MAGNESIUM SUPPLEMENTATION OF RUMINANT DIETS

A thesis submitted to the University of Glasgow

for the degree of

DOCTOR OF PHILOSOPHY

in the Faculty of Veterinary Medicine

by CLARE LESLEY WILSON B.A.

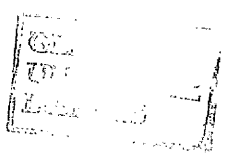
Dept. Animal Husbandry,

University of Glasgow

Veterinary School.

September, 1981.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346



Acknowledgements

I wish to express my sincere thanks to my supervisors, Professor R.G. Hemingway and Dr. N.S. Ritchie, for their help and advice during the course of this study and preparation of this thesis. I also wish to thank sincerely Chance & Hunt (I.C.I. Ltd.) and Magnesitas de Rubian S.A. for the provision of a Research Scholarship which made the project possible, and for the provision of many of the calcined magnesite samples used in the experimental work. I would especially like to thank Mr. S.W. Percival of Chance & Hunt for his interest and helpful advice throughout the study.

I would also like to thank the following: Miss M. Young, Mrs. M. Cunningham, Mr. C. Cameron, Miss M. McIntosh, Miss M. Laidlaw, Mrs. A. McLaughlin and Miss P. Wilson for diligently carrying out the laboratory analyses; Mr. M.J. McColl, Mr. T.I. Collett, Mr. D.G. Gibson and Mr. R.D. Johnston for their help with the experimental animals; Mr. A. Waterston, Mr. J. Fraser and Mr. A. Louden for their co-operation in providing suitable livestock for use in farm situations for Section IV; Mrs. J.C. Mackinnon for typing this thesis and Mr. A. Finney and his staff for the photographic requirements of the thesis.

TABLE OF CONTENTS

SUMMARY

The major objectives of this thesis were to study the variation in the dietary availability of calcined magnesites to ruminants, both indoors and at grass, and to investigate different experimental methods for availability determinations. Calcined magnesite is given to ruminant animals as a preventitive measure against hypomagnesaemic tetany due to magnesium deficiency. Section I describes this and alternative methods of prevention in current use, following a description of normal magnesium physiology and metabolism in ruminants, the symptoms and incidence of hypomagnesaemic tetany, and factors involved in the development of the clinical condition.

In Section II, balance trials with sheep demonstrated that the apparent availability of calcined magnesite was lower in coarse than in fine particles, and that the temperature of calcination was important in so far as "raw" or undercalcined magnesite was poorly available whereas material calcined between 800 - 1,100°C (for 0·75 h) was highly available. An indigestible faecal marker technique for availability determinations was successfully developed and evaluated, and by this method many different magnesium supplements could be "screened" using sheep indoors. Wide differences were detected in the availability of calcined magnesites from different origins, and powdered materials were considerably more available than granular particles.

In Section III a suitable low magnesium diet was developed which induced hypomagnesaemia but which also adequately maintained lactating ewes over long periods. Differences between dietary magnesium supplements were reported in terms of the blood magnesium response as well as availability (determined by indigestible marker). These differences were between calcined magnesites from different origins, and again fine particle grades were better than coarser granular material. In addition, severe heat treatment of calcined magnesite (1300°C for 3 hours) was

shown to be detrimental.

During these experiments with lactating hypomagnesaemic ewes indoors it was observed repeatedly that a dietary magnesium supplement given once daily caused a transient rise in plasma magnesium concentration, with peak concentration after 4 hours and levels thereafter decreasing to the original level by within 12 - 24 hours.

The indigestible faecal marker technique allowed availability determinations to be carried out with livestock at grass (Section IV). Using dairy and beef cattle, differences in availability were reported between calcined magnesites from different origins, and powdered supplements were generally more available than granular materials. The supplements were offered in various forms used in current farm practice and individual intake data in group situations were compared and discussed.

In Section V it was established that the solubility of calcined magnesites in the rumen fluid, as determined *in vivo* using the nylon bag technique, was well correlated with apparent availability (as determined in the feeding trials). However solubility in two different laboratory solvents, ammonium nitrate and citric acid, was less well correlated with availability.

It was concluded that there is some considerable variation in the dietary availability of calcined magnesites with regard to changes in the origin of the material, particle size and temperature of calcination. In addition, some novel methods for assessing availability have been demonstrated.

INTRODUCTION

Ruminant diets are supplemented with magnesium for the prevention of magnesium deficiency, especially in lactating cattle and sheep. Calcined magnesite has, for over 40 years, been the principle material used to provide this supplementary magnesium. The standard recommendations are (and have been since the 1930's) to supply daily 56 g calcined magnesite per cow and 7 - 14 g per sheep. These quantities are greatly in excess of the current recommendations (A.R.C. 1980) for the daily magnesium requirements of ruminants, which are 8 - 23 g Mg per cow and 1·0 - 2·5 Mg per sheep, depending on milk yield, particularly when the magnesium content of the normal feedstuffs is taken into account. However, despite the apparent excess of magnesium supplied, these dietary treatments are not always entirely successful in preventing hypomagnesaemic tetany due to magnesium deficiency. There may be several factors involved in this apparent failure, but one particular aspect which may be involved but which has not previously been studied is the possible variation in the digestibility (or availability) of the magnesium in calcined magnesite.

It is known that the availability of dietary magnesium in ordinary foods varies widely. The Agricultural Research Council (1980), for example, quotes a mean availability for a range of feedstuffs as 29·4 $\pm$ 13·5%, but recommends the use of the lower decile value of 17·0% "to provide a margin of safety" for the calculation of dietary allowances. When calcined magnesite is used as a supplement it is assumed that it has similar average availability, and it is also assumed that this would apply equally to all samples of calcined magnesite whatever the origin, particle size and manufacturing conditions involved. There is no published evidence to justify these assumptions and indeed a single reference on the subject (Jesse, Thomas & Emery, 1981, i.e. since the work reported in this thesis commenced) reports decreased availability of calcined magnesite with increased particle size. Previous studies on

supplementary magnesium availability have largely compared different salts (e.g. Ammerman, Chicco, Loggins & Arrington, 1972; Storry & Rook, 1963) but this present thesis aims to study the variation in availability of calcined magnesite with regard to changes in the origin, particle size and heat treatment during manufacture.

In addition to the traditional balance trial technique for apparent availability determinations, the experimental work investigates alternative methods, notably the use of an indigestible faecal marker which should decrease the time and labour cost involved in such trials. The work also aims to develop a suitable low magnesium diet to induce hypomagnesaemia in lactating ewes. The relative efficacies of different dietary magnesium supplements could then be assessed both in terms of the blood magnesium response and the apparent availability.

The experimental work then investigates supplementary magnesium availability with grazing livestock, as hypomagnesaemic tetany is a problem almost entirely confined to lactating animals at grass, and availability assessments in this situation are made possible by the indigestible marker technique. Results obtained in indoor experiments can then be compared with the outdoor situation. Concurrently, measurements can be made on the variation in individual magnesium intake when supplements are offered in the various forms which are used in farm practice. It is known that intakes can be highly variable and failure to consume a supplement on offer may be one of the main reasons for the apparent failure of a supplement to prevent cases of hypomagnesaemic tetany.

Lastly, <u>in vivo</u> and <u>in vitro</u> solubilities of calcined magnesite samples are investigated in an attempt to establish a suitable test which would correlate with variations in the apparent availability found in the feeding trials.

SECTION I

LITERATURE REVIEW AND BACKGROUND INFORMATION

The following review aims to provide a background for the main topic of the thesis, namely the dietary availability of calcined magnesites to ruminants. The feeding of calcined magnesite is a preventitive measure against hypomagnesaemic tetany in ruminant animals. This condition, which occurs in varying degrees of severity, is associated with a disturbance in magnesium metabolism, and therefore initial consideration is given to normal magnesium physiology and metabolism in the ruminant body. The incidence and symptoms of hypomagnesaemic tetany are described, followed by a consideration of factors implicated in the development of the clinical condition. The various prophylactic measures employed in the prevention of hypomagnesaemic tetany are described, and details are given of the different types of calcined magnesites which may be given to animals.

1. NORMAL MAGNESIUM PHYSIOLOGY AND METABOLISM

Distribution of Magnesium in the body

The animal body contains about 0·05% by weight of magnesium, with about 59% in the skeleton, 40% in the cells of the soft tissues, and 1% in the extracellular fluids (E.C.F.). Thus a 500 kg adult bovine would contain approximately 250 g of magnesium, of which 150 g would be skeletal, 100 g in the soft tissues, and only about 2·5 g in the E.C.F. Similarly, an adult sheep weighing 60 kg would contain about 30 g magnesium, with 18 g skeletal, 12 g in the soft tissue, and as little as 0·3 g magnesium in the E.C.F.

(i) Extracellular Fluids (E.C.F.)

The E.C.F. is composed of the plasma and the interstitial fluid, the latter being an ultrafiltrate of the former, and the presumption is made that their magnesium contents are similar (although the lower protein content of interstitial fluid results in a slightly lower magnesium concentration). The concentration of magnesium in the E.C.F.

in ruminant animals is normally about 1·03 mmol/l and values for the plasma or serum levels for normal healthy animals have been reported from about 0·49 to 1·56 mmol/l (Allcroft & Green, 1934; Maly, 1935; Duncan, Lightfoot & Huffman, 1938; Eveleth & Millen, 1940; Fisher, 1960). Allcroft (1947a), however, classified the lower limit of the normal range of plasma magnesium in cattle at 0·70 mmol/l, values below which can properly be referred to as hypomagnesaemic. This value of 0·70 mmol Mg/l is of particular physiological significance as it is known to be close to the renal threshold.

Plasma magnesium can be divided into ultrafiltrable and protein bound magnesium. Values between 65 and 80 per cent of the total plasma magnesium have been estimated for the ultrafiltrable magnesium (Blaxter, Cowlishaw & Rook, 1960; Walser, 1961). This fraction is in the physiologically active form of free ions, except for a small proportion (13%) combined with phosphate and citrate ions and in other unidentified complexes (Walser, 1961). The fraction which is not ultrafiltrable is bound reversibly to plasma albumin and globulins (Prasad et al., 1959). This binding is modified by pH (Carr & Woods, 1955) and by the concentration of other ions, notably calcium, which compete for binding sites (Carr, 1955).

There is little evidence that the plasma magnesium level is under direct endocrine control, however hormone levels may indirectly affect plasma magnesium during pregnancy and parturition in cattle (Allcroft & Godden, 1934), and during the breeding cycle of sheep (Charton et al., 1959).

(ii) Intracellular Fluids (I.C.F.)

Magnesium is primarily an intracellular ion with most body tissues having a similar value of about 15 mmoles of magnesium per litre of intracellular water (Wilson, 1960). This concentration is much greater

than that in the E.C.F., and so a large concentration gradient exists across the cell membrane. The mechanism concerned in the maintenance of this gradient is not clear but it is likely that magnesium transport into the cell is an active process. Isotope tracer studies have shown that a small proportion of the intracellular magnesium is exchangeable (Brandt, Glaser & Jones, 1958; MacIntyre, 1959). This magnesium is in ionic form, in physico-chemical equilibrium with the ionic E.C.F. magnesium, the remainder inside the cell being bound to protein molecules (Wilson, 1960).

The rate at which ^{28}Mg exchanges across the cell membrane varies in different tissues. Rogers & Mahan (1959) reported the fastest rate of equilibration in heart, liver and kidney, i.e. tissues associated with high metabolic activity, and slower rates in brain,. testis, erythrocyte and skeletal muscle. These results were confirmed by MacIntyre, Davidsson & Leong (1958) and MacIntyre (1959).

Various workers have investigated changes in intracellular magnesium during periods of magnesium deficiency, but their results are somewhat contrasting. For example, MacIntyre & Davidsson (1958) observed a marked fall in the level of magnesium in muscle during the development of magnesium deficiency in rats, and Blaxter et al. (1960) reported a fall in the magnesium content of red blood cells of calves. However, several other workers have observed no measurable decline of magnesium concentration in soft tissues of hypomagnesaemic calves and cattle (e.g. Blaxter, Rook & MacDonald, 1954; Smith, 1957).

The distribution of ^{28}Mg in the tissues of sheep following intravenous administration shows marked variations in the specific activity between different body tissues. Field (1961) reported the order of decreasing specific activity as bile, kidney, plasma, liver, spleen, skeletal muscle and bone.

(iii) Skeleton

Magnesium constitutes 0·5 - 0·7% of bone ash, in a calcium to magnesium ratio of about 55 : 1. It is present in bone as a result of reversible hetero-ionic exchanges between the bone mineral surface and the extracellular fluid. It is present largely as Mg^{2+} and $Mg\ OH^{+}$ ions held by electrostatic attraction within the hydration shell at the apatite crystal surface. Magnesium ions are bound less strongly than calcium ions, and the binding of Mg^{2+} ions *in vitro* has been shown to be proportional to the concentration of magnesium (Gilbert, 1961). Magnesium ions can thus replace calcium ions in the apatite crystal lattice unless the magnesium concentration in the surrounding extracellular fluid is low, when calcium ions replace magnesium ions in the adsorption sites. Taylor (1959) demonstrated that in ruminant bone about 70% of the magnesium could be removed relatively easily by treatment with dilute acid while the remaining 30% appeared to be in much closer association with the bone. Various workers (e.g. Duckworth & Godden, 1941; Blaxter & Rook, 1954) have demonstrated that young animals in magnesium deficiency can mobilise 30% or more of skeletal magnesium. The release of skeletal magnesium is apparently not under hormonal regulation but is probably controlled by the concentration of magnesium in the E.C.F. bathing the bone mineral surface, since bone magnesium and plasma magnesium during depletion are related. (Blaxter, 1956; Smith, 1959; Thomas, 1959). The bone crystal lattice can therefore act as a reservoir of magnesium to be drawn upon at a time when the concentration of magnesium ions in the E.C.F. falls due to an increased tissue demand (such as during growth or lactation) or a reduction in dietary magnesium intake. However the labile portion may vary with the degree of hydration and inversely with the degree of recrystallisation of the bone mineral (Neuman & Weikel, 1955), and

therefore tends to be highest in the young animal, decreasing markedly with age. As the animal matures, a very large part of the skeleton becomes metabolically inert due to the progressive diminution of its blood supply and the mobility of skeletal magnesium is restricted. The adult animal is consequently less able to withdraw bone reserves of magnesium at times of increased demand or dietary deficiency, and this helps to explain the much greater incidence of hypomagnesaemic tetany in older animals. The bones of normal and hypomagnesaemic adult cattle show a similar range of magnesium concentration (Cunningham, 1936a,b; Allcroft, 1960). Field (1960) calculated from the uptake of ^{28}Mg by the skeleton of a 5-year-old sheep that only about 2% of the total skeletal magnesium is available for release in response to physiological demands.

Blaxter (1956) suggested that the whole skeleton of very young magnesium-depleted calves participates in skeletal magnesium mobilization and that there is no particular site where greater depletion occurs. However Smith (1959b), in a more detailed study, reported greater depletion during the early stages of skeletal mobilization from the first phalanges, vertebrae and femur head than from the rib and femur shafts. It should be noted that calves have a higher bone ash magnesium than adults, at 0.80% (Smith, 1959b). The skeletal reserves of magnesium in depleted animals can be restored by oral or intravenous administration of magnesium (e.g. Cunningham 1933; Duckworth & Godden, 1943; Smith, 1959b), but restoration may be incomplete and less rapid than depletion.

Biochemical Functions of Magnesium

Magnesium is an important "activator" or "cofactor" of many enzymes (Lehninger (1950) gives an extensive review). Metallic ion activators are believed to act by aiding the enzyme-substrate linkage via the formation of an enzyme-metal-substrate complex. Upon the removal of these metallic ions by dialysis, the enzyme becomes inactive, but on

their replacement, activity is fully restored. Some enzymes require a specific metal while for others, any one of many metals will suit. Several very important enzyme reactions require magnesium as an essential cofactor whereas for other enzyme systems it may be dispensable as a cofactor. This function seems to be the basic biochemical role for magnesium in living tissues.

There are two main areas of magnesium activity: Firstly in enzyme systems concerned with carbohydrate metabolism and hence with energy production, and secondly in enzyme systems involved in the nervous system. One of the important reactions in carbohydrate metabolism where magnesium is a specific cofactor is in the oxidative decarboxylation of α-ketoglutaric acid to form succinic acid as part of the Kreb's cycle. In the absence of magnesium this reaction could not take place, thereby interrupting Kreb's cycle, causing a build up of intermediary metabolic compounds and a lack of "high energy" phosphate bonds, essential for normal metabolic processes. Magnesium is also one of the cofactors in the oxidative carboxylation of pyruvic acid to acetylcoenzyme A, and any interference in this step would lead to an accumulation of pyruvic acid. The enzyme enolase involved in the conversion of glyceric acid-2-phosphate to enol-pyruvic acid phosphate requires magnesium, manganese or zinc as a metallic cofactor (Douglas, 1960). In this reaction the "low energy" phosphate radical becomes a "high energy" phosphate radical in the product. Another group of enzymes for which magnesium is one of the cofactors is the phosphatases. These are universally distributed in the body and are required to hydrolyse phosphate esters of various types to produce free inorganic phosphate. For example, adenosine triphosphatase breaks down adenosine triphosphate (A T P) to adenosine diphosphate (A D P) and inorganic phosphate, releasing energy. All reactions involving A T P depend on the presence of Mg.

The second important sphere of activity of magnesium as an enzyme cofactor is in the nervous system, where it plays a part in both the production and destruction of acetylcholine, the substance necessary for the transmission of impulses at the neuromuscular junction. Hoff, Smith & Winkler (1940) demonstrated that the site of action of magnesium is the neuromuscular junction, although some evidence suggests a direct effect on the central nervous system (Bryant, Leyman & Knoefel, 1939). The magnitude of the end plate potential at the neuromuscular junction, which is related to the release of acetylcholine by the motor nerve endings, has been shown to be dependant on the relative concentrations of calcium and magnesium (del Castillo & Stark, 1952). Magnesium ions inhibit the release of acetylcholine thus diminishing the end plate potential (del Castillo & Engbaek, 1953), and this depressant action is antagonised by calcium (Malorny & Ohnesorge, 1951). Choline acetylase, the enzyme responsible for the synthesis of acetylcholine, is activated by magnesium ions (Feldberg & Hebb, 1945-46; 1947) but inhibited by calcium ions (Feldberg, 1945), and magnesium is also a cofactor for acetylcholine esterase, which hydrolyses acetylcholine (Nachmansohn, 1940). The rapidity of the synthetic reaction is not of prime importance, but, once released, acetylcholine acts upon a receptor indirectly to trigger off muscular contractions, and therefore the rapid destruction of this transmitting agent is of vital importance to maintain muscular control.

The lack of magnesium will have profound effects on the nervous system and the body as a whole. According to Rook & Storry (1962) in their review of magnesium nutrition, a reduction of extracellular magnesium would:

(1) Increase the release of acetylcholine by motor nerve endings;

(2) Increase the sensitivity of the end plate to liberated acetylcholine;

(3) Decrease the hydrolysis of liberated acetylcholine, with a consequent tendency to delay of recovery from stimulation;

(4) Increase the sensitivity of the muscle membrane to the electrical impulse from the nerve, thus causing a greater "twitch";

(5) Inhibit the relaxing factor of muscle.

In magnesium deficiency in all species the main symptom is derangement of the nervous system. Each of the above changes would tend to increase the irritability of the neuromuscular system and, under extreme conditions, could theoretically lead to the development of tetany. However, the degree of irritability depends not only on the concentration of magnesium ions but also on the concentrations of other ionic constituents, notably calcium (Schmidt & Greenberg, 1935). The involvement of magnesium at the neuromuscular junction may also explain the converse condition where excess magnesium given intravenously produces anaesthesia and eventual death from respiratory failure.

Body Losses of Magnesium

(i) Endogenous faecal losses

Endogenous magnesium in the faeces is derived from desquamated epithelial cells and unabsorbed digestive secretions, notably saliva and gastric juice. Some of the magnesium secreted into the gut is undoubtedly reabsorbed, possibly in preference to that in the diet (Field, 1961), but the conditions controlling the endogenous faecal loss of magnesium are not clearly understood. Since the contribution of magnesium in saliva is relatively important, and saliva output by the cow is affected by the physical fibrousness and bulk of the diet (Bailey, 1961), high-roughage rations may be associated with high endogenous faecal loss of magnesium. Smith (1961) reported that milk fed calves allowed access to wood shavings developed hypomagnesaemia more quickly than muzzled calves, and this he attributed to the increased salivary

output in the unmuzzled calves. Care (1960b) has suggested that during long periods of magnesium deficiency, secretion of magnesium into the gut may be reduced since the concentration of magnesium in bile was found to decrease during development of hypomagnesaemia. This suggestion is supported by observations on the dog (Langley, Grimes & Cockrell, 1962) in which magnesium concentration in the saliva varied directly with that in the plasma, and on the rabbit (Kaya, 1932) in which intravenous magnesium administration caused an increase in the concentration of magnesium in the gastric juice. However, Smith (1959a) reported no increase of endogenous faecal loss in calves after subcutaneous administration of magnesium.

Three principal procedures have been employed for the estimation of the endogenous faecal loss of magnesium in cattle and sheep:-

1. Extrapolation to zero magnesium intake of the regression of faecal loss of magnesium on magnesium intake for natural diets.
2. Measurements of faecal excretion of magnesium on artificial diets extremely low in magnesium.
3. Isotope dilution methods.

Values obtained by the first of these procedures are subject to high errors (see A.R.C., 1965) and can be rejected as unreliable.

The second method is subject to two recognised sources of error. Firstly, artificial diets unavoidably contain small quantities of magnesium and any unabsorbed dietary magnesium will give an error in the estimation of endogenous faecal magnesium. Secondly, the net endogenous faecal loss of magnesium may be reduced in animals receiving a magnesium-deficient diet. Allsop & Rook (1979) reported increased endogenous faecal loss with increased magnesium status of mature sheep when given both natural and artificial diets, and they concluded that this source of error is important.

Isotope dilution techniques have been widely used although their validity has been questioned by Field (1961) because it is assumed that there is no exchange between exogenous and endogenous magnesium in the gastrointestinal tract. However, the values obtained are broadly similar to those given by studies with animals given artifical low magnesium diets.

The Agricultural Research Council (1980) have published a table of endogenous faecal magnesium values estimated using methods 2. and 3. above. They have adopted the value of 3·0 mg/kg live weight per day for both cattle and sheep, except for milk-fed calves of up to 100 kg when a lower value of 2·0 mg/kg live weight per day has been assumed.

