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Studies on the absorption of calcium, phosphorus, magnesium, copper and zinc by sheep fed on roughage-cereal diets

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1. Two groups of four 18-month-old ewes fitted with a rumen cannula and re-entrant cannulas in the proximal duodenum were fed on one of two diets based on varying proportions of dried-grass meal, ground straw and barley.

2. The apparent availability, retention and amount of the mineral reaching the proximal duodenum were measured for calcium, phosphorus, magnesium, copper and zinc. Significant differences were found between diets for Ca only. Net absorption of Mg occurred before the proximal duodenum and also, lower down the alimentary tract. Marked increases were observed in the amount of Cu, Zn and P reaching the proximal duodenum relative to that ingested.

Although the role of minerals in animal nutrition is well recognized, the need for more information on the net changes in the amount of each mineral absorbed or available for absorption in various sections of the gastrointestinal tract has been recognized for some time. This aspect of mineral nutrition has been investigated using surgically-modified animals and a number of diets, including fresh herbage (Grace, Ulyatt & MacRae, 1974), hay-barley (Pfeffer, Thompson & Armstrong, 1970) and concentrate diets (Liebholz, 1974). In addition, semi-purified diets have been used to study particular mineral nutrients (Strachan & Rook, 1975; Field & Munro, 1977). The present study was designed to extend the published information on calcium, magnesium, phosphorus, copper and zinc by using two diets containing differing proportions of ground roughage and cereal.

METHODS

Animals and experimental design

Twelve 18-month-old crossbred ewes (40–45 kg live weight) were randomly allocated to one of three blocks. Within each block two sheep received one of the two diets. Subsequently four of the sheep were discarded from the experiment due to inappetance and lack of thrift, leaving three sheep in blocks 1 and 2 and two sheep in block 3. Nevertheless, each diet was given to four sheep. The sheep were prepared with a rumen cannula and re-entrant cannulas in the proximal duodenum (Brown, Armstrong & MacRae, 1968) except that a thoracic rather than an abdominal approach was used.

All sheep were housed individually. The metabolism crates, feed bins and water containers were coated with a polyvinyl chloride preparation to reduce trace element contamination. The animals were fed once daily in the morning and had free access to tap water which was changed daily.

Diets

The constituents and chemical composition of the diets are shown in Table 1. Diets 1 and 2 contained ground straw, grass meal and ground barley in the following proportions (w/w): 4 : 2 : 3 and 2 : 1 : 6 respectively. Chromic oxide powder (5 g/kg feed) was used as an

Table 1. *The constituents and chemical composition of diets 1 and 2*

	Diet 1	Diet 2
Ground straw (kg/t)	440	220
Ground barley (kg/t)	220	110
Dried-grass meal (kg/t)	330	660
Chromic oxide (kg/t)	5	5
Urea (kg/t)	5	5
Dry matter (g/kg)	853·2	842·4
Ash (g/kg)	62·1	46·6
Calcium (g/kg)	5·4	2·3
Magnesium (g/kg)	1·5	1·5
Phosphorus (g/kg)	2·5	2·8
Copper (mg/kg)	6·9	6·1
Zinc (mg/kg)	36·9	34·4

inert marker and was incorporated into each diet before pelleting. No attempt was made to meet the mineral requirements of the animal by the inclusion of mineral supplements but an injection of retinol, cholecalciferol and α-tocopherol was given.

The diets were fed to the animals at a level slightly above maintenance. This was achieved by feeding 653 g dry matter (DM)/d of diet 1 and 535 g DM/d of diet 2. Sufficient quantities of both diets were weighed into individual bags at the start of each preliminary period. Representative samples of each diet were taken and stored for subsequent chemical analysis.

Digestibility and digesta flow measurements

For each group of animals a 7 d balance period was conducted after a 4-week preliminary period. During the balance period, intake and faecal and urinary output were measured. Representative samples of faeces and urine from each sheep were prepared by bulking one-tenth of the daily output and storing at $-20°$ before preparation of samples for analysis.

After the balance period, a 24 h continuous collection and sampling of duodenal digesta was made using the technique of MacRae & Armstrong (1969). In addition, a further 10% of each sample of digesta collected was taken for each sheep and combined to give a composite fresh sample for each sheep. All samples were stored at $-20°$.

Sample preparation and chemical analysis

Before chemical analysis the representative samples of faeces were thawed and mixed in a Hobart chopper.

Duodenal digesta samples were freeze-dried and milled. A composite sample of the dried digesta was prepared for each sheep in each group on the basis of the observed DM flow for each particular sample.

Food samples were chopped before DM estimation.

