7
TOTAL	11982.4	12978.4	

The period x magnesium interaction sum of squares can be calculated from the above table as follows:-

$$\left[\frac{(2114.8^2 + 2084.8^2 + \ldots + 2814.9^2)}{8}\right] - \left[\frac{(11982.4^2 + 12978.4^2)}{32} - \frac{24960.8^2}{64}\right]$$
$$- \left[\frac{(6163.3^2 + 4296.3^2 + 8864.5^2 + 5636.7^2)}{16} - \frac{24960.8^2}{64}\right] - \frac{24960.8^2}{64}$$

The remaining period x treatment interactions may be calculated in a similar fashion allowing construction of the full analysis of variance table.

Source of Variation	DF	SS	MS	VR
Ca	1	63463	63463	0.400
Mg	1	15521	15521	0.098
P	1	209381	209381	1.321
Ca/Mg	1	244597	244597	1.544
Ca/P	1	237687	237687	1.500
Mg/P	1	12660	12660	0.080
Ca/Mg/P	1	158100	158100	0.998
Period	3	689639	229880	1.451
Ca/Period	3	363608	121203	0.765
Mg/Period	3	289083	96361	0.608
P/Period	3	110934	36978	0.233
Ca/Mg/Period	3	402674	134225	0.847
Ca/P/Period	3	693768	231256	1.459
Mg/P/Period	3	391994	130665	0.825
Ca/Mg/P/Period	3	178714	59571	0.376
Residual	30(2)	4754044	158468	
Total	61	8815864	144522	

Total number of observations 64

Number of missing values 2

8:4. Autopsy Reports

8:4:1. Suspected Urinary calculus

Date	27/1/1972
Animal	12 week old castrate lamb
Experiment	1
Period	3
Treatment	6
Specimen	Urinogenital tract

Macroscopically the urethra was ruptured 5cm from the tip and showed signs of haemorrhage. There was evidence of some necrosis of the urethra mucosa. Apart from urine, the bladder contained milky-white granular material. There was slight distension of the ureters and pelvic areas of the kidneys. No granular material was evident in the ureters or kidneys.

Histologically, there were areas of accumulation of necrotic debris and granular material beneath the pelvic epithelium. One section showed an area of congestion, necrosis and low-grade inflammatory response beneath the pelvic epithelium which had resulted in apparent rupture into the pelvic lumen.

8:4:2. Suspected pneumonias

Date	29/5/1972, 21/6/1972
Animal	$8\frac{1}{2}$ and $11\frac{1}{2}$ week old castrate lambs
Experiment	II
Period	1
Treatment	7 and 4
Specimen	Portions of viscera

Macroscopically, there was pneumonia mostly in the apical lung lobes. There was no evidence of umbilical infection.

Histologically there was evidence of pasteurella pneumonia and suppurative meningitis and choroiditis.

Bacteriologically, Pasteurella haemolytica was isolated from the lungs but not the spleen. Despite this, the indications were of a pasteurella septicaemia.

TABLE 8:4:3.

Summary of autopsy findings on those animals which were killed during Experiment II.

Case No.		21/72	63/72	73/72	40/73	49/73	121/73	125/73	128/73	189/73	195/73
Reason for destruction		Urolithiasis in all cases									
Obstruction	Yes	*	*	*	*	*	*	*	*	*	*
	No										
Site - Urethra	Yes	*	*	*	*	*	*	*	*	*	*
	No										
Bladder deposits	Yes		*	*	*	*	*	*	*	*	*
	No										
	Unclear	*									
Indications of renal origin	Yes		*	*		*	*				
	No										
	Unclear	*			*			*	*	*	*
Indications of bladder/ ureter origin	Yes										* ∅
	No	*	*	*	*	*	*	*	*	*	
Evidence of calcium salts in kidney concretions	Yes		*	*		*					
	No							*	*		
	Unclear	*			*		*			*	*
Mucopolysacchride	Yes		*	*		*					
	No								*		
	Unclear	*			*		*	*		*	*

∅ Haemarrhagic cystitis occurring as well as urolithiasis

8:5 Water Balance Tables
8:5:1

CHANGES IN FLUID ECONOMY ASSOCIATED WITH WEANING LAMBS AT 6 WEEKS OF AGE MEASURED 14 DAYS BEFORE AND 14 DAYS AFTER WEANING