(ii) Urinary excretion

The kidney plays an important role in the regulation of the concentration of magnesium in the extracellular fluid and, ordinarily, urine magnesium represents the excess of absorption over the body's requirements. Urinary excretion involves a filtration-reabsorption mechanism in which magnesium behaves as a threshold substance, appearing in urine only when the magnesium load filtered by the glomeruli exceeds that reabsorbed by the tubules (Wilson, 1960). Tubular reabsorption acts near or at its maximum rate of 2 mg/minute and glomerular filtration occurs at 100 ml/minute. Using these values, Wilson (1960) has calculated the threshold concentration in the plasma of ewes to be about 0·82 mmol/l. This agrees well with direct measurements in cattle which give a value of about 0·74 mmol/l, and above that value, concentrations of plasma magnesium and urinary excretion rates are linearly related (Storry & Rook, 1962). The A.R.C. (1980) give the renal threshold for magnesium as a serum concentration of not greater than 0·74 - 0·83 mmol/l, and values in excess of this may therefore be taken as an index of adequate magnesium nutrition. Conversely, urine excretion of magnesium

by hypomagnesaemic animals falls virtually to zero, as shown by Rook & Balch (1958) when dairy cows were changed abruptly from winter rations to spring grass, thereby causing a drop in plasma magnesium to below 0·74 mmol/l. In addition it can be shown that urinary loss of magnesium is negligible in animals receiving artificial diets low in magnesium.

Urine magnesium level is therefore a useful indication of the magnesium status of an animal. Field, McCallum & Butler (1958) demonstrated a highly significant positive correlation between urinary magnesium and dietary magnesium intake in individual sheep, and there is a positive correlation between net absorption from the gut and urinary excretion, as demonstrated in man by McCance & Widdowson (1942-3). Several other workers have reported close correlation between dietary magnesium input and urinary output (e.g. Rook & Campling, 1962; Storry & Rook, 1963; O'Kelley & Fontenot, 1969).

(iii) Milk

Magnesium excretion via the milk is considerable. The most extensive survey of commercial dairy herds, undertaken by Wernery, Heeschen, Reichmuth & Tolle (1973), reports a mean value of 0·125 g Mg/kg milk, and the A.R.C. (1980) have adopted this value for the calculation of daily magnesium requirements. Other workers have reported values ranging from 0·07 to 0·18 g Mg/kg milk (e.g. Blaxter & Rook, 1954; Blaxter & McGill, 1956; Smith, 1957). A cow yielding 20 kg milk may thus lose 2.5 g magnesium daily via this route, i.e. the equivalent of the total magnesium in the E.C.F.

The magnesium content of colostral milk is high (about 0·4 g Mg/kg; Balch, 1972), but within a few hours it falls to a fairly constant level (Garrett & Overman, 1940; Holmes, Spelman & Wetherbee, 1949) which is not affected by stage of lactation or milk yield (Gueguen & Salmon-Legagneur, 1959; Vanschoubroek, 1959). An important observation is that there is no fall in the magnesium concentration of milk when intake

of magnesium (Groenwald, 1935) or of feed (Robertson, Paver, Barden & Marr, 1960) is reduced, or even if hypomagnesaemia develops (Cunningham, 1936b; Robertson et al., 1960).

The A.R.C. (1980) recommended value to be taken for the magnesium content of the milk of the ewe is higher than that of the cow, at 0·17 g Mg/kg. Thus, the daily output by a ewe yielding 2 kg milk would be about 0·34 g Mg, again approximately equivalent to the total magnesium content of the E.C.F.

Magnesium Absorption

(i) Site of magnesium absorption

Until 1969/70 it was believed that the small intestine was the main absorption site of magnesium. From a comparison of the distribution of ^{28}Mg between the digesta and digestive tract epithelium after its oral or intravenous administration to sheep, Field (1961) concluded that absorption was mainly from the middle third of the small intestine. Phillipson & Storry (1965) reported that absorption could occur throughout the whole of the small intestine of the sheep, and Smith (1962) concluded that young milk fed calves could absorb magnesium from both the small and large intestines up to 3 - 4 weeks of age, and thereafter only from the small intestine. Stewart & Moodie (1956) reported that magnesium could be absorbed throughout the whole length of the alimentary tract but most extensively from the duodenum and small intestines. However, these workers introduced large quantities of magnesium salts into the gut which was not strictly comparable to a practical feeding situation. Care (1960b), using artificial solutions resembling normal digesta, demonstrated that whilst the reticulo-rumen and duodenum of the sheep were permeable to ^{28}Mg there was no significant net uptake from these regions.

During the last decade, the improved techniques employed in absorption studies, with the use of re-entrant cannulae, have led to a better understanding of magnesium absorption from the gastro-intestinal tract. As early as 1963, it was reported that in sheep only part of the ingested magnesium was found in the duodenal contents, and hence some absorption must occur somewhere between the oesophagus and duodenum (Harrison, Hill & Mangan, 1963). In later years this observation was confirmed by several workers whose results show that net absorption of magnesium is mainly localised in the digestive tract proximal to the duodenum in sheep (e.g. Pfeffer, Thompson & Armstrong, 1970; Grace & MacRae, 1972; Axford, Tas, Evans & Ofter, 1975; Strachan & Rook, 1975) and in cattle (e.g. Rogers & van't Klooster, 1969; Kemp, van't Klooster, Rogers & Geurink, 1973; Bertoni, Watson, Savage & Armstrong, 1976).

A number of studies have been undertaken to determine from which part of the proximal digestive tract magnesium is absorbed. Whereas Care & van't Klooster (1965) and Phillipson & Storry (1965) were unable to demonstrate significant absorption of magnesium from the rumen of sheep, Marongui (1971) did find considerable magnesium absorption from this organ. Moreover, Tomas & Potter (1976a) observed evidence of absorption when magnesium was infused into the rumen of sheep, but not when infused into the omasum or abomasum. They concluded that the reticulo-rumen is the principal site of magnesium absorption and that absorption post-ruminally is insufficient to maintain normal magnesium status in the animal. Field & Munro (1977), in a similar infusion experiment, detected some absorption from the omasum but concluded that this was less than from the rumen, and Marongui (1971) found no absorption from the omasum. Ben Ghedalia, Tagari, Zamwel & Bondi (1975) concluded that the omasum is possibly the major site of magnesium absorption for sheep and Fitt, Hutton & Armstrong (1979) using sheep given a magnesium-deficient diet, reported that magnesium infused into the omasum or into

the rumen raised plasma magnesium levels, but administration via the abomasum, proximal duodenum or terminal ileum resulted in steady falls in plasma magnesium to hypomagnesaemic levels. They concluded that the major site of magnesium absorption is the omasum and possibly reticulo-rumen. However, most workers do agree that the sheep abomasum is not an important site, e.g. Pfeffer & Rahman (1974) report an apparent relative lack of absorption of magnesium infused into the abomasum rather than to the rumen.

Magnesium absorption from the stomach of the adult bovine has had less attention than that of the ovine. Smith & Horn (1976) reported that absorption did not occur from the rumen or abomasum of young steers, and Perry, Cragle & Miller (1967) found no magnesium absorption from the rumen of calves. Later, Smith & Edrise (1978) reported significant absorption from the omasum and Horn & Smith (1978) stated that the weight of all their evidence indicated that the omasum is more important than the reticulo-rumen for magnesium absorption in young cattle, but that this has not been shown unequivocably. In addition, the findings reported for the sheep omasum do not necessarily apply to that of the bovine (Edrise, Smith & Buttle, 1977) and extrapolation of results to cattle require caution because of the relatively larger omasum in this species compared with sheep (Phillipson, 1970).

Some workers have employed *in vitro* techniques to study ruminant magnesium absorption. Harrison (1971) demonstrated that the sheep omasal epithelium is permeable to magnesium, with net transport from the lumen to the blood side, and Phillipson & Storry (1965) found the sheep rumen epithelium impermeable to Mg^{2+}. However, Martens, Harmeyer & Breves (1976) demonstrated net uptake of ^{28}Mg from the lumen to the blood side of rumen epithelium in an *in vitro* system. Martens & Rayssiguier (1979) reported that the net flux of magnesium across the sheep rumen mucosa exceeded that across the omasum mucosa by a factor

of 3 - 4 : 1, and they concluded tentatively that the absorptive capacity of the rumen is 25 - 30 times that of the omasum.

Thus there is evidently still some confusion about the principal site of magnesium absorption from the ruminant gut. Certain regions of the intestine, such as the mid-ileum and colon, are probably important areas of net magnesium absorption, but these are largely nullified by the net secretion of magnesium into other sections (Marongui, 1971; Ben-Ghedalia et al., 1975). There seems little doubt that magnesium absorption occurs in the ruminant stomach, and, from all the results obtained for sheep, young cattle and in vitro preparations, magnesium is certainly absorbed from the reticulo-rumen and from the omasum. Considerable uncertainty exists, however, as to which of these fore-stomachs plays the dominant role.

(ii) Mechanism of Mg absorption

The mechanism of Mg absorption is currently believed to be an active process (Martens & Rayssiguier, 1979). However, in the search during the 1960's for sites of Mg absorption it was generally assumed to be a passive diffusion process. From a consideration of electric potentials and concentrations of ultrafiltrable Mg in different parts of the digestive tract, Storry (1961) commented that passive diffusion of Mg could theoretically be expected to occur from the abomasum and duodenum into the blood, but not from the rumen. At that time the duodenum and small intestine were thought to be the main sites of magnesium absorption whereas it is now thought to take place in the ruminant stomach. Thus magnesium is probably absorbed against concentration and electric potential gradients and workers tried to define an active process. Martens et al. (1976) deduced from their observations on ^{28}Mg transfer across isolated rumen epithelium in vitro that Mg absorption occurs by an active process. This and later studies have shown that several criteria for active transport are satisfied (Martens & Rayssiguier, 1979),

i.e. transport against concentration and electric potential gradients, saturation kinetics, competitive inhibition, temperature sensitivity, inhibition by dinitrophenol. They have also shown that magnesium transport is reduced by ouabain the inhibitor of Na/k ATPase, and that a positive correlation exists between the sodium concentration in the buffer and the amount of magnesium transported. It was therefore postulated that magnesium is transported across the ovine rumen mucosa by an active Na-linked process.

Brown, Care & Pickard (1977) were able to demonstrate the existence of an active mechanism in vivo. Having established rumen pouches in sheep, they observed magnesium transport against an electro-chemical gradient between the pouch solution and venous blood, and the absorptive mechanism was saturated at pouch solution magnesium concentrations above 4 mM.

Magnesium Homeostasis

In contrast to calcium, no direct hormonal control of blood magnesium levels in ruminant animals has been found. Some workers have postulated indirect effects of various endocrine glands such as the adrenals (Hanna & MacIntyre, 1960; Oyaert, 1962; Care & Ross, 1963; Scott & Dobson, 1965), thyroid (Allcroft, 1947; Hanna, 1961; Inskeep & Kenny, 1968) and parathyroid (MacIntyre, Ross & Troughton, 1963; Sherwood et al., 1966). More recently the hormone calcitonin has been associated with hypomagnesaemia in experimental rats and goats (Barlet, Rayssiguier & Larvor, 1974). However, magnesium balance is evidently not under close control, unlike calcium which is under such strict control that plasma levels are held within very close limits whatever the dietary calcium intake. Since mobilisation and deposition of magnesium from bone do not play a major part in magnesium homeostasis, especially in older animals, control of the plasma (E.C.F.) magnesium

level depends on the balance between absorption from the gut on the one hand and body losses on the other. Obligatory magnesium outputs from the lactating ruminant, in milk and endogenous losses, are large in relation to the readily available body magnesium pool. Thus a regular input of absorbed magnesium is essential for the maintenance of normal magnesium status, i.e. a regular dietary supply of available magnesium is vital. The animal requires sufficient absorbed magnesium to balance unavoidable losses, and renal excretion removes any excess (over the threshold E.C.F. concentration of 0·7 mmol/l).

2. HYPOMAGNESAEMIA AND HYPOMAGNESAEMIC TETANY

Ruminant animals are susceptible to disorders in their magnesium metabolism. Hypomagnesaemia is the biochemical state in which the blood plasma magnesium level is below 0·74 mmol/l, the lower limit of the normal range. Hypomagnesaemic tetany, also commonly known as magnesium tetany, grass tetany, lactation tetany, or grass staggers, is the term used to describe the clinical condition which may arise in hypomagnesaemic animals. Hypomagnesaemia usually occurs as a herd problem, with the mean plasma magnesium level below 0·74 mmol/l, and some herds of hypomagnesaemic cows may show no obvious signs of ill-health. In other herds, however, some cows may show sub-clinical or clinical symptoms, often preceding death.

Clinical symptoms

The physical symptoms indicate that hypomagnesaemic tetany is a nervous type of disorder (e.g. Sims & Crookshank, 1956; Todd & Horvath, 1970). The onset of the acute tetanic stage can be very rapid in that an apparently healthy animal will suddenly collapse in convulsive fits and unless curative treatment is supplied it may die in under an hour. The animal tends to lie flat on its side with the head thrown back, and the stiffened forelegs pedal periodically. Other symptoms include

frothing at the mouth, grinding of the teeth and retraction of the eyelids. Periods of rest alternate with the attacks, but convulsions may be set off by touch or sudden noise. Pulse and respiration rates are high and body temperature is elevated.

The subacute or chronic form of hypomagnesaemic tetany is essentially similar to the acute form, and only differs in the degree to which the various symptoms are shown. The main symptom again is hyperexcitability of the nervous system, evidenced by muscular twitching and tremors. The affected animal indicates an unusual alertness and nervousness, with head held high, staring eyes, ears pricked, and reacts nervously to external stimuli such as touch or sound. Inco-ordination of movement is evidenced by a stiff staggering gait and exaggerated negotiation of obstacles. Such cases may exhibit these symptoms to a greater or lesser extent for many days until eventual recovery, or, alternatively, progression to the acute form of the disorder.

From studies on neuro-muscular irritability in calves, Todd & Horvath (1970) concluded that the main dysfunction causing tetany is not likely to be in the muscle fibre or in the central nervous system, rather it is a facilitation of the neuro-muscular transmission involving a reduction of the current required to cause contractions to about one-third of normal.

Signs of sub-clinical hypomagnesaemia may also include depression of appetite (Scott, Kelly, Whitaker & Cameron, 1980) and milk yields may fall by 13 - 20% (Thompson, Young & Rys, 1974/75). Anaemia and udder oedema in cows has been associated with hypomagnesaemia in the spring in New Zealand (Hicks & Pauli, 1976), and there is also a suggestion that neonatal calf mortality and prolonged anoestrus may be associated with hypomagnesaemia in older cows (Brodauf, Kopp, Mueller & Schmidt, 1970).

Magnesium deficiency has been extensively studied in laboratory

animals and the classical symptoms in the rat reflect closely those observed in the farm ruminant animal (see review by Rook & Storry, 1962).

Biochemical changes

The most characteristic and consistent change that has been observed in hypomagnesaemic tetany is a sudden drop in blood serum magnesium. The normal range of plasma magnesium is 0·74 - 1·3 mmol/l and Payne (1977) reports "normal" reference standards for dairy herds as 2·4 - 2·6 mg/100 ml, i.e. 0·99 - 1·07 mmol/l. Animals with blood levels below the normal range may properly be referred to as hypomagnesaemic but cases of clinical tetany are more likely if the serum magnesium concentration falls below 1 mg/100 ml, i.e. 0·41 mmol/l (Payne, 1977). The relationship between hypomagnesaemic tetany and blood serum magnesium was originally established by Sjollema (1928, 1930 a & b) and Sjollema & Seekles (1929) in Holland. They reported a mean serum magnesium level of 0·18 mmol/l in 55 cows suffering from hypomagnesaemic tetany. Meanwhile in Great Britain, Dryerre (1932) also reported the association between tetany and blood magnesium deficiency and he found a mean serum level of 0·16 mmol/l in 42 clinical cases. Similarly for sheep (e.g. Inglis, Weipers & Pearce, 1959; Herd & Peebles, 1962) and for calves (e.g. Duncan, Huffman & Robinson, 1935; Todd & Rankin, 1959), workers have demonstrated the existence of extremely low plasma magnesium values in cases of hypomagnesaemic tetany.

Clinical signs, however, have been reported at nearly normal plasma magnesium levels, but Burns & Allcroft (1967) indicate that serum magnesium may actually rise at the onset of convulsions, thus time of blood sampling is an important consideration. Recent research has shown that cows showing clinical signs of hypomagnesaemic tetany have low levels of magnesium in the cerebrospinal fluid (C.S.F.) (Allsop & Pauli, 1975), moreover it has been reported that the onset on tetany is more closely associated with low C.S.F. magnesium than with low serum levels

(Meyer & Scholz, 1972; Pauli & Allsop, 1974). Allsop & Pauli (1975) produced tetany in sheep by perfusion with synthetic C.S.F. solutions containing 0·25 mmol/l, and eliminated signs of tetany using a solution containing normal magnesium concentration. These workers then postulated that a low C.S.F. magnesium level may result in changes in the central nervous system that would produce the nervous clinical symptoms of hypomagnesaemic tetany. There is some evidence that this fall in C.S.F. magnesium is associated with a leakage of magnesium from brain cells (Kolb, Ellrich & Grundel, 1976) due to an increased membrane permeability. Furthermore some evidence suggests that leakage of magnesium from brain cells can be caused by stress, and that agents which tranquilize the central nervous system (such as barbiturates) are of therapeutic value in tetany, (Rogers, 1979).

A large proportion of hypomagnesaemic animals which experience tetany have a concomitant hypocalcaemia. Allcroft (1947a,b) noted that 76% of 406 cows showing clinical hypomagnesaemic tetany were also hypocalcaemic. Later, Hemingway, Ritchie & Brown (1965) reported a rapid fall in both plasma calcium and magnesium after lactating ewes were transferred to grass. Clinical cases were observed to have marked hypocalcaemia (1·50 mmol Ca/l) as well as hypomagnesaemia (0·21 mmol Mg/l). However tetany did not occur in the high proportion of ewes which showed low plasma magnesium, but which maintained normal calcium levels. These findings have largely been confirmed by other workers (e.g. Burns & Allcroft, 1967; Forbes, 1972). It is possible that individual animals can withstand long periods of hypomagnesaemia without clinical signs because a concomitant hypocalcaemia is necessary before tetany occurs. This may be true in some cases but Todd (1969), for example, has reported up to 50% of cattle with the clinical syndrome have normal plasma calcium levels. A constant finding widely reported is that the

urine becomes virtually devoid of magnesium in hypomagnesaemia. Field (1961) observed that transfer of sheep to a spring pasture resulted in an immediate fall in urinary magnesium excretion, even when the change led to an increased magnesium intake, the lowest values occurring within 1 - 2 days. Similarly, Head & Rook (1955) observed an immediate reduction in urine magnesium when stall-fed cows were put to grass, although the magnesium intake was about the same. Simesen (1977) has reported that determination of urine magnesium levels can be a reliable diagnostic test for hypomagnesaemia. An indicator paper which enables a semiquantitative determination of urine magnesium as a quick test on the farm has even been developed (de Groot & Marttin, 1967) and critically tested (Simesen, 1968). Halse (1970) suggests that urinary magnesium would be a better indicator for magnesium status than serum magnesium for individual animals where the "normal" is not known.

The biochemical studies have been extended by some workers to the consideration of the redistribution of magnesium within the body, which could give rise to acute hypomagnesaemia. Larvor (1977) has given a compilation of available data on the relationship between magnesium balance and plasma magnesium level in cows and sheep on young grass or winter diets. In some experiments with sheep, a negative correlation between magnesium retention and blood magnesium has been noted (Larvor & Violette, 1969), i.e. grass induces hypomagnesaemia but with a higher magnesium balance due to a lower urinary output. Larvor (1977) suggested that withdrawal of magnesium from body reserves is reduced by "tetany-inducing" grass, and that there is a shift of body magnesium from the blood into some other tissue. Moreover Care, Ross & Wilson (1965) found, using radioactive magnesium, that the turnover rate between magnesium in the extracellular fluid and the readily exchangeable intracellular magnesium of soft tissues and bone was significantly lower on a fresh grass diet than on hay.

Todd & Thompson (1960) have investigated many major blood electrolytes (Ca, Na, K, Cl) and the blood CO_2-combining power, but could report no consistent alterations in these factors during hypomagnesaemia. Todd, Horvath & Anido (1969) reported that plasma creatine phosphokinase (C.P.K.) levels are considerably elevated for about 24 hours after an acute phase of hypomagnesaemic tetany in calves, and that magnesium therapy reduces these C.P.K. levels but the diagnostic value of plasma C.P.K. is not yet known.

Rumen ammonia levels rise when animals are transferred from dry feed to pasture (Head & Rook, 1955), and administration of a single dose of ammonium acetate or carbonate resulted in ruminal levels comparable to those of cows on fresh grass, with similar changes in urinary excretion and blood magnesium levels.

It has also been shown that hypomagnesaemia in the ruminant may be associated with increased lipolysis (Martens & Rayssiguier, 1979). Stimulation of lipolysis under experimental conditions, for example by fasting, exposure to cold or intravenous infusion of adrenaline, induces hypomagnesaemia in both ewes and cows, associated with a drop in plasma magnesium and an increase in free fatty acids. The relationship between lipolysis and hypomagnesaemia may help in the explanation of hypomagnesaemic tetany resulting from problems of adaptation to a sudden change to outdoor grazing and environmental stress factors.

Incidence of hypomagnesaemic tetany in adult ruminants

Hypomagnesaemic tetany is confined to temperate zones where intensive grassland farming methods are practised, including the heavy use of fertilisers. It occurs in Europe, New Zealand, Australia and parts of North America, where the average temperature and rainfall are conducive to the production of rich pastures which are used for grazing ruminants.

There is considerable seasonal variation in the incidence of hypomagnesaemic tetany. Allcroft (1947a) found that there was a peak of incidence of clinical cases in the month of April in the four years 1937-41, followed by a sharp decline during the summer months when there were very few cases. There was then a secondary peak of incidence in the autumn months of October/November, with other cases reported throughout the winter. These incidence peaks of reported cases coincided with the times when mean serum magnesium levels were lowest in the experimental herds which were being sampled by Allcroft throughout this period. (The incidence peaks may also coincide with "spring-" and "autumn-" calving cows). Many other reports confirmed this pattern of incidence with the greatest number of cases occurring in the spring, very few in the summer, and a smaller outbreak in the autumn and early winter. The spring peak of incidence is associated largely with dairy cows being transferred to grass after the period of winter feeding, when the acute tetanic syndrome may occur after a "latent period" of 5 - 10 days. Similarly, acute cases may occur in association with an autumn flush of rapidly-growing grass, or with inclement weather.