DM was determined by drying in a force-draught oven at 100° for 16 h. Ca, Mg, Cu and Zn were estimated in food, faeces and duodenal digesta by atomic absorption spectrophotometry (Perkin Elmer Model no. 403) in extracts prepared by a macro-version of the wet-digestion procedure described by Thompson & Blanchflower (1971). Hydrogen peroxide (100 vol.) was used to ensure the complete destruction of organic matter (Down & Gorsuch, 1967). P was assayed colorimetrically on the same digest (Donald, Schwehr & Wilson, 1956).

Chromium was determined by atomic absorption spectrophotometry on a digest prepared according to the procedure of Stevenson & De Langen (1960).

Mineral analysis of urine was performed on digests prepared by taking urine samples to

Table 2. *The amounts (g/d) of calcium, magnesium and phosphorus consumed, entering the proximal duodenum, excreted in the faeces and urine and apparently retained for sheep given two diets*†

(Mean values with their standard errors given in parentheses)

	Ca		Mg		P	
Diet	1	2	1	2	1	2
No. animals	4	4	4	4	4	4
Intake (g/d)	3·1* (0·33)	1·1*	0·88 (0·093)	0·76	1·5 (0·02)	1·4
Entering proximal duodenum (g/d)	2·6* (0·27)	1·2*	0·59 (0·073)	0·44	5·5 (0·85)	6·7
Faeces (g/d)	2·6** (0·09)	1·7**	0·59 (0·05)	0·45	1·6 (0·28)	1·6
Urine (g/d)	0·21 (0·082)	0·11	0·16 (0·018)	0·15	0·06 (0·058)	0·11
Amount entering proximal duodenum (g/g ingested)	0·83 (0·668)	1·01	0·68 (0·793)	0·77	3·65 (3·952)	4·70
Apparent availability	+0·14* (0·132)	−0·14*	+0·31 (0·052)	+0·41	−0·05 (0·132)	−0·12
Apparent retention (g/d)	+0·50 (0·330)	−0·62	+0·13 (0·046)	+0·16	−0·15 (0·168)	−0·29

* $P < 0·05$, ** $P < 0·01$ (significant differences only apply within each individual mineral).
† For details, see Table 1.

dryness on a water-bath followed by ashing at 450° for 16 h. The residue was warmed gently with 6 M-hydrochloric acid in block digesters (Thompson & Blanchflower, 1971) until solution was complete.

All glassware used for mineral analysis was acid-washed and rinsed with glass-distilled water and Ultra grade chemicals were used to prepare all reagents.

Statistical analysis

The results were subjected to an analysis of variance to test statistical significance.

RESULTS

Table 2 shows the adjusted mean amounts of Ca, Mg and P ingested, entering the proximal duodenum and excreted in the faeces and urine of the sheep and also the apparent retention of these minerals from diets 1 and 2. Amounts of each mineral entering the proximal duodenum have been corrected to 100% recovery of the Cr_2O_3 given (MacRae & Armstrong, 1969). The mean recovery of Cr_2O_3 fed was 0·80 (SE ± 0·115).

The intake of Ca by sheep fed on diet 1 was significantly higher ($P < 0·05$) than that for sheep fed on diet 2. A significant difference was also found in the amounts entering the proximal duodenum and excreted in the faeces. Although the amounts of Ca entering the proximal duodenum relative to the amount consumed were not significantly different there was a decreased amount entering the proximal duodenum for animals fed on diet 1, thus demonstrating that some absorption of Ca from the forestomachs can occur.

The apparent availability of Ca was significantly different ($P < 0·05$) although the apparent retention was not.

Table 3. *The amounts (mg/d) of copper and zinc consumed, entering the proximal duodenum, excreted in the faeces and urine and apparently retained for sheep given two diets**

(Mean values with their standard errors given in parentheses)

	Cu		Zn	
Diet	1	2	1	2
No. animals	4	4	4	4
Intake (mg/d)	4·0 (0·42)	3·1	21·6 (2·28)	17·4
Entering proximal duodenum (mg/d)	7·5 (1·04)	6·2	26·6 (4·39)	23·4
Faeces (mg/d)	4·6 (0·55)	2·9	28·9 (4·55)	27·4
Urine (mg/d)	0·09 (0·013)	0·08	0·84 (0·203)	0·94
Amount entering proximal duodenum (mg/mg ingested)	1·87 (1·066)	2·00	1·21 (1·101)	1·33
Apparent availability	−0·15 (0·063)	+0·06	−0·36 (0·124)	−0·56
Apparent retention (mg/d)	−0·63 (0·220)	+0·14	−8·15 (2·749)	−10·92

* For details, see Table 1.

There were no significant differences between diets for Mg or P with respect to all measurements made. However, there was a decrease in the amount of Mg and a marked increase in the amount of P entering the proximal duodenum relative to that consumed.