			MEAN DAILY VALUES (ml/24 hr.)														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	SD.
GROUP I (N = 3)	WATER INTAKE (ml/day)	PRE WEANING	1460	1963	1828	1485	1591	1604	1853	1822	1895	1389	1451	1480	1547	2090	225.50
		POST WEANING	622	379	719	660	661	631	240	820	874	838	1053	1000	1131	793	244.03
	URINE OUTPUT (ml/day)	PRE WEANING	467	660	690	624	621	622	610	697	580	570	650	595	553	860	88.87
		POST WEANING	557	192	243	184	248	208	143	187	217	307	203	153	100	157	109.13
	WATER RETENTION (ml/day)	PRE WEANING	993	1303	1138	861	970	982	1243	1125	1315	819	801	885	994	1230	178.21
		POST WEANING	65	187	476	476	413	423	97	633	657	531	850	847	1031	636	283.32
	WATER RETENTION (% of intake)	PRE WEANING	68.5	66.2	66.3	58.0	61.0	61.2	67.0	61.7	69.4	59.0	55.2	59.8	64.2	58.9	4.34
		POST WEANING	10.5	49.3	66.2	72.1	62.5	67.0	40.4	77.2	75.2	63.4	80.7	84.7	91.2	80.2	20.91
GROUP II (N = 2)	WATER INTAKE (ml/day)	PRE WEANING	1833	1888	2054	1939	1349	2176	2310	1660	2145	2035	2032	1802	2072	2185	247.47
		POST WEANING	935	1190	1028	1010	695	825	855	1195	1045	1180	1155	1050	1138	1575	211.17
	URINE OUTPUT (ml/day)	PRE WEANING	550	750	885	1385	574	995	760	693	952	880	740	820	855	1075	213.46
		POST WEANING	855	445	390	263	285	173	255	250	213	545	230	333	510	420	180.74

8:5:2

CHANGES IN FLUID ECONOMY AND DRY MATTER INTAKE ASSOCIATED WITH WEANING LAMBS AT 6 WEEKS OF AGE
MEASURED 14 DAYS BEFORE AND 14 DAYS AFTER WEANING

			1	2	3	4	5	6	7	8	9	10	11	12	13	14	MEANS	SD
DRY MATTER INTAKE (g/24 hr)	PREWEANING	DRIP								65	89	74	112	147	147	147	112	36.2
		NONDRIP								5	32	52	49	112	112	112	68	44.1
	POSTWEANING	DRIP	189	207	138	138	122	158	99	170	198	254	478	300	459	437	239	129.8
		NONDRIP	145	254	214	264	156	269	314	258	316	376	467	204	408	425	291	99.1
WATER INTAKE (ml/24 hr)	PREWEANING	DRIP	1460	1460	1392	1600	1428	1468	1546	1439	1503	1512	1559	1588	1584	1584	1509	68.7
		NONDRIP	928	1216	840	1212	1216	968	1000	1185	1385	1309	1434	1298	1526	1498	1215	214.9
	POSTWEANING	DRIP	2031	2034	2023	2022	2020	2026	2016	2028	2032	2042	2078	2049	2075	2071	2039	21.2
		NONDRIP	602	676	918	846	796	972	971	837	787	812	951	908	941	944	854	112.6
URINE OUTPUT (ml/24 hr)	PREWEANING	DRIP	665	825	705	555	1115	1260	618	620	653	860	748	910	935	553	787	211.9
		NONDRIP	550	310	725	325	580	770	600	690	920	683	775	865	1040	755	685	204.2
	POSTWEANING	DRIP	653	2528	800	1825	2075	1840	883	2065	1165	2370	813	900	1000	1590	1465	647.9
		NONDRIP	465	205	275	345	398	480	533	563	393	198	255	220	360	308	357	120.9
WATER RETENTION (ml/24 hr)	PREWEANING	DRIP	795	635	687	1045	313	208	928	819	850	652	811	678	649	1031	722	237.6
		NONDRIP	378	906	115	887	636	198	400	495	465	626	659	433	486	743	531	230.5
	POSTWEANING	DRIP	1378	−494	1223	197	−55	186	1133	−37	867	−328	1265	1149	1075	481	574	653.4
		NONDRIP	137	471	643	501	398	492	438	274	394	614	696	688	581	636	497	162.1
WATER RETENTION (% of intake)	PREWEANING	DRIP	54.4	43.5	49.4	65.4	22.0	14.2	60.0	56.9	56.6	43.1	52.1	42.6	42.8	65.0	47.7	14.8
		NONDRIP	40.8	74.6	13.7	73.2	52.3	20.45	40	41.7	33.6	47.9	46.0	33.4	31.9	49.6	42.8	17.0
	POSTWEANING	DRIP	67.9	−24.3	60.2	9.8	−2.7	9.2	56.3	−1.9	42.66	−16.0	60.8	56.0	51.9	23.6	28.1	32.0
		NONDRIP	22.8	69.7	70.0	59.3	50.0	50.6	45.2	32.7	50.0	75.6	73.2	75.8	61.8	67.3	57.4	16.4

Appendix 8:6

Environmental Measurements