In beef cattle hypomagnesaemic tetany is prevalent in the autumn and winter, when out-wintered beef herds are grazing poor pasture with little or no supplementary food, or receiving poor winter feeds. Such cases are usually associated with a chronic hypomagnesaemia which has developed over a long period, e.g. Allcroft & Green (1938) observed a winter fall in serum magnesium concentration in Hereford cows at poor winter pasture with no shelter or extra feed, the lowest values being observed in December-April. This may largely be attributable to the low plane of nutrition on which such animals are maintained (Inglis, Weipers & Marr, 1954), and on the severe climatic conditions to which they are exposed (Allcroft & Green, 1938; Allcroft, 1947c).

As climatic factors are important on a seasonal basis, it can be seen that they can influence the animal and its pasture, whether fresh or conserved, to produce yearly variations in the incidence of hypomagnesaemia. That the number of cases varies from year to year is now well known, but it was first pointed out by Green (1939) and Allcroft (1947a).

The pattern of incidence of hypomagnesaemic tetany will depend on the type of farming practised, however White (1953) noted a general tendency for clinical cases to become more prevalent in autumn and winter, whereas in the earlier years the occurrence of tetany in spring was considered to be the major problem. It is the general impression that this trend has continued to date.

Lactating cows are the most susceptible to hypomagnesaemic tetany, but it can occur, though less commonly, in all types of cattle (Burns & Allcroft, 1967). There is a suggestion of different breed susceptibilities in Great Britain, with Ayrshire cattle recording the highest number of cases and Jersey cows the lowest (Burns & Allcroft, 1967). Older cows are more at risk than are heifers, with susceptibility rising continuously with age (Blaxter & McGill, 1956; Leech et al., 1960). Old cows which have had more than 6 calves are 14 times more likely to develop the condition than are heifers in their first lactation (Blaxter & McGill, 1956), and cows in their third and fourth lactations are 6 - 10 times more susceptible. This may not necessarily be related to higher milk production by older cows, as Bartlett et al. (1957) could find no correlation between the incidence of hypomagnesaemic tetany and milk yields, despite the fact that higher milk yields must create an increased demand for magnesium. The increased susceptibility of older animals is probably attributable to the lower labile reserves of body magnesium with the high proportion of body magnesium largely immobilized in the skeleton.

In sheep the condition is mainly confined to lactating ewes with lambs at foot, generally 2 - 6 weeks after lambing. It occurs mainly in the spring season, closely associated with the spring flush of grass, especially where improved pasture is being grazed to enhance milk production for the lambs, but also with the stage of lactation as cases seldom occur directly after parturition nor in the later stages of lactation. Older ewes and those with twins are more susceptible than are gimmers and those with singles, and there is a higher mortality rate from tetany in sheep than in cattle. The growing recognition of the importance of hypomagnesaemic tetany in sheep is reflected in the widespread study of the condition in the 1960's and 1970's and Kelly (1979) has recently reviewed the subject.

The overall incidence of hypomagnesaemic tetany may be relatively low, e.g. about 1 - 2% in Europe (Church & Fontenot, 1979), but individual herds may have a high percentage of affected animals. The mortality rate is often high (30%+) in clinical cases, thus the incidence of this condition can be disastrous to individual farmers. A recent survey of 120 dairy herds in New Zealand (Young, O'Connor & Feyter, 1979) reported hypomagnesaemia to be widespread and severe.

Hypomagnesaemic tetany in calves

Hypomagnesaemic tetany in calves is associated with the feeding of whole milk for an abnormal length of time (Duncan, Huffman & Robinson, 1935). The development of clinical cases is always associated with low serum magnesium levels, but, as with adult ruminants, hypomagnesaemia without signs of tetany does occur. Blaxter & Sharman (1955) reported hypomagnesaemic tetany in suckled calves on beef rearing farms in Invernesshire, with the fastest growing calves showing the greatest decrease in serum magnesium. Milk alone as a food is relatively low in magnesium (0·125 g/kg on a fresh matter basis) and the requirements for a rapidly growing calf are high. In addition there is a gradual decrease

in ability to absorb magnesium with increasing age in calves (Smith, 1958, 1962). Young calves can draw upon their bone reserves of magnesium (0·80% bone ash cf 0·5 - 0·7% in adults) and Smith (1959b) has observed depletion of bone magnesium (by as much as 30%) with decreased plasma levels. It may take several weeks of oral magnesium supplementation to bring the level back to normal. However in most practical calf-rearing systems, some supplementary dry food is available from a relatively early age and the incidence of hypomagnesaemic tetany is not as widespread as in adult ruminants.

3. FACTORS INVOLVED IN THE DEVELOPMENT OF HYPOMAGNESAEMIC TETANY

Some Aetiological Considerations - Factors affecting Magnesium Absorption

Most research has indicated that acute hypomagnesaemia is a conditioned disorder resulting largely from impaired magnesium absorption associated with the intake of spring grass. Much early work involved the study of the characteristics of young "tetany-prone" grass and this has developed into detailed *in vivo* and *in vitro* studies of factors affecting magnesium absorption from the ruminant gut.

Young, rapidly-growing spring grass contains high levels of potassium and phosphorus, and relatively low levels of sodium, calcium and magnesium. It is rich in soluble carbohydrates and crude protein, but low in crude fibre, and lush pastures can contain up to 85% water. During the growing season the content of crude protein decreases (on a dry matter basis), and crude fibre increases. Intensification of grassland production has led to increased incidence of hypomagnesaemic tetany, particularly where heavy applications of fertilisers have been employed.

There is conflicting evidence in the literature from grazing trials as to the importance of high dietary potassium in relation to the development of hypomagnesaemia. Increased incidence of the condition

has been associated with high potassium levels in the pasture by some workers (e.g. t'Hart & Kemp, 1956; Kemp, 1958) and the situation is exacerbated by applications of potassic fertilisers (e.g. Hvidsten et al., 1959). Bartlett et al. (1954), however, reported severe decreases in serum magnesium from potassium fertilisation in pastures in which clover was controlled, but they observed no such effect on a sward predominantly of clover. Other workers have reported no substantial effect of high potassium levels in pasture (e.g. Alten, Rosenberger & Welte, 1958; Smyth, Conway & Walsh, 1958; Hemingway et al., 1963; Lomba et al., 1971).

However, in feeding experiments, dietary additions of potassium salts have been shown to decrease magnesium absorption in sheep (Suttle & Field, 1967; Suttle & Field, 1969; House & van Canpen, 1971; Newton et al., 1972) and cattle (Kemp et al., 1961).

There are indications that high dietary potassium levels may alter magnesium metabolism in other ways. For example, House & Bird (1975) found that the decrease in serum magnesium levels following intravenous injection of magnesium was more rapid in goats fed a 4% potassium diet than those fed a 0·9% potassium diet. They postulated that supplementary potassium may have increased the cellular uptake and retention of magnesium. Other research workers, however, have been unable to report an effect of high dietary potassium on the development of hypomagnesaemia (e.g. Green & Allcroft, 1951; Eaton & Avampto, 1952; Daniel et al., 1952).

Infusions of potassium salts into the rumen severely reduce magnesium absorption from that organ (Tomas & Potter, 1976; MacGregor & Armstrong, 1979), and in vitro studies using isolated rumen mucosa suggested that the inhibitory action of potassium was dependent on the Na : K ratio rather than the potassium concentration per se (Käsebieter, 1978, reported in Martens & Rayssiguier, 1979). Further in vivo studies have complemented this finding as Marten & Rayssiguier (1979) found a linear

increase in magnesium absorption from the sheep rumen with an increase in the Na : K ratio of the rumen solution, up to a maximum of 5 : 1; beyond this there was no further increase in absorption. Similar importance of the Na : K ratio in the stomach has been reported for steers (Horn & Smith, 1978) and sheep (MacGregor & Armstrong, 1979). Young "tetany-prone" grass has a low Na : K ratio, especially where the pasture is intensively fertilised, thus creating unfavourable rumen conditions in the grazing animal for magnesium absorption. Patterson & Crichton (1960) demonstrated that under commercial conditions the incidence of hypomagnesaemia could be drastically reduced by feeding additional sodium chloride to dairy cows grazing spring grass. However, the mechanism of the Na : K effect is as yet unknown.

Other mineral ratios have also been suggested as indices for the relationship between a given sward and the degree of hypomagnesaemia produced, e.g. K/(Ca + Mg) (Kemp & t'Hart, 1957; Butler, 1963); K x 100/(K + Ca + Mg) (Verdeyen, 1953); Na x 100/K + Na + Ca + Mg (t'Hart & Kemp, 1957). However, no consistent findings have been reported.

Nitrogen levels are high in young grass, and fertiliser applications of nitrogen and potassium together usually produce lower serum magnesium in cows than either fertiliser applied alone (Smyth et al., 1958; Kemp, 1960). McIntosh, Crooks & Simpson (1973) observed that increasing potassium fertiliser reduced total and water-soluble magnesium in grass, while increasing nitrogen fertiliser increased total and water-soluble magnesium, but only at a low potassium level. Fontenot et al. (1960) reported a severe reduction in magnesium absorption by sheep given a high protein, high potassium ration, but high levels of dietary nitrogen in addition to high levels of dietary potassium generally do not adversely affect magnesium absorption to a greater extent than high levels of potassium alone (Fontenot, Wise & Webb, 1973).

Fertilisation of pastures with high levels of nitrogen increases the crude protein content and has generally resulted in lowered serum magnesium in ruminants grazing the pastures (Bartlett et al., 1954, 1957; Kemp, 1958, 1960; O'Kelley & Fontenot, 1969) even though the magnesium intake may be higher (L'Estrange, Owen & Wilman, 1967; Lomba et al., 1971). Some workers have reported an inverse relationship between magnesium absorption or apparent digestibility and dietary protein content (Rook, Balch & Line, 1958; Kemp et al., 1961), but Moore et al. (1972) reported similar magnesium absorption by lambs receiving diets containing either 10% or 33% crude protein. Larvor & Gueguen (1963) have reported a negative relationship between the soluble nitrogen fraction in grass and blood magnesium levels in sheep. There are contrasting results comparing the effects of different rates of nitrogenous fertiliser application. For example, Stillings et al. (1964) observed a highly significant reduction in magnesium absorption by sheep when ammonium nitrate was applied to pasture, whereas L'Estrange & Axford (1966) reported no such effect when nitrochalk (a mixture of ammonium nitrate and chalk) was used. Thus results are conflicting as to the importance of dietary nitrogen levels in the aetiology of hypomagnesaemia. However, the high crude protein content in young grass (20 - 30%) is readily fermented and ammonia concentrations in the rumen increase markedly as a result. Head & Rook (1955) were the first to suggest a possible role of high ruminal ammonia production in the development of hypomagnesaemia. The results of Martens & Rayssiguier (1979) support this concept since they observed reduced magnesium absorption from the sheep rumen when the ammonia concentration was raised. It had been suggested that increased ammonia levels in the rumen fluid decrease the pH and lead to the precipitation of magnesium ammonium phosphate (Simesen, 1970; Kemp et al., 1961). However, this theory does not agree with a report by Hemingway & Brown (1967) that magnesium

ammonium phosphate is in fact a good dietary magnesium source. In addition, it has been suggested that high ammonia or urea levels administered artificially may depress appetite and this is the reason for depressed magnesium absorption and lowered blood magnesium concentrations. No significant changes in pH of ruminal, abomasal or small intestinal contents have been observed in association with spring grass (Simesen, 1970). Martens & Rayssiguier (1979) suggest that depression of rumen absorption of magnesium is possibly an ammonia toxicity effect on the mucosa, as magnesium absorption is impaired only with an acute rise in ammonia levels and the animal adapts over an extended period of time.

High levels of dietary calcium and phosphorus appear to lower magnesium absorption (Chicco, Ammerman & Loggins, 1973; Pless, Fontenot & Webb, 1975; Nel, 1976), but the effect does not appear to be as severe as high levels of potassium (Newton et al., 1972).

There has been considerable interest in the relationship of plant organic acids to the incidence of hypomagnesaemic tetany. The organic acid concentrations have been shown to increase in response to nitrogenous and potassic fertilisers (Grunes, Stout & Brownell, 1970) and some grasses associated with tetany have high levels of trans-aconitic acid (Burau and Stout, 1965; Stout, Brownell & Burau, 1967), especially in cold environmental temperatures, and aconitate and citrate are known to form insoluble chelates with calcium and magnesium. However, field trial results are conflicting as Kenedy (1968) reported that plasma and urine magnesium levels were unaffected by trans-aconitic or citric acids, whereas other workers (Bohman et al., 1969; Scotto, Bohman & Lesperance, 1971) could induce tetany by drenching cattle with potassium chloride together with citrate or trans-aconitate (potassium chloride alone reduced plasma magnesium only over an extended time period in this trial).

Experiments investigating the possible effects of readily available carbohydrates also give inconsistent results. Starch supplementation was found to lessen the depression of plasma magnesium in grazing dairy cattle (Wilson et al., 1969) but did not influence magnesium absorption in lambs on a semi-purified diet (House & Mayland, 1976b). Glucose supplementation to hay was found to increase apparent magnesium absorption in sheep, but had no effect when added to grass (Madsen et al., 1976). Mayland et al. (1974) observed that a rapid increase in the ratio of nitrogen to total water-soluble carbohydrates in grass coincided with increased occurrence of tetany, and the imbalance between crude protein and readily available carbohydrates may give rise to an energy deficit (Dishington, 1965; Martens & Rayssiguier, 1979). Such an energy deficiency would lead to increased rumen ammonia production, argue Martens & Rayssiguier (1979), and reduced volatile fatty acid (V.F.A.) formation. The adverse affects of ammonia have already been indicated, and it has also been shown that magnesium absorption increases with increased V.F.A. concentration in artificial rumen fluid (Martens & Rayssiguier, 1979). Raising the energy supply in line with the dietary protein should abolish the inhibiting action on magnesium absorption.

A close relationship has been reported between higher fatty acids and crude protein content in grasses (Kemp et al., 1966; Molloy et al., 1973). Nitrogen fertilisers are reported to increase the lipid content of herbage and this might be important in the reduction of herbage magnesium absorption (Kemp et al., 1966). Various workers have supplemented ruminant diets with lipids but their results are inconsistent. Decreased magnesium utilisation in cattle has been associated with dietary additions of animal fat (Kemp et al., 1966), hydrogenated marine fat (Sundstol, 1974), and peanut oil (Wilson et al., 1969). For sheep, however, corn oil was found to decrease magnesium

utilisation, by Devendra & Lewis (1974), whereas Grace & Body (1979) could find no effect of corn oil on apparent magnesium absorption or plasma magnesium levels. Whatever the net effect on magnesium balance, all these workers report that an increased intake of long chain fatty acids inevitably increases the precipitation of insoluble soaps, largely with magnesium or calcium, which are excreted in the faeces; thus a proportion of magnesium in the gut is rendered unavailable.

Fitt and his co-workers (1972) have investigated the possible relation between the binding of magnesium ions by bacterial cell walls of rumen bacteria and the development of hypomagnesaemia. They found magnesium uptake by isolated rumen bacteria to increase with increased magnesium concentration and with increased pH, and that at low magnesium concentrations (when the levels may be critical with reference to the animal's requirement) magnesium uptake is substantial and directly proportional to the magnesium concentration. They suggest that further studies on the subsequent fate of the "bound" magnesium and its relation to the aetiology of hypomagnesaemia should be undertaken.

Magnesium content of feeds and pasture

Cereal and protein concentrate feeds have varying contents of magnesium (see, for example, A.D.A.S., 1976), but the normal indoor diets for cattle and sheep usually have comparatively high levels, i.e. over 1·5 - 2·0 g Mg/kg DM, and animals given such diets very rarely develop hypomagnesaemia. Good feed sources of magnesium are wheat bran (5 g/kg DM) and most vegetable protein concentrates, especially linseed cake and cottonseed cake (6 g/kg DM). Indeed, it is difficult to devise a magnesium-deficient diet without recourse to artificial dietary constituents (see Section IIIA).

Hypomagnesaemia and hypomagnesaemic tetany are problems almost entirely confined to animals at grass, thus much attention has been

focussed on the magnesium content of pastures. Many workers have reported the same, if not higher, magnesium content in so-called "tetany-prone" pastures compared with normal ones (e.g. Sjollema, 1932; Blakemore & Stewart, 1934-35). However, t'Hart & Kemp (1956) demonstrated that the magnesium content in "tetany pastures" was about 10% below that in "non-tetany" pastures. Kemp (1960), and later Allcroft & Burns (1968) reported that hypomagnesaemia did not occur on pastures containing over 2 g Mg/kg DM. This value has since been adopted by many workers as the desired herbage magnesium concentration for control of hypomagnesaemia.

The magnesium content of pastures in temperate regions is lowest in the spring, averaging about 1·5 Mg/kg DM, and increases during the summer to levels generally about 2·0 g/kg DM (Stewart & Holmes, 1953; Todd, 1961; Wolton, 1960). These levels are usually maintained during the autumn and fall during the winter. This seasonal variation may in part be due to temperature changes, since Dijkshoorn & T'Hart (1957) found that the magnesium content of grass grown at 40°F was 30% lower than a comparable sward grown at 70°F. Amounts of solar radiation may also be important, as Mayland & Grunes (1974) suggested that shaded forage contains less magnesium than forage grown in full sunlight.

In addition to seasonal variation, the magnesium content of pastures can be affected by other factors, notably the botanic composition of the sward. Herbs and legumes generally have higher magnesium contents than grasses and are said to reduce the "tetany-proneness" of pasture (T'Hart, 1960). An example of this difference is given by Thompson (1960) as grasses 2·4, legumes 6·9 and herbs 7·5 g Mg/kg DM. Red clover usually has a higher magnesium content than white clover, but legumes begin growth later in the season and therefore cannot make a significant contribution to the magnesium content of the earlier spring pastures. Indeed, hypomagnesaemic tetany occurs most frequently on pastures where

grasses predominate. Among the grasses the ryegrasses (Lolium spp.) and cocksfoot (Dactylis glomerata) do not differ greatly in magnesium content, but Timothy (Phleum pratense) generally has lower magnesium levels than most other grasses (Thomas et al., 1952; Parr & Allcroft, 1957).

Fertiliser treatment of pasture can also affect its magnesium content. The use of magnesium-containing fertilisers, notably magnesium sulphate and magnesium oxide, to increase the magnesium content of herbage has been the subject of many investigations (e.g. Bartlett et al., 1954; Birch & Wolton, 1961; Hemingway, 1961; Smyth et al., 1958). Results suggest that there is a more or less proportional increase in herbage magnesium content for increasing amounts of magnesium fertiliser applied. However, the response to such fertiliser treatment is partly dependent on the soil type and pH (Collins, 1960); acid soils respond well whereas alkaline or heavier soils respond poorly. The treatment is required to increase the soil exchangeable magnesium to raise the magnesium uptake by the herbage to the point where the herbage magnesium content is above about 2 g/kg DM. Thompson (1960) points out that there are few cases of hypomagnesaemic tetany among animals at grass on soils such as the magnesium limestone soils in Durham, reflecting the high magnesium levels in the pasture. Conversely, light soils deficient in magnesium will produce herbage containing insufficient magnesium.

The use of nitrogenous and potassic fertilisers can also influence the mineral composition of pasure. In addition, nitrogen fertilisers often reduce the proportion of clover in the sward, whereas applications of potassium and phosphorus may quickly produce a clover-dominant sward. As clover is high in magnesium these alterations to the botanical composition of the sward can be significant. Most plants absorb potassium in preference to magnesium from the soil, therefore potassium applications generally decrease pasture magnesium levels (e.g.

Blaxter et al., 1960; Kemp, 1960; Wolton, 1960; Hemingway, 1961). Nitrogen applications tend to increase the magnesium content of grass (e.g. Wolton, 1960; Hemingway, 1961) but not when potassium is also applied (Kemp, 1960). Fertiliser applications can also influence the absorption of the magnesium from herbage by the grazing animal, and this important aetiological aspect will be considered later.

Some workers are investigating the possibility of breeding grasses with a higher magnesium level, i.e. 2 g Mg/kg DM in the spring, and Hides & Thomas (1981) report on increasing the magnesium content of Italian ryegrass species by selection.

Availability of dietary magnesium

In the study of mineral metabolism, it is generally recognised that the total content of a mineral element in a particular diet has little significance unless it is qualified by a factor indicating the biological availability of the element to the animal. No element is ever completely absorbed and utilised. However, there is some confusion in the literature as to what constitutes "utilisation" and "availability", and how they should be expressed and applied in nutrition. Peeler (1972) has discussed several terms and criteria that have been used, e.g. percent utilisation, apparent absorption, net retention, true availability, apparent availability, biological availability etc. Generally the most reliable values express the amount of a dietary mineral element that is absorbed from the gut, expressed as a percentage of the dietary intake. The only direct measurements, then, involve the use of radioactive tracer techniques in which the fate of a dietary mineral can be followed directly. However, in the case of magnesium, the only available tracer is magnesium-28 which, apart from being very expensive, has a short half-life of only 21·3 hours. Its use in nutrition experiments has therefore been limited, although some workers have reported "true availability" figures for dietary magnesium using such methods, e.g. Field (1959),

Care (1960c), Simesen et al. (1962), Hjerpe (1968). It is also not really feasible to manufacture calcined magnesite which contains Mg-28.

Most magnesium availability values have been obtained using complete balance trial data, i.e. involving detailed measurements of dietary inputs, and faecal and urinary outputs. However, workers have differed in their interpretation of such data. Some have used urinary magnesium loss expressed as a percentage of intake as apparent availability (e.g. Rook & Campling, 1962), or the output in milk plus urine as a percentage of intake (Rook, Campling & Johnson, 1964), or the regression coefficient of urine magnesium on dietary magnesium intake (Field et al., 1958, Field, 1967). The theory behind this approach is that magnesium behaves as a renal threshold substance, and that urinary magnesium has been shown to be sensitive to magnesium intake (e.g. Halse, 1970). An increase in the amount of dietary magnesium, and therefore in the quantity absorbed, is reflected in a proportional increase in urinary excretion. Expressed as a percentage of intake, urine magnesium is thus thought to give an estimate of apparent absorption, or apparent availability. Field et al. (1958) discuss the validity of the approach, but Lomba et al. (1968), from their statistical treatment of extensive metabolic data, report that urinary losses are not related to magnesium intake or magnesium digestibility.

However the most direct use of metabolic data is to express apparent availability as the percentage of dietary magnesium intake not present in the faeces (i.e. apparently absorbed), (e.g. Kemp et al. 1966; Chicco et al. 1972). Rook & Campling (1962) considered that an indirect assessment of apparent availability using percentage loss in urine was more accureate than the direct use of faecal data. However it is well known that individual animals differ in their ability to absorb magnesium, and the variability in faecal magnesium and hence in apparent availabilities calculated from faecal data is a feature of magnesium

studies. Throughout this thesis the word "availability" is used to express the percentage of dietary magnesium not present in the faeces, unless otherwise stated.