Table 3 shows the adjusted mean amounts of Cu and Zn consumed, entering the proximal duodenum and excreted in the faeces and urine and apparently retained for sheep fed on diets 1 and 2. There were no significant differences between diets for Cu and Zn with respect to all measurements made. However, there were considerable increases in the amounts of both Cu and Zn entering the proximal duodenum relative to that consumed.

DISCUSSION

One of the most interesting aspects of this work was the marked increases in the amounts of Cu entering the proximal duodenum relative to that ingested. Analysis of the drinking-water for the animals showed that it would contribute approximately 0·1 mg Cu/d, assuming a water intake of 2 l/d and having measured the Cu concentration (0·06 mg/l). Therefore, a considerable secretion of Cu into the lumen of the gut must have occurred before the duodenum. This Cu could have come either from the saliva or from secretions into the forestomachs of the animal. Saliva taken from an anaesthetized sheep fed on silage gave a Cu concentration of 0·2 mg/l and since sheep can produce 5–6 l saliva/d (Kay, 1960), the contribution of Cu from this source would be approximately 1·2 mg/d. Although this would explain part of the increase, an additional net secretion of Cu through the gut wall must have occurred, as had been shown for Zn (Weston & Kastelic, 1967) and sodium (Grace *et al.* 1974). Binnerts (1978) has reported that Cu levels in the rumen are increased after intravenous or intramuscular injections of Cu but does not explain the mechanism by which this increase occurs. Bertoni, Watson, Savage & Armstrong (1976) have shown that considerable secretion of Cu can occur before the proximal duodenum in cows fed on forage-cereal diets. In addition, Ivan & Grieve (1976) using a slaughter technique have

reported that copper secretion occurs prior to the proximal duodenum in concentrate fed calves. The marked increases in the level of Zn entering the proximal duodenum relative to that ingested have also been observed by Grace (1975) and Bertoni *et al.* (1976). The contribution of Zn from drinking-water would have been minimal, approximately 0·3 mg/d, but the Zn concentration of sheep saliva (obtained as for the Cu estimation) was 1·5 mg/d. Therefore, the increase in the amount of Zn in duodenal digesta could have been accounted for by the secretion of saliva. On the other hand, Weston & Kastelic (1967) have reported that Zn can be secreted into the rumen through the rumen epithelium. From the present results, it is impossible to say which additional source of Zn contributed most to the increased level of Zn entering the proximal duodenum relative to that ingested.

The marked increase in the amount of P entering the proximal duodenum found in the present study has been reported previously (Pfeffer *et al.* 1970; Grace *et al.* 1974) and is undoubtedly a reflexion of the quantity of P present in and the amount of saliva produced by the animals (Kay, 1960).

Although there were no significant differences between diets for Mg there was an apparent absorption (25–30%) of Mg before the proximal duodenum. This supports the findings of Forbes & Keith (1914), Pfeffer *et al.* (1970), Grace *et al.* (1974) and Field & Munro (1977) but is contrary to the earlier reports of Care & Van't Klooster (1965) and Phillipson & Storry (1965), who suggested that the rumen was of negligible importance in Mg absorption.

The significant differences observed between diets 1 and 2 with respect to Ca are probably a reflexion of the composition of the diets. Diet 2 contained 660 g ground barley/kg compared with 330 g/kg in diet 1 and as a result the Ca content of diet 1 was twice that of diet 2. Furthermore, the greater proportion of barley in diet 2 would increase the phytate content of the diet and this may have contributed to the findings that the Ca availability on diet 2 was much less than on diet 1. On the other hand, Pfeffer *et al.* (1970) have reported results contrary to this when the proportion of barley fed to sheep increased from 300 to 600 g in a hay-barley diet.

Considerable absorption of P and Cu occurred distal to the proximal duodenum as has been shown by Pfeffer *et al.* (1970), Grace *et al.* (1974), Grace (1975) and Bertoni *et al.* (1976). In this study, little or no net absorption of Mg occurred in the intestines which is contrary to the findings of Phillipson & Storry (1965) and Pfeffer *et al.* (1970). The amounts of Ca excreted in the faeces were similar to or greater than those entering the proximal duodenum. Secretion of Ca into the small intestine is known to occur (Grace *et al.* 1974) and this confirms results from sheep given diet 2. The apparent lack of net absorption of Zn in the intestines is contrary to the findings of Grace (1975) and Bertoni *et al.* (1976). The observed increases may have been due to secretion of Zn-containing enzymes in the succus entericus but no satisfactory explanation can be advanced for the apparent lack of absorption of Zn.

Generally, this work confirms previous findings on the digestion and absorption of the minerals studied. Diet, except in the instance of Ca, had little effect on the apparent availability and retention of the other minerals examined. It would appear that turnover rates of minerals within the animal are an aspect of mineral metabolism requiring further investigation. Until the dynamics of these processes both within the animal and between the animal and gut contents are fully elucidated, mineral requirements for animals will continue to be difficult to assess.

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