A number of research workers have looked at dietary magnesium utilisation over the years but this discussion will be limited to the most comprehensive recent review and derived standards published by the Agricultural Research Council (A.R.C., 1980). In 1965, the A.R.C. defined the availability of a mineral element as the percentage of the element supplied by the food that can be used by the body to make good endogenous loss or promote storage. This is effectively the percentage absorbed, and in 1980 the A.R.C. have rejected the sometimes ambiguous word "availability" in favour of "coefficient of absorption". They define "absorption" as the amount of a mineral supplied in the diet that enters the body from the gut, and "apparent absorption" is this entity less the net endogenous secretion into the gut. The "coefficient of absorption" (or of "apparent absorption") is the amount absorbed (or apparently absorbed) divided by the amount ingested. However most of the literature refers to this same coefficient, usually expressed as a percentage, as availability.

The A.R.C. (1980) have collated extensive information on the faecal loss of magnesium in individual animals from metabolic trials, and, assuming an endogenous faecal loss of 3 mg Mg/kg liveweight, the coefficients of absorption for dietary magnesium have been calculated. For three categories of feed (mixed forage and concentrate diets, hay and grass), they report values which are relatively low and extremely variable (0·184 - 0·459). Coefficients of magnesium absorption are given for a range of mixed diets (forage/concentrates) and classes of stock. Most information in the literature is, however, for lactating cows, the overall mean for this type of stock being 0·267. The only significant difference was a reduction in the coefficient of absorption when silage

was included in the mixed ration (0·245 against 0·289). The mean coefficient of absorption for sheep on mixed rations was summarised to be 0·405.

Published values for the coefficient of absorption in grass are variable but do not differ significantly from mixed diets for cattle (0.266 for lactating cows on grass), however they are appreciably lower for sheep (0·230), according to A.R.C. (1980). L'Estrange, Owen & Wilman (1967) reported an increase in magnesium utilisation by sheep as the grazing season progressed, whereas the results of Rook & Balch (1958) show no significant effect of stage of maturity of grass on magnesium absorption. Non-lactating cows at grass were found to have slightly higher values for coefficient of absorption than lactating cows (0·347 against 0·266) (A.R.C., 1980).

It is a widely held view that the availability of magnesium in grasses and forages is unusually low but the A.R.C. working party (1980), after careful consideration of all relevant data, found that they could not support this idea. They do, however, draw attention to the fact that magnesium absorption from grass may be especially low in certain swards, e.g. those receiving heavy nitrogenous or potassic fertiliser applications (see Section entitled "Factors affection magnesium absorption"). Thus, for all ruminant diets and classes of stock the A.R.C. (1980) consider there is insufficient information to permit reliable categorisation. For the calculation of dietary requirements, therefore, they have taken the overall mean of all their collated values for coefficient of absorption, i.e. 0·294. However, for the calculation of allowances which provide a margin of safety the lower decile value of 0·17 is recommended.

Calves and lambs receiving milk diets can be considered in a separate class from other ruminant animals. The coefficient of magnesium absorption is very high in the young animal, but falls away rapidly with

age, especially when the animals are unmuzzled and therefore able to consume some fibrous bedding (Smith, 1957, 1958, 1961). The A.R.C. (1980) propose that values of 0·70 for a 50 kg calf (or 5 kg lamb), 0·30 for a 75 kg calf (or 7·5 kg lamb), and 0·20 for a 100 kg calf be adopted.

Dietary magnesium requirements for ruminants

The published work of the A.R.C. Working Party (1980) gives as accurate an assessment as can be found to date of the nutrient requirements of ruminant livestock. They have used the factorial approach to estimate the requirements for the major mineral elements, and they point out that the application of standards is most critical in relation to magnesium as a dietary deficiency can quickly result in hypomagnesaemia. The A.R.C. publication gives tables of the estimates of average requirement and recommended dietary allowances for maintenance, growth, pregnancy and lactation, for both cattle and sheep; (separate estimates are given for milk-fed calves). The relative requirements, i.e. g Mg/kg liveweight, are similar for the two species, the major net requirements being those for maintenance and for lactation. For example, the daily requirement (in g/day) for magnesium (or recommended dietary allowance) for a non-pregnant dry 500 kg Friesian cow is 5·1 (8·8)*, but when she is yielding 20 kg milk day, the requirement (and allowance) is raised to 14·6 (25·3). However, a 500 kg steer gaining 1·0 kg/day requires less at 6·5 (11·3).

* Throughout this present discussion, and in the A.R.C. published tables the dietary requirements for magnesium are given, using a coefficient of absorption of 0·294, with the recommended dietary allowance given in parenthesis, using a coefficient of absorption of 0·17 (see Section on "Availability of dietary magnesium").

Lactating ewes, 40 - 75 kg liveweight, require 0·99 (1·71) - 2·5 (4·32) g Mg/day, depending on milk yield, and growing sheep, from 5 - 60 kg liveweight, gaining at 0·1 - 0·4 kg daily, require 0·18 (0·32) - 1·18 (2·04) g Mg/day. Growing cattle, from 100 - 600 kg liveweight and gaining at 0·5 - 1·5 kg/day, require amounts ranging from 1·7 (3·0) to 8·2 (14·2) g Mg daily. Requirements for pregnant cows gradually increase throughout gestation from 6·2 (10·8) up to 8·9 (15·5) g Mg/day, and similarly they increase for pregnant ewes from 1·34 (2·32) to 2·5 (4·32) g Mg/day for a 75 kg ewe and 0·99 (1·71) - 2·14 (3·71) g Mg/day for a 40 kg ewe.

4. TREATMENT AND PREVENTION OF HYPOMAGNESAEMIC TETANY

Treatment

Treatment for an animal showing clinical signs of hypomagnesaemic tetany must be prompt. It involves the parenteral administration of a solution containing a magnesium salt, such as magnesium sulphate, and often an additional injection of a calcium solution, usually calcium borogluconate. Administration is usually subcutaneous as the rapid introduction of ionic magnesium into the blood is liable to be acutely toxic and fatal. It is perhaps surprising that no satisfactory form of bound magnesium has yet been proved. Meyer & Busse (1975) reported a unique method of magnesium chloride administration via the rectum, and Bell et al. (1978) report the efficiency of magnesium chloride enemas as emergency treatment for cows in tetany; magnesium sulphate enemas were found not to be effective. In severe cases the administration of tranquilisers such as chlorpromazine or barbiturate can also help to ease the animal's condition.

However, treatment is often ineffective, perhaps only about 25% successful (Black's Veterinary Dictionary 1979). Extreme care is necessary when handling animals in tetany due to their hyperexcitable state, and death may result from any kind of stress, even the prick of

a needle. In addition, cases of tetany often occur during the night, when an animal which was apparently healthy the previous day is found dead in the morning. Treatment must be immediate to have a chance of being successful, and animals are therefore vulnerable during the long unsupervised dark hours. Thus, since the mortality from tetany is high, even when cases are treated, emphasis must be placed on prevention.

Prevention

Prevention or control of hypomagnesaemia and hypomagnesaemic tetany generally involves increasing the intake of magnesium by the animal. It is assumed that depression of magnesium absorption associated with the condition can be overcome simply by additional dietary magnesium intake.

It should be noted here that husbandry can play an important role. Useful practices are the provision of shelter for out-wintered animals and the gradual "turn-out" of animals from winter stall diets to spring pasture. Suckled calves should be weaned by early October at the latest (e.g. Todd, 1967) to decrease the risk of tetany among the cows in late October/November, and lactating ewes should be allowed access to old unimproved pastures rather than being confined to new improved pasture (e.g. Rees, 1975). Alteration of fertiliser programmes so that a minimum of only nitrogenous fertilisers are applied in the spring to provide the "early bite", and potassic fertilisers are applied later in the year, or a predominance of clover is encouraged in the pasture. However, this conflicts with attempts towards intensive grassland production, and altogether husbandry techniques can only alleviate the problem, not being sufficient alone to prevent hypomagnesaemia with the modern farming methods employed today.

Various methods are used to increase magnesium intake by animals. These can be divided into three broad categories; (i) increasing

the magnesium content of herbage; (ii) individual administration of magnesium alloy rumen "bullets"; and (iii) the oral provision of supplementary magnesium salts.

(i) Increasing the magnesium content of herbage

This approach to the control of hypomagnesaemia aims at increasing spring pasture magnesium levels to 2 g/kg DM, a fairly arbitrary level above which the condition is supposed not to occur (Kemp, 1960). Several experiments have shown that tetany can be prevented by increasing the pasture magnesium content by applications of magnesium fertilisers to the soil (e.g. Parr & Allcroft, 1956; Smyth et al., 1958; Todd, 1965; Campbell, 1972). Applications of magnesium oxide (usually as calcined magnesite), dolomite ($CaCO_3MgCO_3$), or magnesium sulphate have been shown to be effective in a number of trials, but the magnitude and duration of the response depends on the type of soil (see section entitled "Magnesium content of feeds and pasture"). Some reports suggest a poorer response from dolomite than from calcined magnesite or magnesium sulphate (M.A.F.F., 1974) but others report its apparent effectiveness (Todd, 1976). M.A.F.F.(1974) recommends that the application of 650 kg MgO/ha on coarse, acidic soil can give protection against hypomagnesaemia for up to 3 years, and only pastures to be grazed in the spring need be dressed. However this treatment is very expensive at such a high rate of application and is ineffective on alkaline or fine textured soils. As a long-term measure, the use of magnesium limestone (preferably with over 40% $MgCO_3$) as a routine liming agent, irrespective of soil type can be recommended as a cheaper control treatment. However the application of magnesium fertiliser is an unsuitable method of control where animals graze extensively over large areas, as the cost would be prohibitive.

Attempts at breeding species of grass with higher magnesium content are still in the early stages, but Hides & Thomas (1981) have demonstrated

that the magnesium content of Italian ryegrass varieties can be increased by selection and their work is continuing. However it must be noted that some workers have reported no relation between incidence of hypomagnesaemic tetany and grass magnesium content per se (e.g. Blakemore & Stewart, 1933; Hopkirk, Marshall & Blake, 1933; Nicholson & Shearer, 1938).

(ii) Use of metallic magnesium bullets

Magnesium bullets are solid cylinders composed of 86% magnesium metal alloyed with 12% aluminium and 2% copper. They are weighted with uniformly dispersed iron shot particles to ensure good retention within the animal's reticulo-rumen where they are intended to lodge, releasing magnesium slowly into the digesta. They are designed to release 1 - 1·7 g magnesium/day in cattle uniformly over a 28 - 50 day period when at grass, and 0·3 - 0·5 g/day over 20 - 40 days in sheep at grass (and in calves on milk). The recommended treatment for a cow is two bullets given 1 - 2 days prior to going out to pasture in the spring, and the effectiveness of this method of control of spring tetany has been reported (e.g. Ritchie & Hemingway, 1968; Smythe, 1969). However bullets do not always afford complete protection against hypomagnesaemia, especially in areas where tetany is a serious problem (e.g. Kemp & Todd, 1970). Bullet treatment is not really suitable for seasonal (autumn/winter) hypomagnesaemia because of the protracted period of risk (and such animals may also be considerably depleted in magnesium).

The recommended treatment for sheep (and calves) is 1 bullet per animal, administered to ewes with young lambs two days prior to any change of grazing or cessation of supplementary food. Prevention of tetany by this method has been reported (e.g. Davey, 1968; Egan, 1969), but later experience has shown that sometimes many of these bullets are regurgitated and lost at pasture (Kelly, 1979).

Thus bullets are effective in preventing hypomagnesaemic tetany in some situations but are less able to prevent subclinical hypomagnesaemia. Disadvantages also include the expense, and the labour involved in "bulleting" large numbers of individual animals.

(iii) The use of oral magnesium supplements

As early as 1934, Cunningham demonstrated that serum magnesium levels of cattle and sheep could be increased by oral administration of magnesium compounds. In the following year, Blakemore & Stewart (1935) confirmed that 31 g magnesium oxide (18 g Mg)/head/day prevented the occurrence of seasonal hypomagnesaemia in beef cattle at grass. However in 1938, Allcroft & Green found that 45 g MgO (27 g Mg)/cow/day had only a slightly beneficial effect on low serum magnesium levels found in December, and Allcroft (1947c) reported that 160 g MgO (96 g Mg)/cow/day raised serum magnesium levels but not always to within the normal range. Later work demonstrated that a daily dose of 2 oz magnesium oxide as commercial calcined magnesite (= approx. 30 g Mg) was an effective method of controlling hypomagnesaemia in cows (Allcroft, 1954; Line et al., 1958). Allcroft (1954) states that "the supplement of 2 oz was an arbitrary amount and it has not yet been possible to investigate minimum effective doses under varying conditions". However the possible use of smaller quantities of calcined magnesite has seldom been investigated. O'Moore (1955) reported that an intake of just 12 g magnesium/head/day reduced considerably the incidence of hypomagnesaemic tetany in dairy herds, whereas Allcroft (1953) recorded the failure of 2 oz (56 g) calcined magnesite to maintain normal plasma magnesium and to prevent deaths from hypomagnesaemic tetany in some herds. The fact that a supplement of 2 oz calcined magnesite does not afford complete protection in cattle has presumably engendered the belief that any less a quantity would inevitably provide less protection, although there is no convincing

scientific evidence to support this. Nor indeed is there evidence to suggest that a greater quantity would afford more protection, but the dangers of excess magnesium intake have been reported. Care (1960d), for example, reports severe scouring in cattle receiving 6 oz - 12 oz calcined magnesite/head/day, but daily supplements of up to 4 oz did not have this effect.

Irrespective of the quantity of supplementary magnesium, it is essential that it be given daily since there is no carry over of a protective effect from day-to-day. For example, Allcroft (1960) reports that under conditions conducive to hypomagnesaemia serum magnesium levels in cows fell to dangerously low levels within 48 hours of ceasing oral supplementation, even though a high intake of magnesium had been given daily for several weeks previously. It follows that acute hypomagnesaemia cannot be prevented just by giving extra magnesium before spring turn-out. (However it is generally considered advisable to commence supplementation in good time to ensure acceptability by the animals).

It is important to note that the response of serum magnesium levels to supplementary magnesium varies among individuals. It is often greater in animals with normal values and can be negligible in animals with pronounced hypomagnesaemia (Ender, Dishington & Helgebostad, 1957; Hemingway & Ritchie, 1963; Rook & Storry, 1962b). Ritchie & Hemingway (1963) report the failure of daily supplements of up to 4 g magnesium as magnesium oxide or magnesium nitrate to increase plasma magnesium levels in lactating ewes at grass. However, increases were recorded 4 hours after administration which were not sustained for the full 24 hours, indicating that some absorption did take place.

The current standard preventive recommendation is still 2 oz (56 g) calcined magnesite (about 87% MgO)/day for cattle (i.e. about 30 g Mg), and for sheep $\frac{1}{4}$ - $\frac{1}{2}$ oz (7 - 14 g)/day is recommended (i.e. about 4 - 8 g Mg). Again, there is no experimental evidence to confirm the most effective

prophylactic dose for sheep and this standard range of values was probably derived from a consideration of the body size of sheep relative to cattle. Calcined magnesite is the cheapest magnesium supplement available with the highest magnesium content (48 - 52%). Traditionally the aim of supplementation is to provide the quoted levels of calcined magnesite, or equivalent if another salt is used.

a) Methods of providing supplementary magnesium salts

The provision of an inorganic magnesium supplement in some shape or form is the most widespread prophylactic measure against hypomagnesaemia and there are various methods of administration.

"Pasture dusting" is a common practice in some situations. Fine calcined magnestie powder is spread as a surface dust which adheres to the grass (especially if the leaves are damp with dew) immediately prior to grazing. Magnesium oxide applied at the rate of 25 - 30 kg/ha is effective in preventing hypomagnesaemic tetany in grazing cows (e.g. McConaghy et al., 1963; Todd & Morrison, 1964). However, each treatment provides "once-over" protection only, lasting little over one week, and routine treatments on intensive pastures should be repeated every 2 - 3 weeks during periods of risk. Pasture dusting is a reliable method for the control of hypomagnesaemia as it ensures that all animals receive supplementary magnesium, including the shy feeders, and the palatability of the grass is apparently unimpaired. Disadvantages, though, are that granular calcined magnesite, which is more common than powdered, cannot be used, and the method is ineffective if the pasture grass is short (less than about 10 cm; Rogers, 1979). It is an unsuitable method for control of seasonal hypomagnesaemia and tetany in beef cows at grass in winter, and generally only suits a system employing intensive grazing in the spring. The calcined magnesite generally adheres well to the leaf but exceptional wind or rain could dislodge it.

Using the same principle, surface sprays of magnesium sulphate solution (Epsom salts) repeated at fortnightly intervals can be effective in preventing hypomagnesaemia and hypomagnesaemic tetany (Bould 1964), though it has been reported that this is less effective than magnesium oxide applications (Grund, 1961).

Administration of supplementary magnesium via the drinking water is another possibility, and Simpson (1971) demonstrated the efficacy of adding either magnesium acetate or magnesium chloride to the water supply for dairy cows. Water medication usually involves extensive plumbing (see Rogers, 1979) and the soluble magnesium salts (sulphate, acetate and chloride being the most common) are expensive compared with the insoluble calcined magnesite. The system can only be recommended where pasture dusting or direct magnesium feeding cannot be used.

Conserved forages can be treated with magnesium salts for the prevention of hypomagnesaemic tetany. The successful feeding to sheep of hay to which magnesium oxide was added prior to baling has been reported (Campbell, 1972), and tetany was prevented in beef cows during the winter when magnesium oxide, in aqueous suspension, was sprayed on to hay at the time of feeding (Herd, Schuster & Coltman, 1965). Similarly, magnesium oxide can be incorporated into silage at the time of making (McDonald & Jackson, 1955; Thompson, 1960) or sprinkled onto cut-down silage at time of feeding.

When concentrates are being given, the incorporation of 56 g calcined magnesite/cow/day in the ration (or 7 - 14 g/sheep/day) is the most direct means of providing supplementary magnesium. Most animal feed compounders produce magnesium enriched cakes for feeding during high risk periods, for different classes of stock, and when fed at the recommended level they are an efficient method of supplying the required amount of magnesium. For example, a dairy cow concentrate nut containing

125 g/kg calcined magnesite (c.7·5 Mg) can be fed at a rate of 0·5 kg/cow/day in the milking parlour, with or without other concentrates. Where farmers feed according to milk yield, this small inclusion of magnesium-rich concentrates is advisable, as replacing the whole ration with a concentrate containing slightly less magnesium will result in widely differing magnesium intakes. Where farmers feed regardless of yield, or there is a group of similar animals (cattle or sheep) a concentrate containing a fixed percentage of magnesium (consistent with the level of feeding) can provide the whole ration. Home produced rolled cereals can also be supplemented with calcined magnesite. The inclusion of molasses with the ration is recommended to decrease the dustiness and hence to overcome selectivity and reduced palatability, especially when feeding to sheep.

Direct feeding of supplementary magnesium is often impractical as animals at grass, especially in the spring, may require no extra feeding. In addition, group-feeding is disadvantageous in that some may eat too little and others an excess of the supplement, the former gaining no protection and the latter possibly developing a scour (Care, 1960d).

The methods for control of hypomagnesaemia considered so far are only suitable for use with intensive farming systems. In many areas, hypomagnesaemic tetany is a serious problem among suckler herds on extensive hill grazing, where concentrate feeding is minimal. In these situations the only economic solution is to provide "free-acess" mineral mixtures containing magnesium compounds. Such mixtures should ideally contain about 50% calcined magnesite, but the palatability of magnesium supplements, notably calcined magnesite, is low and the mixture must also contain more palatable, acceptable constituents. Frye et al. (1977) reported satisfactory (free-access) intakes of supplemental magnesium oxide by cows if a palatable ingredient, such as cottonseed meal or dried

molasses, was included. It has been shown that mixtures of calcined magnesite and molasses in equal proportions when put out in tubs are readily taken by most cows (Todd, Scally & Ingram, 1966) and this is effective in reducing the incidence of tetany in the herd. Other researchers have reported improvements in serum magnesium in cows allowed access to palatable high magnesium mineral mixtures (e.g. Smith et al., 1974).

Free access liquid feeds dispensed from a ball feeder have also been used successfully for cows. For example, Ross & Gibson (1969) showed that a 5% solution of magnesium acetate in molasses was effective in maintaining normal serum magnesium levels in dairy cows.

Supplements for free-access feeding can be provided in the form of feed blocks (Horvath, 1964). Some commercial companies produce blocks with special formulations, both for cattle and for sheep, for use at times when hypomagnesaemic tetany is likely to occur. It is claimed that feed block intakes are regulated by the quantity and digestibility of herbage, but this means that intake in the spring would be negligible as there is an abundance of highly digestible grass. Several manufacturers claim to overcome this problem with their supposedly attractive and palatable products, but generally blocks are of more use for outwintered herds and flocks when they may be used for several months over prolonged periods of low magnesium intake. However there are many factors influencing feed block intakes by animals (Kendal, 1977) and they generally cannot ensure adequate magnesium intake by all individuals. Indeed, no ad lib feeding method can be expected to be 100% effective as some animals may receive no supplement at all, and the dangers of excess have already been pointed out (Care 1960d). (In the case of feed blocks, though, animals are usually prevented from eating too much by the hardness of the block).

Thus it can be seen that there are many different methods, suiting different farming systems, to administer supplementary magnesium to

ruminants. However the principles remain the same, i.e. to ensure regular intake by all animals in the herd or flock.

b) Availability of supplementary magnesium salts

There is relatively little published work on the availability of supplementary (inorganic) magnesium. As with studies on dietary magnesium availability, some workers have expressed supplemental availability as the percentage loss in the urine. There is supposedly a linear increase in urinary magnesium output over a basal level when supplementary magnesium is added to the basal diet, hence availability estimates are potentially fairly accurate. However other workers have expressed availability as the percentage of supplementary magnesium not present in the faeces and this meaning will be adopted in this review. This apparent availability (or absorption) percentage has been calculated or drawn out from the literature wherever possible if the worker has expressed his conclusions in a slightly different way. However in some cases, where complete balance data is not presented, this is not possible, and also various other criteria, such as plasma magnesium levels, have been used to assess the efficacy of different inorganic magnesium sources.

Table X shows the apparent availabilities of different magnesium salts, as estimated by different workers. All values are given as percentage supplementary magnesium intake not present in faeces, except for the results of Storry & Rook (1963) where they give their results as percentage supplementary magnesium intake excreted in the urine. It can be seen that reagent grade magnesium oxide is often used as a reference standard and it has a reasonably high availability (32 - 75%). Table Y presents some of these results again, and some more (qualitative) data, expressed as "relative biological availabilities" compared with reagent grade magnesium oxide (100%).

Table X: Apparent availabilities of different magnesium salts estimated by different workers

Reference	Ammerman et al., 1972	Gerken & Fontenot, 1967	Storry & Rook, 1963	Chicco et al.,1972	Fishwick & Hemingway, 1973	Fishwick, 1978	Hemingway & Brown, 1967	Hemingway & McLaughlin 1979
	sheep	bullocks	dry cows	sheep	sheep	sheep	sheep	sheep
Magnesium Salt								
Reagent Grade MgO	73	51	32	75	47	48	-	42
Reagent Grade $MgCO_3$	72	-	-	-	-	-	-	-
Magnesite($MgCO_3$ ore)	14	-	-	-	-	-	-	-
Dolomitic limestone	-	14	-	-	-	-	-	-
Sulphate	78	-	19	-	-	-	-	-
chloride	-	-	30	-	-	-	-	-
nitrate	-	-	31	-	-	-	-	-
acetate	-	-	34	-	-	-	-	-
citrate	-	-	47	-	-	-	-	-
lactate	-	-	32	-	-	-	-	-
silicate	-	-	22	-	-	-	-	-
phosphate	-	-	-	-	33	26-44	-	33
Calcium magnesium phosphate	-	-	-	-	42	-	-	-
Magnesium ammonium phosphate	-	-	-	-	-	-	66	-

Table Y: Relative biological availabilities of different magnesium salts estimated by different workers

Reference	Ammerman et al., 1972		Gerken & Fontenot, 1967	Storry & Rook, 1963	Fishwick & Hemingway, 1973	Fishwick 1978	Hemmingway & McLaughlin 1979	Huffman et. al., 1941	Moore et. al., 1971
Class of stock	sheep		bullocks	dry cows	sheep	sheep	sheep	calves	bullocks
Criterion	balance trial	feed intake response	balance	% Loss Mg in urine	balance	balance	balance	plasma Mg level	balance
Magnesium Salt									
Reagent Grade MgO	100	100	100	100	100	100	100	100	100
Reagent Grade $MgCO_3$	99	113	-	-	-	-	-	100	100
Feed Grade MgO	-	85	-	-	-	-	-	-	-
Magnesite($MgCO_3$ ore)	19	-	-	-	-	-	-	-	-
Dolomitic Limestone	-	-	27	-	-	-	-	-	< 100
sulphate	107	65	-	59	-	-	-	< 100	-
chloride	-	-	-	90	-	-	-	100	-
nitrate	-	-	-	97	-	-	-	-	-
acetate	-	-	-	107	-	-	-	-	-
citrate	-	-	-	148	-	-	-	< 100	-
lactate	-	-	-	98	-	-	-	-	-
silicate	-	-	-	66	-	-	-	< 100	-
phosphate	-	-	-	-	70	54-92	79	100	-
Calcium magnesium phosphate	-	-	-	-	89	-	-	-	-
magnesium metal	-	-	-	-	-	-	-	< 100	-

Based on the response of plasma magnesium levels in magnesium-deficient, milk-fed calves to supplementary magnesium salts, Huffman et al. (1941) reported the oxide, carbonate, chloride and phosphate to be better than citrate, silicate, sulphate and metallic magnesium, whereas Thomas (1959) reported no difference in availability between carbonate, sulphate and acetate forms for calves. Storry & Rook (1963) reported similar availabilities for the oxide, chloride, acetate, lactate and nitrate as supplements to a low magnesium diet (0·1 - 0·2 g Mg/kg DM) for dry cows, but the sulphate and silicate were less available, while the citrate was more so. Magnesium nitrate has also been reported to be more rapidly absorbed than the sulphate by sheep (Stewart & Moodie, 1956), and House & Mayland (1976a) reported greater availability to sheep of magnesium from the sulphate compared with metallic magnesium from magnesium alloy rumen bullets. However Hemingway & Ritchie (1969) have reported good utilisation of magnesium from bullets for calves.

Meyer & Grund (1963) found greater availability of magnesium from the oxide and chloride than from carbonate by cattle, but carbonate has been shown to be an effective source by other workers (Huffman et al., 1941; Barrentine & Morrison, 1953; Thomas, 1959). Moore et al. (1971) reported similar availabilities for magnesium oxide and carbonate, but dolomitic limestone was considerably poorer as a magnesium supplement for cattle, probably (these workers suggest) partly due to an increased rate of passage of digesta on this treatment. Gerken & Fontenot (1967) also report the magnesium in dolomitic limestone to be less available than the oxide for cattle, but Fontenot et al. (1965) report the apparent effectiveness of dolomitic limestone as a magnesium source in field studies with beef cows.

Ammerman et al. (1972) evaluated different magnesium salts by balance data and in terms of feed intake response following magnesium administration to magnesium-deprived sheep (voluntary food intake in

these animals was reduced by about 60% in 4 days on a purified magnesium-deficient diet). Based on feed intake response the order of increasing merit was sulphate, feed grade magnesium oxide, reagent grade magnesium oxide, and magnesium carbonate, but based on balance (availability) data the oxide, carbonate and sulphate were equally well utilised while magnesite (magnesium carbonate natural ore) was very much less available.

Magnesium in magnesium phosphate was generally found to be less available than reagent grade magnesium oxide for sheep given natural diets (Fishwick & Hemingway, 1973; Fishwick, 1978; Hemingway & McLaughlin, 1979), though Huffman et al. (1941) had reported phosphate to be as good as the oxide in raising calf plasma magnesium levels.

The actual type of magnesium salt tested is not always clear in the literature and this can be an important consideration. For example, Ammerman et al. (1972) reported that magnesium carbonate as magnesite was utilised to a very limited extent whereas reagent grade magnesium carbonate was well utilised. Magnesite is a natural ore with a definite crystalline structure compared with reagent grade magnesium carbonate which is a powdery compound of indefinite crystallisation. Similarly reagent grade magnesium oxide, a fine white powder containing 99·99%MgO, is very different in nature from the feed grade magnesium oxide which can be anything from powder to coarse granules and generally contains 85-87% MgO. Most workers have used reagent grade magnesium oxide, the underlying assumption being that this would represent all magnesium oxide, but there is notably little published information on feed grade magnesium oxide availability. This is surprising as this source of magnesium is used extensively by the animal feed industry.

In addition, there is remarkably little information on magnesium salt availability to animals at pasture. Rook & Storry (1962b) estimated from blood data an availability of 5 - 10% for the magnesium in magnesium oxide given to dairy cows at spring grass, but there is a

conspicuous absence in the literature of direct measurements of apparent availability. Much of the published work reports supplementation of artificial magnesium-deficient diets. This is valuable information in itself but the results cannot necessarily be extrapolated to natural diets, including grass, where a different kind of rumen fermentation is likely to exist.

The experimental data on the relative availability of magnesium from its different salts vary considerably and more research is needed to define more accurately their relative efficiencies. Furthermore it would be desirable to study feed grade magnesium oxide in greater detail as this is of greatest commercial importance. Some workers in the U.S.A. (Jesse, Thomas & Emery, 1979, 1981) have reported decreased availability to cows of magnesium from magnesium oxide with increased particle size of the oxide, as assessed by urinary magnesium output. However this is the only published report on work of this kind to date.

c) Additional reported effects of magnesium supplementation

Reports in the literature that cannot be disregarded here indicate that magnesium supplementation can have "side" effects on production by ruminants. Wilson & Grace (1978) reported that cows at grass suffering from hypomagnesaemia produced milk containing a lowered fat concentration, and Young & Rys (1977) reported significant responses in milk yield and fat concentration following magnesium supplementation of hypomagnesaemic cows. However, Wilson (1980) reported increased yields with no increase in fat levels in response to oral magnesium chloride, whereas Jesse et al. (1979) found milk yields depressed and fat levels increased following oral magnesium oxide. Erdman et al. (1980) reported large increases in milk production by cows supplemented with sodium bicarbonate together with magnesium oxide.

Apparent digestibility of rations for cattle have been shown to be depressed by dolomitic limestone, but not by magnesium oxide or magnesium carbonate supplementation (Gerken & Fontenot, 1962; Moore et al., 1971). However, Wilson (1980) reports an increase in apparent digestibility of hay for sheep when supplemented with magnesium sulphate.

These reported effects on production occur only when the supplementary magnesium is administered orally, indicating that the effect is possibly mediated by an influence on rumen fermentation. A dietary magnesium deficiency may result in rumen microbial magnesium deficiency, reduced rumen fermentation and hence poor production. This may be rectified by increasing magnesium intake, but the inconsistency of published results indicate that this is probably an oversimplification of a complex situation.

Commercial Magnesium Oxide

Commercial magnesium oxide is produced by two fundamentally different methods.

The most common commercial material in the animal feed trade is produced by the calcination (or burning) of natural mineral magnesite (magnesium carbonate), the reaction equation being

$$MgCO_3 \longrightarrow MgO + CO_2.$$

The quality of the calcined magnesite so produced is therefore determined largely by the composition and purity of the original ore deposit. Common impurities are iron (as ferric oxide), which gives the calcined magnesite a brownish colour, and silica.

A less common magnesium oxide in the animal feed trade is a white material produced by the calcination of magnesium hydroxide

$$Mg(OH)_2 \longrightarrow MgO + H_2O.$$

The magnesium hydroxide is produced when natural dolomite ($MgCO_3 . CaCO_3$) is calcined (to MgO + CaO) and allowed to react with sea-water, when the

highly reactive magnesium oxide is hydrated and the calcium oxide reacts with the soluble magnesium salts present in sea-water. Magnesium is thus extracted simultaneously from sea-water and dolomite, and is precipitated as the hydroxide:

$$\underline{I} \quad MgCO_3 . CaCO_3 \longrightarrow MgO + CaO + 2CO_2$$

$$\underline{II} \quad MgO + H_2O \longrightarrow Mg(OH)_2$$

$$\underline{III} \quad CaO + Mg^{2+} + H_2O \longrightarrow Ca^{2+} + Mg(OH)_2$$

High quality magnesium oxide can also be made from magnesium chloride extracted selectively from sea-water. Sea-water magnesias are generally more consistent in composition and quality than are natural magnesias. They can be made within very close chemical and physical tolerances and are consequently in demand from industries requiring magnesia of specifically high purity, e.g. refractory and steel industries, ceramics, chemical processing, synthetic rubbers and paper.

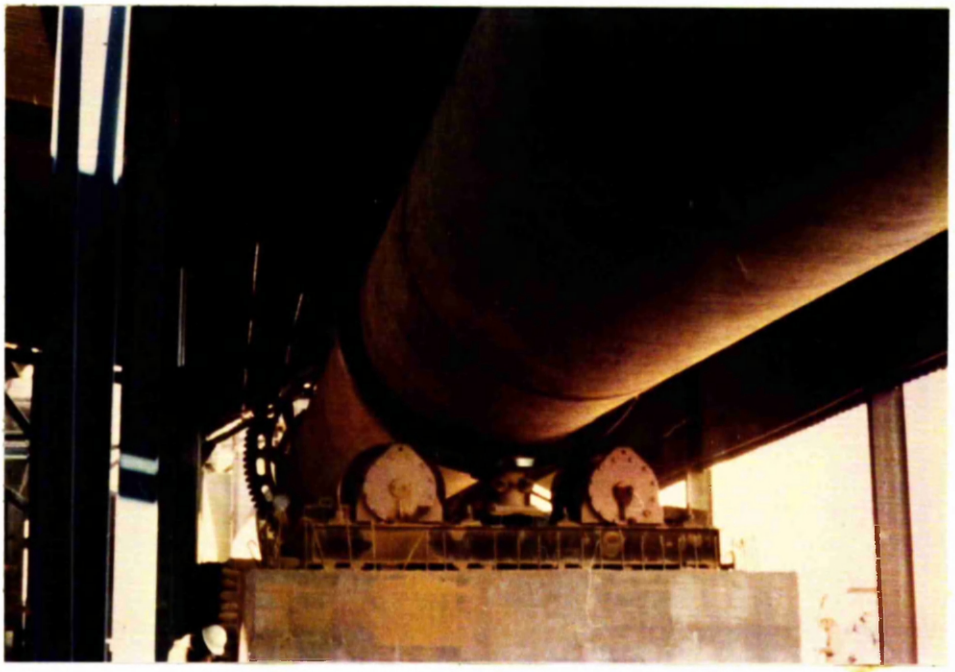
Details of production processes

1. Natural magnesia

The calcining of mineral magnesite by a particular producer in Spain (Magnesitas de Rubian S.A.) will be described as an example of the stages in the production of natural calcined magnesite. The magnesite is quarried from ore deposits in Galicia, N.W. Spain (see Plate 1). The rock is then crushed and screened to below 10 mm particle size before being fed at a constant rate into a large rotary kiln for calcination. A typical kiln is roughly 65 m long, 2·6 m diameter, (see Plate 2) and rotates at approximately 0·75 revolutions/minute, so that the magnesite takes, on average, about 3 hours to pass through. The heat is produced by a flame about 15 m long at one end of the kiln, and the temperature is recorded by an I.R. pyrophotometer at this point. The temperature is normally registered between 800° and 900°C. However it should be noted that where the magnesite enters the kiln, the temperature is only about 480°C, with an even gradation of increasing

Plate 1. Natural magnesite quarry in Galicia, N.W. Spain.

Plate 2. A calcining kiln in N.W. Spain.



temperature along towards the flame. Some material will inevitably pass through the flame itself and will therefore experience much higher temperatures. Such material can thus be rendered relatively unreactive and becomes what is known in the trade as "deadburnt". The calcination reaction commences only above about 400°C, but the speed and extent of calcination increases with temperature to an optimum between 800° and 900°C when the most (chemically) reactive oxide is produced.

The freshly calcined magnesite passes from the kiln to a cooler, of similar dimensions, before being screened to below 4 mm particle size, rejected material (about 7% of the total) being recycled through the kiln. A second screen separates calcined magnesite of over 2 mm particle size as fertiliser grade, and below 2 mm as animal feed grade.* An automatic sampler operates at this point, and subsequent analyses for the main elements in the calcined magnesite, i.e. magnesium, silica, alumina, iron and lime, are carried out. In addition, the "Loss on Ignition" (L.O.I.) is determined, by burning the material at 950°C to constant weight. This includes carbon dioxide, hydroxide and free water, and is thus a measure of the extent of calcination of the produce. The L.O.I. is usually about 1-2% for the animal feed grade calcined magnesite. Provided the material is not rejected on the results of analysis, it is then bagged off into 50 kg bags ready for sale.

The magnesite ore varies in colour and appearance according to various impurities. For example, there are quartz crystals in some rocks (silica), mica chips, and iron in the ferrous (Fe^{II}) state (white colour) or ferric (Fe^{III}) (giving a red colour) present as a mixed iron/

*This screen mesh size was 1 mm until about September 1980 when a 2 mm screen was introduced in order to decrease the dustiness of the fertiliser calcined magnesite.

magnesium carbonate. The size of the magnesite crystals is therefore variable, and the rocks may be relatively compact or friable. Particles are broken down during calcination but their size may well determine the rate of passage through the kiln, and hence the extent of calcination. Much fine dust is produced at all stages and this can pass through the kiln in as little as $\frac{1}{2}$ hour. Such dust emerging from the kiln is generally under-calcined (60-70% MgO with 13-16% L.O.I.) and is separated off as "Cyclone Dust TBH". The possible commercial use of this product is thought to be extremely limited due to its high variability and consequently it is discarded as waste. (It has been used on a very small scale for pasture dusting in Spain but its variable magnesium content makes the optimum application rate difficult to ascertain).

The calcination of Greek and Chinese natural magnesites is also carried out at 800-900°C in rotary kilns, in a production process broadly similar to that described.

2. Sea-water magnesia

The production processes employed by an English producer of sea-water magnesia (Steetley Minerals Ltd., Magnesia Division) will be described as an example, although this particular producer does not have a recognised animal feed grade at the time of writing.

Natural quarried dolomite is crushed, graded and burnt in rotary kilns to produce "Dolime". The "Dolime" is first slaked to a fine dry powder in hydrators, then the hydrated product is formed into a slurry, which is classified to remove impurities. Sea-water, pretreated with sulphuric acid to remove calcium bicarbonate, is mixed well with the Dolime slurry in agitated reaction vessels. Magnesium hydroxide is precipitated and the dilute suspension is allowed to settle in large settling tanks. The concentrated magnesium hydroxide is drawn off and extracted by pumps as a slurry. After washing with fresh water or

pretreated sea-water, the slurry is filtered on rotary vacuum disc filters, and the filter cake (50% solids) is fed either into rotary kilns (which deadburn the product at over 1800°C to produce refractory grade magnesia) or into a calcining furnace (to produce more reactive products, at 800-900°C).

It can be seen that this production process allows much stricter control on the quality and nature of the magnesium oxide than does the previous method, but the production costs are inevitably greater. The method also applies to the extraction of magnesia from concentrated brines.

Calcined magnesites used in U.K. agriculture

Approximately 40,000 tonnes of animal feed grade calcined magnesite are used annually in the U.K. Virtually all is imported from three countries, namely Spain, China and Greece, in decreasing order of magnitude of imports. (Their respective shares of the market in 1980 were about 60%, 30% and 10%). The major Spanish producer is Magnesitas de Rubian S.A., which quarries and calcines natural magnesite in Galicia. There are two magnesite quarries (of similar composition), at Shantung and Tallien, accounting for 90% of the total Chinese output, and in Greece the main producer, Grecian Magnesite Co. S.A., quarries at two sites, Yerakini and Castri. Thus all three counties supply natural mineral calcined magnesite to the U.K. The product grades available on the market are as follows:-

Spain

1. Granular, 0-2 mm, approx. 85/87% MgO. Animal feed grade (trade name Agma FG85).
2. Powder, approx. 85/87% MgO. For pasture dusting only.
3. Coarse granules, 2-4 mm, approx. 80% Mg. Fertiliser grade.

China

1. Granular, 0·2-0·3 mm, min. 85% MgO.
2. "Gritty", 30 mesh (i.e. about 0-550 μm), min. 85% MgO.
3. Powder, 60 mesh (i.e. about 0-250 μm), min. 87% MgO.
4. Powder, 120 mesh (i.e. about 0-125 μm), 90+ % MgO.

Greece

1. Granular, 0-2 mm, approx. 80-85% MgO.
2. Powder, approx. 80-85% MgO.

Over 90% of the U.K. animal feed market is for granular products, powders being used almost exclusively for pasture dusting. Virtually all the calcined magnesite is sold to animal feed compounders and mineral supplement manufacturers, with under 2% going for direct retail sale to farmers.

The three major calcined magnesites used in the U.K. are therefore the Spanish, Chinese and Greek granular materials. There are differences in their particle size distributions, the percentage of particles below 250 μm being 50-60 for Spanish, 20-30 for Greek and only 10-20 for Chinese granular calcined magnesites, with their upper particle size limits being 2,000, 2,000-3,500 and 2,000-2,800 μm, respectively. For a detailed particle size analysis of these products, see Appendix 1. There are also differences in appearance and magnesium content (although all are produced by the same process), due to differences in the nature of the original magnesite deposits. For example, Greek magnesite is whiter in colour as it contains very little iron, unlike Spanish magnesite which is reddish-brown due to the presence of iron. (See Plate 3). Greek and Chinese rock magnesites comprise a finer grained magnesite, which tends to produce more powder when calcined, whereas the Spanish material is macro-crystalline. Thus there are differences in the physical as well as the chemical properties of different calcined magnesites.

Plate 3. Typical examples of Greek and Spanish calcined magnesites.


Greek
Spanish

SECTION II

AVAILABILITY DATA OBTAINED USING INDOOR DIETS

A. Balance Trials.

The principal behind the balance trial technique in nutritional studies (also called metabolism or digestibility trials) is to obtain accurate measurements of total inputs and total outputs of particular feed components or supplements, in this case the element magnesium. The experiments to be described here each employ a Latin square design with 14-day periods, the first seven days being treated as changeover periods, and complete collections of faeces and urine being made during the second 7 days. The equipment and routine procedures employed are as follows:-

Metabolism cages. These are metal, of a standard size and design (after Duthie, 1959). The cages are arranged side-by-side in two long rows facing one another, in a well ventilated farm building. Each has a wire mesh floor beneath which is a sloping metal tray, or chute, designed to collect and channel the urine towards an aperture at the back.

Animals. Wether sheep are used in order to facilitate separate collections of faeces and urine. Their hind-quarters are clipped to allow "cleaner" collections of faeces, decreasing the chance of faeces adhering to the wool. These animals settle quickly in metabolism cages and become relatively calm to handle.

Diets. Daily quantities of the basal diet are carefully weighed out into paper bags in advance for each 14-day period, and representative samples for each period are taken for general analysis. The diet is given in two approximately equal portions at about 07.30 h and 16.00 h each day. Any spillage of food by the sheep while eating is carefully collected and replaced in front of the animal for consumption, as it is essential that precise food intakes are maintained.

Supplements. Similarly, enough daily magnesium supplements are weighed out into small plastic tubs in advance for each 14-day period, with

representative samples taken for analysis. Supplements are added to the surface of the morning feed.

Water. Throughout the experiments the sheep are allowed tap water ad libitum. This was found to contain minimal magnesium, at about 2 µgs/ml.

Faeces collection. The sheep are fitted with a standard type of leather harness which includes a chest strap and two straps encircling the body, one just behind the forelegs and the other immediately in front of the hind quarters. Four spring-loaded scissor-grip metal hooks are attached by string at four well-spaced points on this rear strap. When faeces are to be collected, a semi-rigid rubberised bag (Avon Rubber Co. Ltd.) can be held closely positioned over the rear of the sheep by the scissor-grip hooks. Each day during a collection period, the contents of the faecal bag are emptied into a large, numbered, heavy-duty polythene bag behind the cage. The total faeces from each sheep collected over seven days are weighed, then thoroughly mixed on a clean concrete floor and subsequently sampled. All samples are dried (giving the dry matter content), ground, and subsequent subsamples are analysed for magnesium.

Urine collection. Urine is collected directly from the sloping metal tray below the cage floor into a polypropylene jug. The urine passes through a glass wool filter to prevent particulate matter entering the jug (e.g. loose wool, "spilled" faeces). Each day the chute is rinsed with a minimal quantity of water and the washings are also filtered into the jug. The actual volume of urine produced is not important in these balance trials and the washing picks up any urine which has not drained completely from the chute. During a collection period, the jug is emptied daily (or more often as required) into a large (c. 25 litre) numbered polypropylene container situated behind the cage. Dilute hydrochloric acid is added to the jug and/or bulk container to prevent

the precipitation of salts from the urine during storage. After the seven days, the total urine (plus acid and chute washings) from each sheep is weighed, well shaken and sampled for specific gravity and magnesium analyses.

In some cases a hard pale brown precipitate is deposited on the chute of the metabolism cage, and/or in the urine collection jug. The deposit in the jugs can usually be dissolved in hydrochloric acid and added to the urine, but it is often impossible to remove and collect all of the deposit from the chute. The degree of deposition is unrelated to magnesium supplementation of the diet but some individual sheep tend to produce more than others. In one trial, a visibly large quantity of deposit in one cage which accumulated over a seven-day collection period was carefully removed (by scraping) for separate analysis. It was found to contain 0·16 g Mg, equivalent to an average of 0·02 Mg/day. This represents roughly the maximum error in daily urine magnesium output figures for individual sheep, and is thus reasonably small in percentage terms.

Blood samples. Venous blood samples are taken towards the end of the 14-day periods, by jugular puncture, using evacuated heparinised tubes, and the plasma magnesium concentrations are determined. Plasma is also analysed for calcium as the concentrations of these two cations are often interrelated (especially in cases of hypomagnesaemic tetany, e.g. Hemingway & Ritchie, 1965).

Changeover periods. During the changeover week, urine and cage washings are allowed to run to waste. For convenience, faeces are still collected daily in bags but discarded. The sheep receive their diets, with or without supplements, as during collection periods.

Acclimatisation. The sheep are fitted with their harnesses and are introduced into the metabolism cages at least two weeks prior to the start of a balance trial. They are thus well acclimatised during this

period to the normal balance trial routine and environment before the experiment begins.

Treatment of balance trial results. The direct data obtained in a balance trial are the total magnesium intake (basal diet with or without supplement) and total magnesium output in the faeces and in the urine, over each 7-day collection period, for each individual sheep. The mean balance data, in terms of gMg/day, are presented for each experimental treatment, together with the mean "retention". Retention is a figure calculated from the difference between total magnesium input and total magnesium output (faeces and urine) for each sheep.

In order to calculate the availabilities of magnesium supplements, it is necessary first to calculate the basal diet magnesium availability, i.e. the percentage of magnesium from the basal diet not present in the faeces, for each individual sheep, using balance data from periods when no supplementary magnesium was fed. This figure is then used to calculate the amount of faecal magnesium presumed to be derived from the basal diet during periods when magnesium supplements are being fed, and hence the quantity of faecal magnesium derived from the supplement. Apparent availability of a supplement is then calculated, for each sheep, as $\frac{\text{supplement Mg intake - faecal Mg derived from supplement}}{\text{supplement Mg intake}}$. This method of calculation assumes a constant availability for basal diet magnesium whether supplements are being given or not.

Latin square statistical analyses are carried out on all balance trial data to obtain means, standard errors (SEM) and significance of any differences between mean results.

The four balance trial experiments to be described in this section investigate the possible effects of particle size, temperature of calcination and level of supplementation on the availability of natural and sea-water derived calcined magnesites.

Experiment 1.1. A comparison of the availability of the magnesium in different particle size fractions of Spanish calcined magnesite.

Introduction

Preliminary experiments with Spanish calcined magnesite had shown that some of the larger particles (500-1,000 μm diameter) pass through the intestinal tract of sheep and appear in the faeces apparently unchanged. This suggests that the magnesium in particles of this size may only be poorly available to the animal. Moreover it is noticeable during laboratory analysis for magnesium content that finer particles of calcined magnesite can dissolve more quickly in the digestion acid (see "Analytical Methods") than do coarser fractions. As commercial calcined magnesites contain a fairly wide range of particle sizes (under 75 μm to over 2,000μm in some products), it is possible that the overall availability is the mean effect of several different availability values for each different particle size.

The present experiment describes a balance trial in which wether sheep were fed a basal diet of dried grass supplemented with one of seven particle size fractions of Spanish calcined magnesite.

Materials and Methods

Magnesium supplements

At the time of this experiment, animal feed grade Spanish calcined magnesite (trade name Agma FG85) comprised material below 1·0 mm †, and the fertiliser grade (trade name Agma CG80) consisted of the coarser particles over 1·0 mm †. Bulk samples (50 kg) of both products were obtained from the same production batch, thus ensuring that both were subjected to the same commercial calcining conditions of temperature and

† Note that in September 1980 this "cut-off" point was raised to 2·0 mm.

duration. The producers, Magnesitas de Rubian S.A., also provided the "laboratory bulletins" relative to these particular bags, and these gave the mean analyses for the animal feed grade at 84·7% MgO, 1·1% L.O.I., and for the fertiliser grade at 78·2% MgO, 2·0% L.O.I. The coarser fertiliser calcined magnesite thus has a lower magnesium content and higher L.O.I. than the corresponding animal feed grade, in spite of the fact that both were produced at the same time in the kiln. It is probable that particles of different sizes travel at different rates through the rotary kiln and are thus subjected to slightly different calcining conditions.

The seven particle size ranges studied were (μm): A, under 75; B, 75-150; C, 150-250; D, 250-500; E, 500-1,000; P, 1,000-2,000; Q, 2,000-3,300. Fractions A to E were obtained by hand sieving the animal feed grade calcined magnesite, and P and Q by sieving the fertiliser grade. The separation was carried out by means of a series of brass laboratory test sieves (Endecotts Ltd., London) until complete separation was achieved. An estimate of the percentage particle size distribution was made (by weight) for the animal feed and fertiliser grade product samples used in this trial †† (Table 1). The presence of a large proportion of particles below 1·0 mm in the fertiliser grade is indicative of considerable particle breakdown in transit from the production plant. The maximum particle size in this grade was found to be just under 3·3 mm. Table 1 also shows the magnesium contents and L.O.I. for each supplement. It can be seen that L.O.I. decreases with decreasing particle size, except for the smallest particle fraction (under 75 μm) which had a relatively high value.

†† It will be seen that this particle size distribution for animal feed calcined magnesite differs slightly from the average of several samples (see Appendix 1), and differs greatly from the more recent product which includes particles up to 2 mm.

Table 1. Expt.1.1. Magnesium supplements: Particle size distributions in animal feed and fertiliser products, magnesium contents, L.O.I., and daily amounts fed (gMg) to sheep.

Fraction	Particle size (μm)	% in commercial product	gMg/kg	% L.O.I.	gMg/sheep/day
Animal feed grade					
A	< 75	6	512·7	1·51	0·988
B	75-150	21	509·0	0·60	0·981
C	150-250	26	527·5	0·72	1·017
D	250-500	31	538·4	1·00	1·038
E	500-1,000	16	524·1	1·28	1·022
		100			
Fertiliser grade					
P	1,000-2,000	47	493·4	3·32	1·022
Q	2,000-3,300	12	436·0	3·78	0·990
		59†			

† The remaining 41% of the fertiliser grade was under 1,000 μm and was discarded.

Table 2. Expt.1.1. Mean composition of dried grass nuts (g/kg DM).

Dry Matter	892·7 g/kg FM
Crude Protein	116·3
Crude Fibre	241·4
Ether Extract	18·8
Ash	112·8
Magnesium	2·34

Experimental design

Twenty growing wether sheep (Suffolk ♂ x Greyface ♀) aged about 7 months and with a mean initial live weight of 35 kg were maintained in metabolism cages. For an initial acclimatisation period of 14 days and throughout the subsequent eight week experiment, the sheep were given a basal diet of 1·2 kg/day dried grass nuts (Table 2). This alone provided more than adequate magnesium, at 2·5 g daily, in view of the initial liveweight and daily liveweight gain (0·09 kg/day) of the sheep, when compared with the Agricultural Research Council (1980) recommended dietary magnesium allowance of 0·90 g.

The experiment was conducted in the form of two replicated 4 x 4 Latin squares in which sixteen sheep were used to test particle size fractions A,B,C,D,E and P; and an additional four sheep were used to test fraction Q in a simpler experimental design.

Each sheep received a daily supplement of Spanish calcined magnesite, weighed accurately to give about 1 g Mg, for 14 consecutive days (see Table 1). Each sheep was given a different supplement in each of the four 14-day periods including one period during which no supplement was received. The sheep were grouped into the four Latin squares as uniformly as possible on a live weight basis. Sheep in two replicate squares received supplements A,C and E (forming section (a) of the experiment), while the other two replicate squares tested B,D and P (section (b)). Thus, within each square, each sheep received each of three supplements being tested, as well as undergoing a period with no magnesium supplement. The remaining four sheep were used to assess supplement Q (section (c) of the experiment). They were treated in the same manner as the sheep in sections (a) and (b), receiving no supplement for 14 days and supplement Q for the following 14 days.

The first 7 days of each 14-day period were treated as changeover periods, and during the second 7 days total outputs of faeces and urine

were collected, weighed and sampled for analysis.

Blood samples were obtained at 10.00 h on the last day of each 14-day feeding period.

In addition to the faecal samples dried for analysis in the usual way, samples of fresh faeces were also taken in order to isolate any particulate mineral residue. Each 1 kg sample was blended with water in a laboratory liquidiser and the mixture allowed to settle in a polypropylene bucket. Time was given for the dense mineral particles to settle to the bottom before the organic matter suspension was carefully decanted. More fresh water was added and the washing and decanting process repeated several times until a residue of particulate mineral matter remained. This was analysed for total magnesium content. It was considered unnecessary to carry out this washing process with faeces from sheep receiving the smallest particles of calcined magnesite (A) as there would be very little chance of recovering such small particles in this way. This was carried out purely as a qualitative test in order to discover whether whole particles of calcined magnesite did pass through the animal. In an attempt to calibrate this rather rough method, known amounts of each supplement (B,C,D,E,P and Q) were added to fresh faeces which were then treated in the same manner in order to ascertain the proportion of the calcined magnesite recoverable by this method. Six 1 kg samples of fresh faeces from sheep which had been receiving no supplement to their basal diet were taken, and to each was added about 2 g (1 g Mg) of one of the six supplements. These were then blended with water and treated as above, the particulate mineral residue being analysed for total magnesium content.

Results

The principal results for each section of the experiment ((a)(b) and (c)) are detailed in Table 3.

Table 3. Expt.1.1. Mean daily magnesium (g Mg) balance data and the concentration of magnesium and calcium (mmol/l) in the blood of the sheep at the end of each 14-day feeding period.

(a) SUPPLEMENT	Nil	A	C	E	SEM	Significance
Intake	2.51	3.50	3.53	3.52		
Urine	0.54	0.73	0.70	0.68	0.022	A,C,E > nil***
Faeces	1.92	2.68	2.72	2.89	0.044	A,C,E > nil*** E > C*; E > A**
Retention	0.05	0.09	0.11	-0.04	0.051	NS
Availability(%)	-	23.1	21.1	2.7	4.85	A,C > E**
Blood Mg	0.92	0.94	0.95	0.94	0.009	NS
Blood Ca	2.63	2.59	2.61	2.65	0.032	NS

(b) SUPPLEMENT	Nil	B	D	P	SEM	Significance
Intake	2.51	3.49	3.55	3.53		
Urine	0.55	0.67	0.68	0.64	0.029	B,D,P > nil*
Faeces	1.93	2.75	2.80	2.77	0.024	B,D,P > nil***
Retention	0.03	0.08	0.08	0.12	0.028	NS
Availability(%)	-	16.5	16.8	17.8	2.39	NS(P > E**; B,D > E*)
Blood Mg	0.92	0.95	0.94	0.95	0.014	NS
Blood Ca	2.69	2.61	2.65	2.66	0.021	NS

(c) SUPPLEMENT	Nil	Q
Intake	2.42	3.59
Urine	0.65	0.77
Faeces	1.77	2.92
Retention	0	-0.10
Availability(%)	-	-2.8
Blood Mg	1.03	1.02
Blood Ca	2.62	2.70

The diet was well consumed throughout. However two of the four sheep in section (c) used to assess the largest particle size fraction of calcined magnesite (Q, 2,000-3,300 μm) failed to consume any appreciable amounts of this supplement, even when additionally coated with palatable molasses. Accordingly, urine and faeces collections were not made and results from only two sheep for this particle size were obtained. In a subsequent balance trial two more sheep were offered this same supplement (to the same diet) and again it was not well consumed, so that no further results could be obtained.

The basal diet provided a mean of 2·51 g Mg/day in sections (a) and (b), and 2·42 g Mg/day in section (c). The mean daily amounts present in the urine and faeces of the eight sheep in section (a) receiving basal diet alone were 0·54 and 1·92 g respectively, and retention was calculated (by difference, intake minus faeces and urine) to be 0·05 g/day. In section (b) the mean amounts present in urine and faeces were 0·55 and 1·93 g (respectively) giving a retention of 0·03 g/day. Accordingly sections (a) and (b) were excellently duplicated in respect of the basal diet. In section (c) the mean daily urine and faecal magnesium outputs for the two sheep on the basal diet were 0·65 and 1·77 g respectively, giving apparently zero retention.

All supplements significantly increased both faecal and urinary magnesium outputs. However, none of the supplements significantly affected magnesium retention, values being in the range -0·04 to +0·12 g/day for sheep in sections (a) and (b). The mean retention for the two sheep in section (c) was -0·10 g/day. The faecal magnesium output by sheep given supplement E was significantly greater than that of sheep given supplements A ($P < 0·01$) and C($P < 0·05$), and that of the two sheep receiving supplement Q was also larger, although statistical significance cannot be demonstrated.

Apparent availabilities of the supplements, i.e. the percentage of supplementary magnesium not recovered in the faeces, were in the range 16·5 to 23·1% for particle sizes A, B, C, D and P, with no significant differences. However the availability of supplement E was significantly lower, at 2·7% than A, C, P ($P<0·01$), B and D ($P<0·05$). The availability of magnesium in the basal diet was calculated to be 23·6% and as this was closely duplicated in the different Latin squares in sections (a) and (b), the statistical comparisons of these two sections in respect of the supplements was considered justified. Figure 1 shows the mean availability of each supplement plotted against the median particle diameter of each fraction. From this it is suggested that the feed grade calcined magnesite, providing supplements A to E, and fertiliser grade, providing P and Q, may be fundamentally different materials. Thus a regression analysis was carried out for all the individual availability values for supplements A to E only against median particle size. The following regression equation was obtained:-

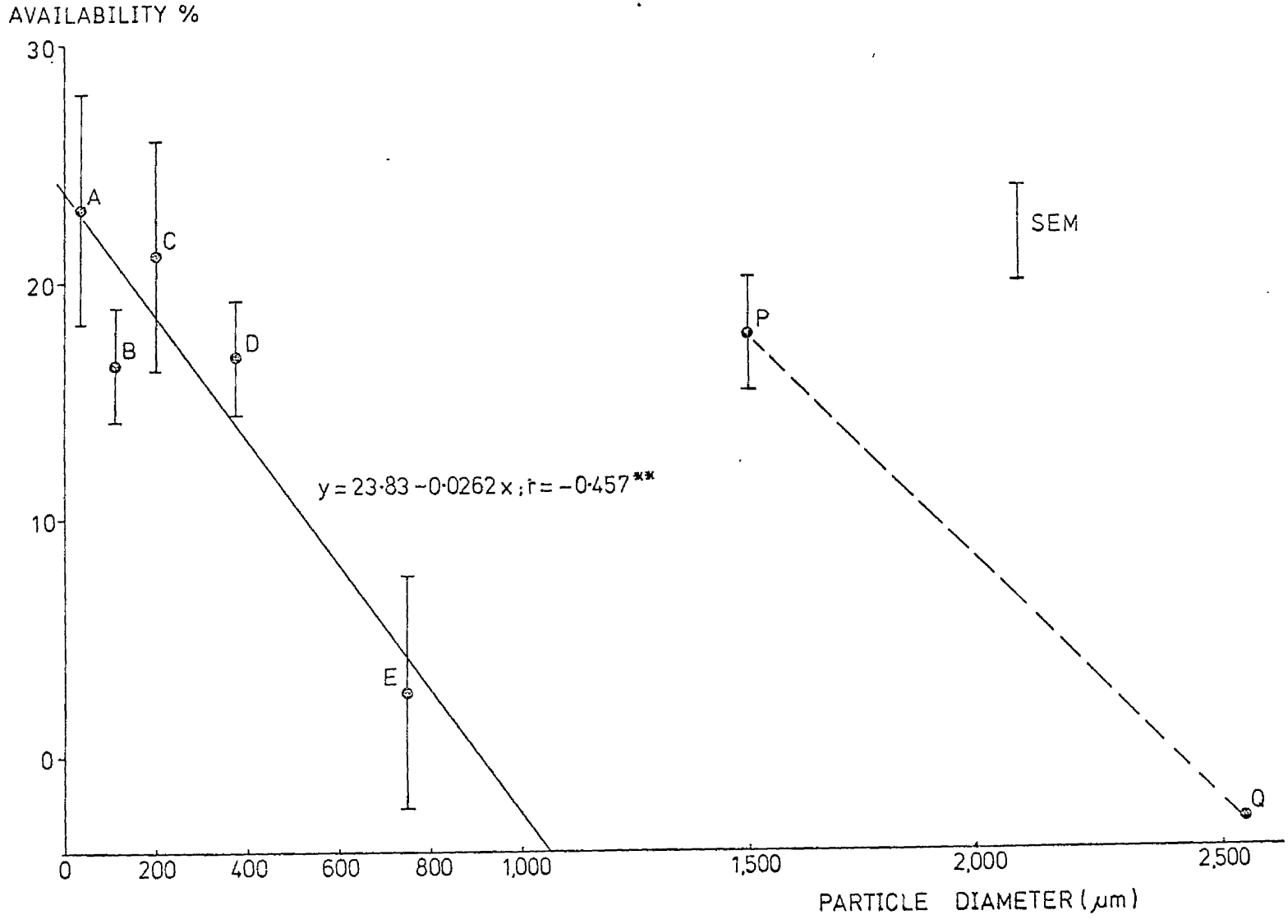
$y = 23·83-0·0262x$; $r = -0·4571$ ($P<0·01$)

where y = % availability, and x = particle diameter (μm)

The availability results for supplements P and Q appear to show that a similar linear relationship between availability and particle size may exist within fertiliser grade calcined magnesite, but there were insufficient results to justify a regression analysis.

The faecal residues obtained from "washing" through fresh faeces contained reasonable amounts of magnesium (Table 4), and the larger particles, notably P and Q, could be seen intact in the residues. The daily quantity of magnesium in the residue increased progressively with increased particle size of the magnesium supplement from 0·011 g (zero supplement) to 0·298 g (supplement Q). The magnesium in the residues derived from the supplement alone was estimated by subtracting the amount of magnesium recovered on zero supplementation (i.e. 0·011 g).

Figure 1. Expt.1.1. Apparent availabilities (%) of different particle sizes (μm) of Spanish calcined magnesite (median particle diameter of each fraction).


AVAILABILITY %
30
20
10
0
A
B
C
D
E
P
Q
SEM
y = 23·83 −0·0262 x ; r = −0·457**
0
200
400
600
800
1,000
1,500
2,000
2,500
PARTICLE DIAMETER (μm)

This was then expressed as a percentage of the supplementary magnesium intake. The rough "calibration" of this method gave the appropriate percentage recoveries one could expect for each particle size fraction, the highest recoveries being only about 60%, and the lowest 25% for supplement B. The calculated daily magnesium outputs in the particulate faecal residues were then adjusted using these estimated recovery coefficients, and the results are given in Table 4.

Table 4. Expt.1.1. Amounts of magnesium recoverable in particulate mineral residues from fresh faeces.

Supplement	gMg/day in fresh faecal residue	gMg derived from supplement/day in faecal residues (x)	(x) as % of supplementary Mg intake	"Calibration" % recoveries	g supplementary Mg in faecal residue as % of intake (adjusted by % recovery)
nil	0.011	-	-	-	-
B	0.018	0.007	0.7	25.0	2.7
C	0.029	0.018	1.8	34.3	5.2
D	0.069	0.058	5.6	66.3	8.5
E	0.139	0.127	12.6	67.5	18.7
P	0.180	0.169	16.5	57.3	28.8
Q	0.298	0.287	29.0	61.3	47.4

None of the supplementary treatments significantly increased the concentration of magnesium in the blood, values remaining in the fully normal range 0·92 - 0·95 mmol/litre for sheep in sections (a) and (b), and 1·02 - 1·03 mmol/litre for sheep in section (c). Similarly none of the supplements significantly affected blood calcium concentrations, values remaining between 2·59 - 2·69 mmol/litre for sheep in sections (a) and (b), and 2·62 - 2·70 mmol/litre in section (c).

Discussion

A significant negative relationship between magnesium availability and increasing particle size in Spanish animal feed grade calcined magnesite has been shown. There were no statistically significant differences between the availabilities of particle size ranges up to 500 μm (16·5 - 23·1%), but the upper particle size fraction 500 - 1,000μm gave significantly greater faecal magnesium outputs and hence a much lower availability at 2·7%. It is therefore possible that the relationship between availability and particle size may be curvilinear, and not linear as drawn in Figure 1. However, the particle size fraction 500 - 1,000 μm only constitutes 16% of the particular sample of the animal feed product used (Table 1) whereas particles up to 500 μm constitute 84%. The mean availability of the latter is 18·5%, whereas the mean availability of the whole product, including particles over 500 μm, is lowered only to 16·0% (Table 5). From these results, then, there would be little advantage in the extra effort involved to screen off particles over 500 μm from the animal feed product. However, it must be emphasised that this conclusion can only be applied to the particular sample of material supplied, and, as mentioned earlier, the commercial product now contains a larger proportion of coarser particles (500 - 2,000 μm) (see Appendix 1).

Table 5. Expt.1.1. Availability of Spanish animal feed grade calcined magnesite.

a) Excluding particles 500 - 1,000 µm

Fraction	% in whole product	% availability	Contribution of each fraction to whole product availability	Mean availability
A	6	23.1	1.363	
B	21	16.5	3.465	15.522 = 18.5%
C	26	21.1	5.486	0.84
D	31	16.8	5.208	
	84		15.522	

b) Including particles 500 - 1,000 µm

E	16	2.7	0.427	16.0%
	100		15.949	

The availability results obtained for the particle size fractions derived from Spanish fertiliser grade calcined magnesite are slightly anomalous. Particles 1,000 - 2,000 µm have a relatively high availability (17.8%) which is not significantly different from that for particles up to 500 µm, but greater than that for the range 500 - 1,000 µm. However, particles over 2,000 µm appeared totally unavailable, as expected, and in addition these particles are less acceptable to sheep, as indicated by the refusal of four out of six sheep to consume them.

The animal feed and fertiliser products appear to be fundamentally different materials, even though both samples used in the trial were derived from the same production batch. The fertiliser grade has a much greater L.O.I., indicating less severe or incomplete calcination, which might be due to a faster passage rate for larger particles through the calcining kiln. The calcination process may be seen as a balance between the production of magnesium oxide from the carbonate and the

"deactivation" of already produced oxide, by heating. The lower L.O.I.'s for the animal feed grade particle fractions, especially 75 - 150, 150 - 250 and 250 - 500 μm, suggest some measure of "deactivation" of the oxide and hence their availabilities appear no different from that of the fertiliser fraction 1,000 - 2,000 μm; i.e. the oxide in the latter fraction may be more reactive than that in animal feed grade particles up to 500 μm, so that it maintains a high availability despite its large particle size. If the availability/particle size relationship within the fertiliser grade mirrors that of the animal feed grade, the grinding of the fertiliser product may produce a material with even higher availability. However this is speculation and it is clear from this experiment that not only is particle size important but also the precise calcination conditions are critical. The ideal situation would be to compare different particle sizes of calcined magnesite each with identical magnesium content and identical L.O.I. In addition it is important to note that "particle diameter" may be relatively meaningless per se as there is a large internal surface area where carbon dioxide has been driven out from the particles leaving them fairly porous.

It is clear from this trial that a proportion of calcined magnesite particles may pass apparently unaltered through the animal and may be visible following "washing" the faeces with water. Although the method employed to isolate the particles in the faeces was only a qualitative assessment, the percentage of supplementary particles recovered was relatively small despite the low availability values obtained. However this proportion did increase with increasing particle size.

Retention of magnesium by the sheep remained low throughout and was unaffected by any supplementary treatments. This was to be expected in view of the mean weight gain of the sheep (0.09 kg/day) and the value

of 0·41 g Mg/kg given by the Agricultural Research Council (1980) as the magnesium content of empty body weight increase (i.e. about 0·37 g Mg/kg live weight gain). From these figures an average retention of 0·03 g Mg/day could be expected.

Blood magnesium and calcium levels remained high and normal throughout, and urinary magnesium excretion was increased as expected by magnesium supplementation.

A general conclusion from this balance trial is therefore that finer particles of calcined magnesite are more available to sheep than are coarse particles and this is in agreement with more recent work with cows (Jesse et al., 1981).

Experiment 1.2. A comparison of the availability of the magnesium in Spanish magnesite calcined at different temperatures.

Introduction

Various workers in the past have investigated the effect of calcination conditions on the chemical properties of magnesium oxides. For example, Britton, Gregg and Winsor (1952) calcined natural rock magnesite samples for two hours at a series of fixed temperatures in the range 600 - 1,400°C, and they found the most chemically active oxide was produced at 700°C. In 1967, Harper reported that the most active magnesium oxide produced from Indian and Greek rock magnesites was calcined at 800°C for 2 hours, whereas that derived from basic (reagent grade) magnesium carbonate was calcined at 600°C for 2 hours. In each case the critical temperature is reported to be the minimum (associated with the particular time period) for complete calcination of carbonate to oxide, and the chemical reactivity of the product declines if the temperature is raised or lowered.

Experiments carried out on the use of calcined magnesite as a magnesium fertiliser for sugar beet have shown that changes in the calcining conditions can affect the reactivity of the product. Draycott, Durrant and Bennett (1975) found that on neutral or alkaline soils the availability to crops of the magnesium in so-called "lightly burnt" (800°C) Spanish magnesite was greater than that in "over-burnt" (850°C) material. In a later trial, Durrant and Draycott (1976) tried to establish the conditions required to produce the most reactive magnesium oxide by varying the temperature and duration of calcination of Spanish magnesite ore. They found that oxides with the highest proportion of readily exchangeable magnesium † were produced by calcining either for 3 hours at 700°C, or 0·5 hour at 800°C, i.e. the minimum time at each temperature for complete conversion of carbonate to oxide, whereas prolonged heating at higher temperatures was detrimental. In addition, data on the exchangeable magnesium content of calcined magnesites gave some guidance over the extent of, and rate at which, magnesium was available to plants. Subsequent work (Draycott & Hutchison, 1979) demonstrated, in field trials and glasshouse experiments, differences in magnesium availability to plants related to different temperatures of calcination. The range of calcination temperatures tested (650 - 900°C) showed that at the lower and upper ends of the range, availability declined.

It is therefore a reasonable assumption that calcining conditions may also influence the dietary availability of calcined magnesite to ruminant animals. The present experiment describes a balance trial in which wether sheep were given a basal diet supplemented with Spanish magnesite calcined at different temperatures (500 - 1,100°C), in a laboratory kiln, for a given length of time (0·75 hour). The different

† i.e. NH_4^+ - exchangeable magnesium - see section V B.

supplements fed comprised the same particle size range to eliminate any particle size availability differences. Three of the six supplements studied were given as additions to a second basal diet, of higher magnesium content, to investigate any possible differences in supplement availability that may be attributable to the type of diet fed.

Materials and Methods

Magnesium Supplements

Samples of magnesite ore ($MgCO_3$) from N.W. Spain†, crushed and screened to 75 - 1,000 μm, were calcined for 0·75 hour at temperatures between 500°C and 1,100°C in a small electric laboratory furnace. The samples of roughly 300 - 400 g were spread evenly on unglazed pottery trays and preheated to 100°C in a drying oven. The trays were introduced to the furnace at 350°C and heated as quickly as possible to the required temperature. To reach 500°, 650°, 800°, 900° and 1,100°C, it took 20, 35, 50, 75 and 110 minutes respectively. After 0·75 hour at the chosen temperature, the furnace was switched off, opened and cooled (with the aid of a cold air blower) to below 400°C. This took 30, 45 and 70 minutes respectively from 500°, 650°C and 800°C, while samples at 900° and 1,100°C had to be left overnight to cool. The calcined sample was removed from the furnace at 350 - 400°C and allowed to continue cooling at room temperature. All calcined samples were hand sieved to 75 - 500μm when cool and stored in air tight jars for use in the feeding trial. There was evidence of some particle breakdown during calcination especially at the three higher temperatures as there was more powder created (< 75 μm) and a smaller proportion of particles 0·5 - 1·0 mm to be sieved out. The six magnesium supplements used in the trial were (A) raw magnesite, and magnesites calcined for 0·75 hour at (B) 500°,

† Supplied by Magnesitas de Rubian S.A.

(C) 650°, (D) 800°, (E) 900° and (F) 1,100°C. The magnesium analysis, loss on ignition (L.O.I.) and particle size distributions are given in Table 1. The chosen particle size range was fairly wide but an earlier trial (Experiment 1.1.) indicated similar availabilities for particle sizes within this fraction and, as Table 1 shows, the particle size distributions within this range remained similar for each supplement (in spite of some particle breakdown during calcination as reported above).

Table 1. Expt.1.2. Magnesium supplements.

Supplement	gMg/kg	%L.O.I.	Particle size (μm) distribution(%)			Amount given	
			75-150	150-250	250-500	g/sheep/day	g Mg/sheep/day
A Raw magnesite	250.1	47.71	22	26	52	4.224	1.105+
Magnesite calcined at							
B 500°C	256.2	47.62	27	27	46	2.457	0.629
C 650°C	298.2	31.53	23	27	50	1.752	0.522
D 800°C	460.8	3.12	23	25	52	1.001	0.461
E 900°C	482.4	1.38	26	28	46	1.044	0.504
F 1,100°C	498.0	0.32	22	27	51	0.935	0.466

+ The intention was to give about 0.5 g Mg daily for all supplements but precise, repeatable magnesium analysis for raw magnesite was unavailable at the start of the experiment and the actual figure used initially was evidently too low.

Diets

The two basal diets used in the balance trial were (i) dried grass nuts, and (ii) concentrate cubes (hereafter referred to as "maize cubes")

made from a mixture of cooked flaked maize, maize dark grains and barley husk siftings in a 4 : 3 : 3 ratio, containing 1% salt, 1% limestone, plus some molasses to aid the cubing process. The mean general analyses of these diets are given in Table 2. The grass nuts were fed at 1·2 kg/sheep/day and the maize cubes at 1·0 kg/day, to provide similar energy intakes and hence similar sheep growth rates and magnesium requirements. The magnesium content was lower in the maize cubes than in grass nuts (1·54 against 2·42 g Mg/kg DM), but both provided adequate magnesium, in view of initial liveweight (38 kg) and liveweight gain (0·05 kg/day), to satisfy individual sheep requirements (0·84 g Mg/day; A.R.C., 1980). The calcium concentration was higher in the maize cubes than grass nuts (8·54 against 4·75 g Ca/kg DM). The diets were also analysed for sodium and potassium as it is thought that the Na : k ratio in the rumen affects magnesium absorption from that organ (Martens & Rayssiguier, 1979), and hence magnesium availability. The maize diet has a much higher Na/k ratio of 1·53, compared with only 0·22 for the grass nuts, and this might lead to different supplementary magnesium availabilities on the two different diets.

Table 2. Expt.1.2. Composition of Diets (g/kg DM)

	(i) Dried Grass nuts	(ii) Maize cubes
Dry Matter (g/kg FM)	871.4	864.7
Crude Protein	174.1	162.0
Crude Fibre	244.3	121.4
Ether Extract	17.4	12.9
Ash	113.9	87.2
Magnesium	2.42	1.54
Calcium	4.75	8.54
Sodium	2.81	5.32
Potassium	12.65	3.47

Experimental Design

Twenty-four growing wether sheep (Suffolk ♂ x Greyface ♀) aged about 7 months, with initial live weight 38 kg, were maintained in metabolism cages over the eight week experiment. After the initial two week acclimatisation period, the experiment was conducted in the form of three replicated 4 x 4 Latin Squares. Sixteen sheep in two replicated squares were given 1·0 kg/day maize cubes, with or without one of supplements A, B and C (section (a)) or D, E and F (section (b)). The eight sheep in the third replicated square (section (c)) received 1·2 kg/day dried grass nuts with or without one of supplements D, E or F.

Each sheep received a given daily supplement of calcined magnesite, accurately weighed (see Table 1), for 14 consecutive days. Each sheep was given a different supplement in each of the four 14-day periods, including one period during which no supplement was received. The first week of each period was a changeover period, and during the second week total outputs of faeces and urine were collected, weighed and sampled for appropriate analysis.

Blood samples were taken from the sheep at 10.00 h on the penultimate day of each feeding period.

Results

Diets and supplements were well consumed throughout the trial and the principal results are detailed in Table 3.

Sheep in section (a) received 1·33 g Mg/day from the basal maize diet alone and corresponding mean daily urinary and faecal outputs were 0·45 and 0·84 gMg, respectively, giving a retention of 0·04 g. Only supplement C increased urinary output ($P < 0·01$) but all supplements significantly raised daily faecal magnesium output ($P < 0·001$), this being greater for supplement A, at 1·73 g, than for B and C, at 1·41 and 1·33 g Mg, respectively ($P < 0·001$). However daily magnesium intakes differed between

the three supplements (see Table 1) and their apparent availabilities were not significantly different, at 16·0, 9·2 and 6·4% respectively for A, B and C. The mean basal diet magnesium availability in this section was 36·8%. Retention when given supplement A was greater, at 0·26 g/day, than with supplement C at -0·04 g/day ($P<0·01$), and B at 0·11 g/day and the control group at 0·04 g/day ($P<0·05$).

Table 3. Expt.1.2. Daily magnesium (g Mg) balance data and the concentration of magnesium and calcium (mmol/l) in the blood of the sheep.

SHEEP RECEIVING MAIZE CUBES

(a) Supplement	Nil	A	B	C	SEM	Significance
Intake	1.33	2.39	1.96	1.85		
Urine	0.45	0.45	0.44	0.56	0.020	Nil,A,B < C**
Faeces	0.84	1.73	1.41	1.33	0.038	Nil < A,B,C***; B,C < A***
Retention	0.04	0.26	0.11	-0.04	0.046	Nil,B < A*; C < A**
Availability (%)	-	16.0	9.2	6.4	4.29	NS
Blood Mg	1.05	1.01	1.00	1.06	0.023	NS
Blood Ca	2.60	2.65	2.60	2.59	0.047	NS

(b) Supplement	Nil	D	E	F	SEM	Significance
Intake	1.33	1.79	1.83	1.80		
Urine	0.52	0.73	0.77	0.72	0.034	Nil < D,E,F***
Faeces	0.74	1.00	1.02	0.99	0.023	Nil < D,E,F***
Retention	0.07	0.06	0.04	0.09	0.031	NS
Availability (%)	-	45.0	46.2	47.2	4.73	NS
Blood Mg	1.06	1.08	1.07	1.06	0.018	NS
Blood Ca	2.72	2.71	2.66	2.66	0.031	NS

SHEEP RECEIVING GRASS NUTS

(c) Supplement	Nil	D	E	F	SEM	Significance
Intake	2.53	2.99	3.03	3.00		
Urine	0.58	0.68	0.66	0.69	0.026	Nil < D.E.F*
Faeces	1.86	2.18	2.14	2.14	0.044	Nil < D,E,F***
Retention	0.09	0.13	0.23	0.17	0.036	Nil < E*
Availability (%)	-	28.4	43.1	38.4	6.68	NS
Blood Mg	0.91	0.96	0.92	0.92	0.016	NS
Blood Ca	2.58	2.60	2.57	2.50	0.032	NS

Sheep in section (b) of the trial received 1·33 g Mg/day from the basal diet alone, with corresponding daily outputs in urine and faeces of 0·52 and 0·74 g respectively, giving a daily retention of 0·07 g Mg. All supplements significantly raised urinary ($P<0·001$) and faecal ($P<0·001$) magnesium outputs, though differences between supplements were not apparent. Retentions remained in the range 0·04 - 0·09 g/day for all treatments, including the unsupplemented controls, and availabilities were not significantly different for all supplements at 45·0, 46·2 and 47·2% respectively for D,E and F. The apparent availability of magnesium in the basal diet was 44·4% in this section.

The basal diet of dried grass nuts used in section (c) supplied 2·53 g Mg/day, with corresponding daily urinary and faecal outputs of 0·58 and 1·86 g respectively, giving a retention of 0·09 g/day. All supplements increased urinary ($P<0·05$) and faecal ($P<0·001$) magnesium concentrations. Retention was raised ($P<0·05$) only with treatment E, at 0·23 g/day, while with supplements D and F it remained at 0·13 and 0·17 g/day, respectively. There were no statistically significant differences between apparent availabilities of the supplements, at 28·4, 43·1 and 38·4% respectively for D, E and F, and the mean availability of the magnesium in the grass nuts was 26·5%.

Availability values for supplements A to F are also shown in Figure 1. The availabilities of D, E and F on the two different diets are compared in Table 4 and none are significantly different.

None of the supplementary magnesium treatments, in all sections, significantly affected blood magnesium or calcium levels. Magnesium concentrations remained in the range 1·00 to 1·08 mmol/litre in sections (a) and (b), and 0·91 to 0·96 mmol/litre in section (c), while blood calcium concentrations remained between 2·50 - 2·72 mmol/litre throughout.

Figure 1. Expt 1.2. Apparent availabilities (%) of magnesites calcined (for 0.75 h) at different temperatures (^{o}C), when given to sheep receiving the two diets.

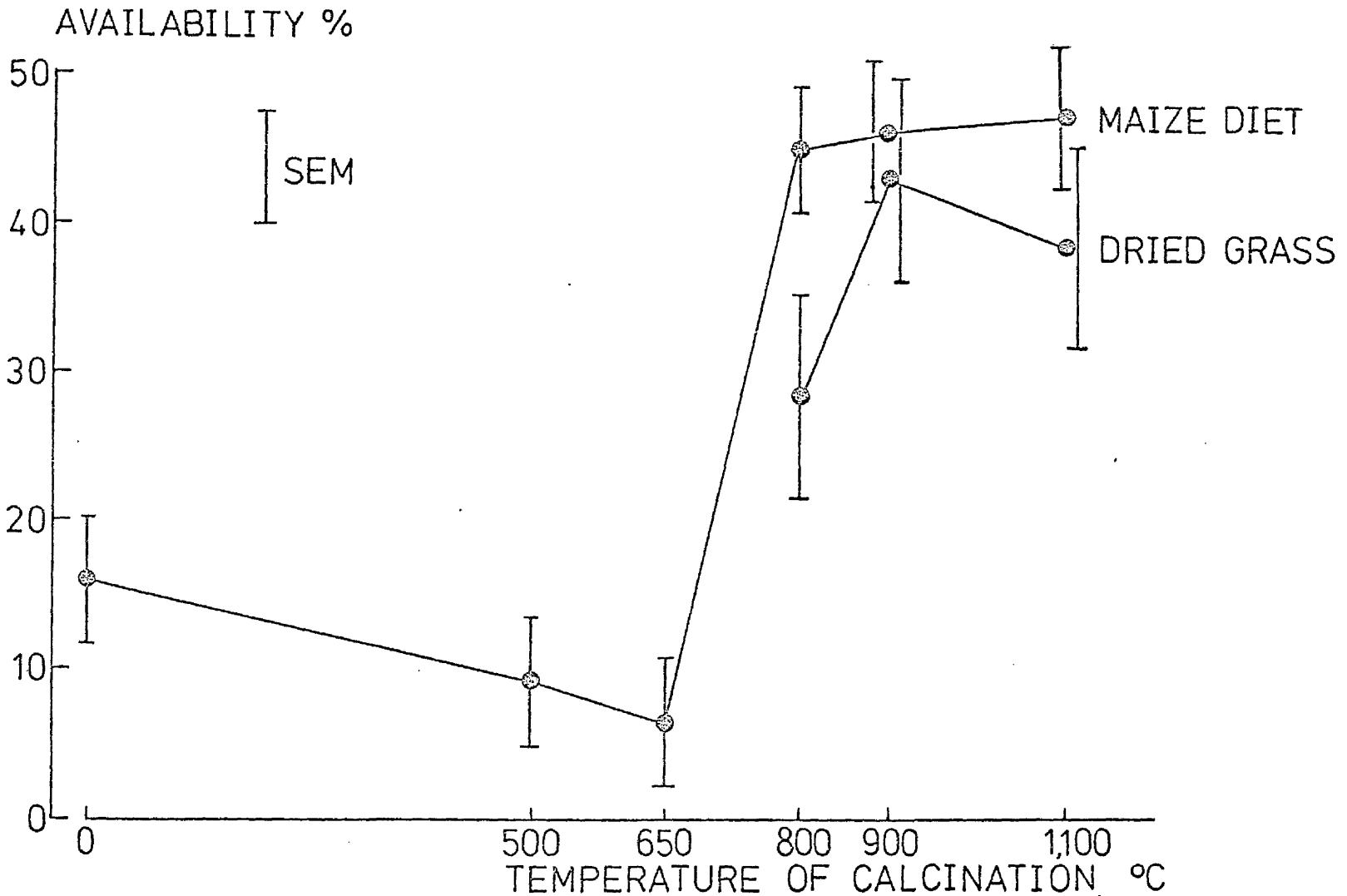

AVAILABILITY %
50
40
30
20
10
0
SEM
MAIZE DIET
DRIED GRASS
0
500
650
800
900
1,100
TEMPERATURE OF CALCINATION °C

Table 4. Expt.1.2. Comparison of availability figures for supplements D, E and F when given with the two different diets.

	Availability when Given:-				
Supplement	MAIZE DIET	SEM	GRASS NUTS	SEM	Significance
D. (800°)	45.01	4.734	28.38	6.685	NS
E. (900°)	46.18	4.734	43.11	6.685	NS
F. (1100°)	47.25	4.734	38.35	6.685	NS

Discussion

The experimental results broadly indicate that magnesite calcined for 0·75 hour at 650°C or below appears to be poorly available to sheep, whereas magnesite calcined at 800°C or up to 1,100°C is highly available.

During the calcination process, carbon dioxide is expelled from the magnesite crystal lattice by heating. The reaction commences at about 400°C, above which the speed and extent of calcination increases. Calcination may be incomplete (and the product unreactive) at low temperatures, but excessive temperatures, or prolonging the process, causes sintering of the particles and decreased activation. Draycott and Hutchison (1979) found that the magnesium in untreated magnesite was relatively unavailable to plants (14%), magnesite calcined at 650°C was significantly less effective (60%) than that calcined at 700°C (80%), and availability declined as the temperature was increased to 900°C (67%). (In each case the magnesite was brought to temperature over a 3 hour period and maintained at temperature for a further 0·5 hour). The present trial demonstrated that raw magnesite is similarly poorly available to sheep (16%). The magnesite calcined at 500°C is hardly calcined at all in the 0·75 hour, maintaining virtually the same (high) L.O.I. and with only a slightly higher magnesium content (Table 1) than untreated

magnesite, and they are visibly indistinguishable. A low availability for this product was therefore understandable. At 650°C calcination was incomplete, as indicated by its high L.O.I, low magnesium content, (Table 1) and paler brown colour compared with the calcined magnesites produced at higher temperatures. Availability to sheep was accordingly depressed, but the actual value obtained (6%) is surprisingly low as the product contains some (presumably active) MgO as well as $MgCO_3$. This availability did not differ significantly from that of raw magnesite, unlike the situation for plants quoted above. The products calcined at 800°C and 900°C were darker in colour, more akin to the commercial product. Calcination was complete and the L.O.I. figures (Table 1) indicate a reasonably active MgO, reflected in high availability to sheep. The magnesite calcined at 1,100°C had a very low L.O.I. and was darker still in appearance. It was expected that some sintering, with associated decreased activity, would have occurred, but the dietary availability to sheep remained high. Thus, unlike the situation with plants, availability of calcined magnesite to animals remains high and does not decline with temperature over the range 800 - 1,100°C.

Many workers have looked at urine magnesium levels as an indication of supplementary magnesium availability (e.g. Storry & Rook, 1963; Gerken & Fontenot, 1967). In this present trial the sheep had normal plasma magnesium concentrations and some urine magnesium excretion at all times, indicating normal magnesium status. The increase in urine magnesium output over the basal level was most marked for the three most available supplementary treatments, 800°, 900° and 1,100°C calcined magnesites. This increase expressed as a percentage of supplementary magnesium intake was 46, 50 and 43% respectively (section (b), calculated on mean values in Table 3). These values agree closely to the apparent availabilities calculated on faecal data. In section (a) "availabilities" calculated on urine magnesium outputs are 0, 0 and 21% respectively for

raw magnesite and those calcined at 500°C and 650°C. Treatment of data in this way therefore differentiates between the partly calcined and untreated magnesites, unlike the apparent availabilities from faecal data. However in both cases all three products are apparently poorly available to sheep.

Magnesium retentions remained low throughout the trial. This is only to be expected when the magnesium content of growing sheep is considered. The Agricultural Research Council (1980) quote this as 0·41 g Mg/kg empty-body weight increase, i.e. about 0·37 g/kg liveweight gain. The sheep in the present trial gained weight at an average of 0·05 kg/day, thus one could expect retention to be 0·02 g Mg/day. In a balance trial, the actual figure obtained for retention includes cumulative errors as it is calculated by difference. The author therefore attaches little significance to the slightly higher retentions obtained on some treatments, e.g. raw magnesite (where availability is low in any case).

The three supplements investigated on both the maize diet and grass nuts diet gave similar apparent availability results in both cases. Values tended to be slightly lower when the sheep were given grass nuts but individual variation was always greater on this diet (as evidenced by a higher SEM, Table 3) and the differences were not statistically significant. It is also interesting to note that the "availabilities" calculated on urine magnesium output data differ somewhat from the values quoted on faecal data in section (c) where the sheep are fed grass nuts. These are 22, 16 and 23% respectively for magnesites calcined at 800°C, 900° and 1,100°C. The mean basal diet magnesium availability was surprisingly different for the two sections of sheep receiving maize cubes (36·8% and 44·4%) but it was considerably higher than that for sheep given grass nuts (26·5%), despite the lower magnesium content of the former diet. It is possible that the higher Na/k ratio of the maize

diet is a contributory factor, or the difference is due to the different nature of the dietary constituents, resulting in different types of rumen fermentation. However it was not unequivocably demonstrated in this trial that the basal diet affected supplementary magnesium availability, but it is perhaps significant that supplement availability tended to be lower on the diet with the lowest basal magnesium availability.

Experiment 1.3. A comparison of the availability of the magnesium in some English sea-water magnesia products.

Introduction

The present experiment investigates the availability of the magnesium in a range of sea-water magnesias. Magnesium oxides produced at different temperatures by the calcination of magnesium hydorixde are studied, including a deadburnt commercial product. As discussed in the previous experiment (1.2.), differences in the calcination temperature for natural rock magnesite result in differences in availability of the magnesium oxide to plants and, to a lesser extent, animals. It would therefore be interesting to detect any similar relationship for sea-water magnesia products.

Particle size is believed to be important in determining availability (Experiment 1.1.) for natural magnesias and it would be appropriate to establish whether the same applies to sea-water magnesias. Therefore different particle size ranges of sea-water derived magnesium oxide were included in the trial.

Lastly, a purified magnesium hydroxide was included as representative for the parent material of all of the oxides studied.

Table 1. Expt.1.3. Magnesium Supplements.

Supplement	Particle size (μm)	gMg/kg	g/sheep/day to give 1·0 g Mg
MgO calcined for 1 hour at			
A 600°C	< 150	498.8	2.005
B 900°C	"	535.8	1.866
C 1,100°C	"	537.9	1.859
D 1,400°C	"	577.6	1.731
E $Mg(OH)_2$	< 75	376.9	2.653
F Deadburnt MgO	< 200	598.0	1.672
G Granular MgO	500 - 1,000	540.3	1.851
H Granular MgO	200 - 500	563.5	1.775

Materials and Methods

Magnesium supplements

Eight different English sea-water magnesia products (supplied by Steetley Minerals Ltd., Hartlepool) were studied in this trial. Four magnesium oxides were specially produced by the calcination of the magnesium hydroxide at four different temperatures (A, 600°C; B, 900°C; C, 1,100°C; D, 1,400°C) for one hour, and each sample was ground to below 150 μm particle size (i.e. powdered). Commercial magnesium hydroxide (trade name Lycal 93HS) itself was tested (supplement E). This is a high quality, free flowing, finely powdered product (99·10% under 75 μm) used, for example, as a combustion additive in oil-fired steam raising plants and other industrial applications requiring careful metering. Supplement F was a "deadburnt" commercial refractory grade magnesium oxide produced at about 1800°C (trade name Britmag 222), zero to 200 μm particle size range. Lastly two experimental samples of sea-water derived, granular magnesium oxide of two different particle size ranges were included in the trial: G, 500 - 1,000μm, and H, 200 - 500μm.

Experimental Design

Ten growing wether sheep (Suffolk ♂ x Greyface ♀) aged about 10 months, with mean initial liveweight 45 kg, were kept in metabolism cages throughout the ten week trial. Each sheep was given 1·5 kg/day dried grass nuts (mean analysis Table 2) providing 2·93 g Mg daily which, in view of the initial liveweight and daily liveweight gain (0·12 kg/day), was alone adequate for the sheep's requirements (1·14g Mg/day; A.R.C., 1980).

The experiment was conducted in the form of two 5 x 5 Latin squares. Supplements A, B, C and D were tested in one square (a) while supplements E, F, G and H were tested in square (b). Each sheep received a different daily supplement, containing 1·0 g Mg, for each of the five 14-day periods, including one period during which no supplement was given. The first 7 days were treated as a changeover week and during the second week of each 14-day period total faecal and urinary outputs were collected for weighing and sampling for appropriate analysis.

Blood samples were obtained at about 14.00 h on the twelfth day of each feeding period.

Table 2. Exot.1.3. Composition of dried grass nuts (g/kg DM).

Dry matter (g/kg FM)	887.6
Crude protein	166.0
Crude fibre	256.9
Ether extract	18.8
Ash	111.0
Magnesium	2.20

Table 3. Expt.1.3. Mean daily magnesium (g Mg) balance data, the concentration of magnesium and calcium (mmol/litre) in the blood of the sheep and the availability of the supplementary magnesium (%).

(a)

Supplement	Nil	A	B	C	D	SEM	Significance
Intake	2.93	3.93	3.93	3.93	3.93	-	
Urine	0.63	0.64	0.71	0.76	0.78	0.055	NS
Faeces	2.13	3.36	3.12	3.20	3.35	0.097	A,B,C,D>0***
Retention	0.17	- 0.07	0.10	-0.03	- 0.20	0.110	NS
Availability (%)	-	-23.0	1.3	-7.1	-21.7	9.78	NS
Blood Mg	0.86	0.90	0.91	0.90	0.91	0.009	NS
Blood Ca	2.64	2.64	2.68	2.65	2.61	0.019	NS

(b)

Supplement	Nil	E	F	G	H	SEM	Significance
Intake	2.93	3.93	3.93	3.93	3.93	-	
Urine	0.60	0.58	0.58	0.69	0.71	0.048	NS
Faeces	2.26	3.47	3.43	3.34	3.19	0.136	E,F,G,H>0***
Retention	0.07	- 0.12	- 0.08	-0.10	0.03	0.140	NS
Availability (%)	-	-21.0	-16.9	-8.4	14.4	13.25	NS
Blood Mg	0.88	0.88	0.89	0.90	0.88	0.015	NS
Blood Ca	2.73	2.65	2.64	2.71	2.62	0.035	NS

Results

The principal results are given in Table 3.

The basal diet alone provided 2·93 g Mg/day, and the corresponding mean daily amounts present in the urine and faeces of the five sheep in section (a) were 0·63 and 2·13 g Mg respectively, giving a retention of

Figure 1. Expt.1.3. Apparent availabilities (%) of supplements

E $Mg(OH)_2$

MgO produced from $Mg(OH)_2$ at

A 600°C

B 900°C

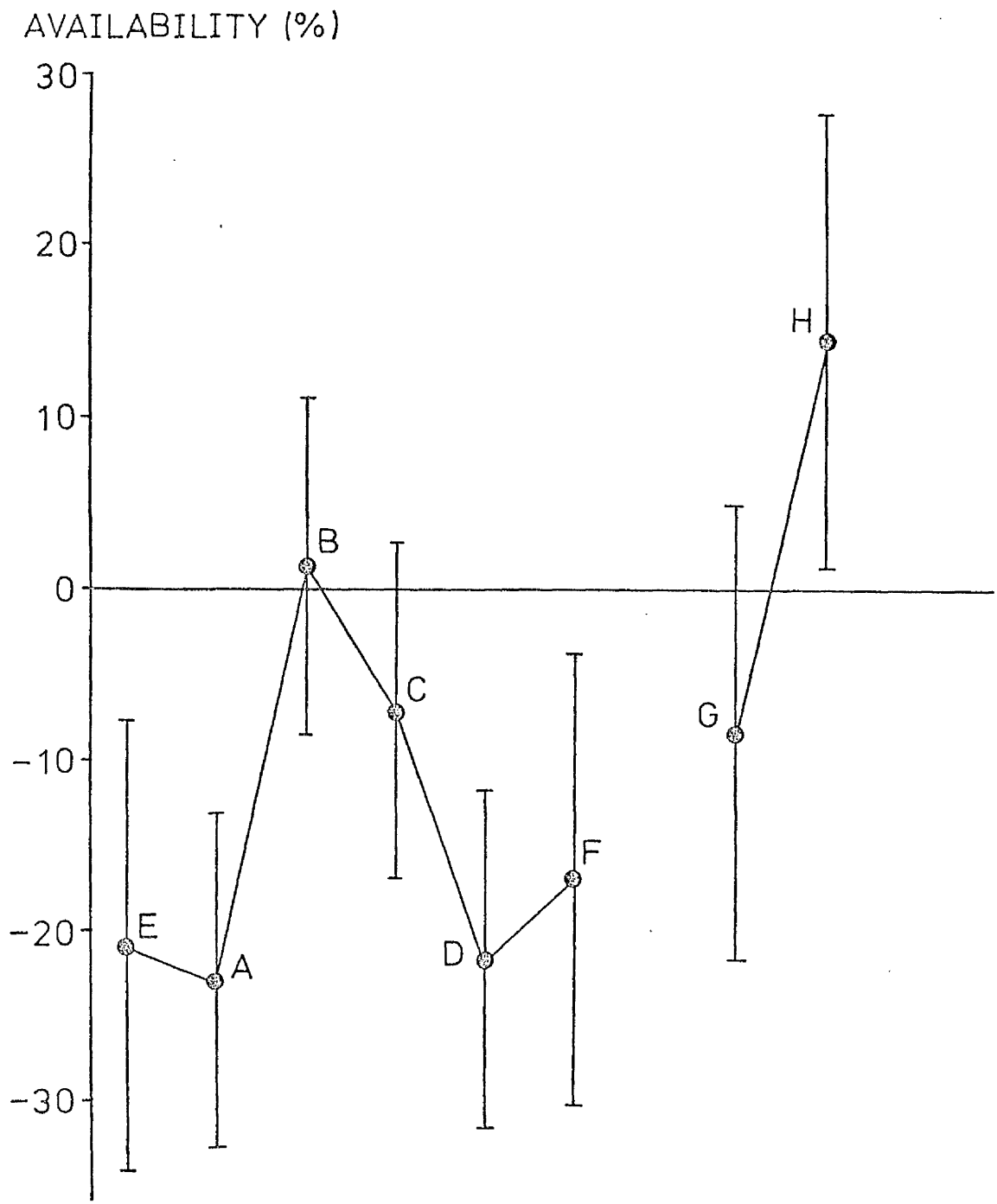
C 1,100°C

D 1,400°C

F c.1800°C "deadburnt"

G 500-1,000 μm MgO

H 200-500 μm MgO


AVAILABILITY (%)
30
20
10
0
-10
-20
-30
E
A
B
C
D
F
G
H

0·17 g/day. In section (b) the corresponding mean urinary and faecal outputs were 0·60 and 2·26 g respectively, with a retention of 0·07 g/day. All supplements significantly increased faecal magnesium output ($P < 0·001$) but none significantly affected urine outputs or magnesium retention, retention values being in the range -0·20 to + 0·17 g/day. Faecal magnesium levels were not significantly different between supplements and hence apparent availabilities were not significantly different, ranging from -23·0% to + 14·4% (see also Figure 1). The mean availability for the magnesium in the grass nuts was 25·1%.

None of the supplementary treatments significantly increased the concentration of magnesium in the blood, values remaining in the fully normal range 0·86 to 0·91 mmol/litre. Similarly no supplement significantly affected blood calcium concentrations, values being in the range 2·61 to 2·73 mmol/litre.

Discussion

The experimental results show no statistically significant differences between the availabilities of the range of English sea-water magnesium products investigated, values being surprisingly low in this particular trial. The basal diet magnesium availability of 25·1% agreed well with the value obtained for similar sheep on the same diet in previous experiments (23·6% in Expt.1.1.; 26·5% in Expt.1.2.). However, the reasons for such low supplement availabilities, either negative or small and positive, are not immediately apparent. Mean urine magnesium outputs remained low throughout and did not increase significantly above the basal level when supplementary magnesium was fed, thus confirming the low availability figures derived from faecal data. There was some severely cold weather during the course of the trial which might have affected magnesium availability to sheep (Meyer, personal communication), but differences between feeding periods were not evident in statistical analysis. As usually observed, there was wide individual variation, but

this was not consistent over all treatments and thus did not appear statistically significant. The standard errors of the mean availability values remain large in spite of the relative "precision" of the Latin square experimental design, thus rendering all values non-significantly different.

The expected daily magnesium retention of the sheep was about 0·04 g, in view of the mean growth rate (0·12 kg/day) and the Agricultural Research Council (1980) value of 0·41 g Mg/kg empty-body weight gain. However, in all cases in this trial, magnesium supplementation appeared to decrease retention (though non-significantly). Slight negative retentions may be explained by cumulative errors in their calculation, but paired with negative magnesium availabilities the situation is apparently inexplicable. The sheep retained normal plasma magnesium (and calcium) status throughout which could not be expected if negative retention occurred. It therefore appears that there is a large consistent error associated with all supplementary treatments, producing these anomalous results. One possibility, which has not been verified, is that the basal diet magnesium availability does not remain constant but is somehow reduced when supplementary magnesium is administered.

Broadly it is concluded from this trial that all the supplements had zero availability. However, some trends in availability are apparent (Figure 1). The calcining of the magnesium hydroxide at 600°C did not improve on the availability of hydroxide itself, but calcining at 900°C produced the material with the highest availability. Availability thereafter declined with further increases in calcination temperature, being poorer at 1100°C and much reduced at 1400°C and for the deadburnt refractory grade magnesia (c. 1800°C). Also the smaller of the two particle size ranges of the granular magnesium oxide (200 - 500μm) appeared to be more available than the larger size (500 - 1,000 μm), which agrees well with the findings in Experiment 1.1.

Experiment 1.4. The availability of Spanish calcined magnesite at four levels of supplementation.

Introduction

The present experiment describes a nutritional balance trial in which wether sheep received different levels of magnesium supplementation to a basal diet of dried grass nuts. It is generally assumed that magnesium availability remains constant over a range of intakes up to the point where excess magnesium intake results in the animal scouring, but there is little published evidence on this point. A previous trial has indicated that particle size may be important in determining availability (Experiment 1.1.), thus a particular particle size fraction (150 - 250 μm) of Spanish animal feed grade calcined magnesite was chosen for this trial.

Materials and Methods

Magnesium supplements

A "medium" particle size range, 150 - 250 μm, containing 534·6 g Mg/kg, was obtained by hand-sieving from the same bulk sample of Spanish calcined magnesite as used in Experiment 1.1. The experimental treatments comprised four different levels of supplementary magnesium given in this calcined magnesite fraction : A, 1 g Mg; B, 2 g Mg; C, 4 g Mg; D, 6 g Mg.

Experimental Design

Five growing wether sheep (Suffolk ♂ x Greyface ♀) aged about 10 months, with mean initial live weight 43 kg, were used in this trial which was run concurrently with the previous experiment (1.3.). The sheep, in metabolism cages, were given 1·5 kg/day dried grass nuts (see Experiment 1.3., Table 2) which alone provided a more than adequate magnesium intake for sheep growing at 0·12 kg/day when compared with the

ARC (1980) recommended daily allowance of 1·10 g.

The experimental design was one 5 x 5 Latin square.

Each sheep received a different daily supplement (A, B, C and D) in each 14-day period, including one period when no supplement was fed, during the ten week trial. The first week of each feeding period was a changeover period, and complete collections of faeces and urine were made during the second week for weighing, sampling and appropriate analysis.

Blood samples were obtained at 14.00 h on the twelfth day of each feeding period.

Results

The principal balance results are shown in Table 1.

Table 1. Expt.1.4. Mean daily magnesium (g Mg) balance data and the concentration of magnesium and calcium (mmol/litre) in the blood of the sheep and the availability of the supplementary Mg (%).

Supplement	Nil	A	B	C	D	SEM	Significance
Intake	2.93	3.93	4.93	6.93	8.93		
Urine	0.55	0.72	0.69	0.98	0.89	0.073	0,A,B<C*; 0<D*
Faeces	2.36	3.37	4.28	6.02	7.15	0.113	0<A<B<C<D***
Retention	0.02	-0.16	-0.04	-0.06	0.83	0.151	0,A,C,C<D**
Availability	-	-1.0	3.7	8.4	20.1	10.69	NS
Blood Mg	0.86	0.86	0.89	0.90	0.90	0.016	NS
Blood Ca	2.66	2.62	2.63	2.64	2.62	0.028	NS

The basal diet alone provided 2·93 g Mg/day and the mean daily amounts present in corresponding urine and faeces were 0·55 and 2·36 g respectively, giving a retention of 0·02 g/day. All levels of magnesium supplementation significantly increased faecal magnesium output

($P < 0.001$), but urine outputs were only increased by the two higher levels. Retention was only increased by feeding the highest level, 6 g Mg/day, when it was 0·83 g/day, compared with -0·16 to +0·02 g/day for the four other treatments. The mean apparent magnesium availability for the calcined magnesite at increasing levels of supplementation appeared to increase from -1·0 to 20·1%, but these differences were not significant. Thus it was justified to calculate a mean availability over all levels. Using the mean increased faecal magnesium output due to the supplement, 2·84 g/day, and the mean amount of magnesium given over the four amounts of supplement, 3·25 g/day, the mean availability for the calcined magnesite (150 - 250 μm) was 12·6%. The basal diet magnesium availability in this trial was 19·6%.

None of the supplementary treatments significantly affected the concentration of magnesium in the blood, mean values remaining in the range 0·86 to 0·90 mmol/litre, nor that of calcium, values remaining between 2·62 and 2·66 mmol/litre.

Discussion

As in Experiment 1.3., the standard error associated with availability figures obtained in the present trial is quite large. Hence no statistically significant differences could be reported for different levels of magnesium supplementation and the mean availability for the calcined magnesite (150 - 250 μm) was 12·6%. However, this is slightly lower than the value obtained in a previous trial (Experiment 1.1.) for this particle size range (21·1%). Indeed a more direct comparison would be between the previous result of 21·1% and that obtained in the present trial when the supplement was given at the same rate of 1 g Mg/day, i.e. only -1·0%. The two trials were almost identical, in terms of animals and basal diet, and the unrepeatability of this result remains anomalous.

Although no differences are significant, there appears to be a trend for availability to increase with increased amount of magnesium fed. However, the increases in urinary magnesium outputs associated with each level of supplementation do not confirm this. The percentage of supplementary intake present in the urine for 1, 2, 4 and 6 g Mg daily supplementary treatments are, respectively, 17, 7, 11 and 6% (giving a mean of 10·3%).

Blood magnesium concentrations remained high, indicating normal magnesium status, but retention was significantly higher with the highest level of supplementation. Retention could be expected to be 0·04 g Mg/day, as in Experiment 1.3., but in this experiment mean retentions were subject to wide variations (as indicated by the relatively large standard error). Little significance is therefore attached to the slight negative values for retention obtained, but the large positive retention calculated for the highest level of magnesium supplementation is entirely unrealistic and must be attributed to accumulation of errors.

Thus it appears from this trial that the availability of the magnesium in calcined magnesite to sheep remains relatively constant at all levels of intake (in the range 1 - 6 g Mg daily).

B. Chromic oxide technique

The balance trial technique for the determination of availability has some major disadvantages. The Latin square design is usually employed, in order to minimise the effects of very considerable individual animal variation, and this is time consuming and laborious (as can be seen from the earlier section). Even then variation and relatively large standard errors can be associated with the results obtained. Thus an adaptation of the indigestible faecal marker technique has been investigated for the determination of supplementary magnesium availability.

Markers, otherwise known as indicators, tracers, reference substances or index substances are widely used by workers in nutritional studies as the techniques are generally more convenient, less costly, and sometimes more precise than methods involving total measurements. Certain criteria must be met by a nutritional marker for its effective use as a faecal index (Kotb & Luckey, 1972). Notably, it must be inert, non-toxic and completely recoverable in the faeces. It should have no appreciable bulk, and must lend itself to ready, precise, quantitative measurement. Chromic oxide (Cr_2O_3; chromium sesquioxide) is a dark green, powdered compound which has the characteristics of an inert indicator. Normal foods and ruminant dietary constituents contain negligible amounts of chromium and its use as a faecal marker was first proposed by Edin (1918). It is relatively cheap, available, non-toxic and has given recoveries close to 100% in many experiments with ruminants (e.g. Kane et al., 1950; Putnam, Loosli & Warner, 1958; MacRae & Armstrong, 1969). Thus chromic oxide was a convenient marker to choose for the present availability determinations.

Chromic oxide is closely admixed in known precise quantities with the magnesium supplement to be fed. A proportion of the magnesium will be digested and absorbed while the inert chromium passes undigested

through the animal's gut. Thus the ratio of magnesium derived from the supplement to chromium in the faeces will be lower than that in the supplement as fed, and the supplementary magnesium availability is given by the difference in these ratios expressed as a percentage of the intake ratio; i.e.

$$\frac{\text{(Intake ratio supplementary Mg : Cr) - (Faecal ratio Mg derived from supplement : Cr)}}{\text{(Intake ratio supplementary Mg : Cr)}} \times 100\%$$

Animals receiving no supplement therefore have no chromium in their faeces, and their faecal magnesium corresponds to the basal diet output alone. Any increase in the faecal magnesium that occurs when a supplement is administered is associated with an output of chromium, and is assumed to be undigested supplementary magnesium. Using this method of calculation it can be seen that total input and output data for magnesium and chromium are unnecessary, and concentrations alone are sufficient to determine the relevant ratios.

To test the comparative accuracy of the chromium marker technique, chromic oxide was administered along with the magnesium supplements fed to sheep in the traditional balance trials in Experiments 1.2. and 1.4.

Experiment 1.2.

0·200 g chromic oxide, containing 635·75 g Cr/kg, was added to each daily magnesium supplement, and the chromium concentration of the faeces was determined along with the magnesium content. Supplementary ratios of magnesium to chromium for the six calcined magnesites (C.M.'s) investigated are given in Table (i). Faecal ratios of magnesium derived from the supplement (i.e. the increase over the basal level) to chromium are calculated for each of the eight sheep on a given treatment. Individual availabilities can then be calculated (as shown above) and the mean results (with standard errors) are given in Table (i). As supplements to the maize diet, availabilities for A, B, C, D, E and F

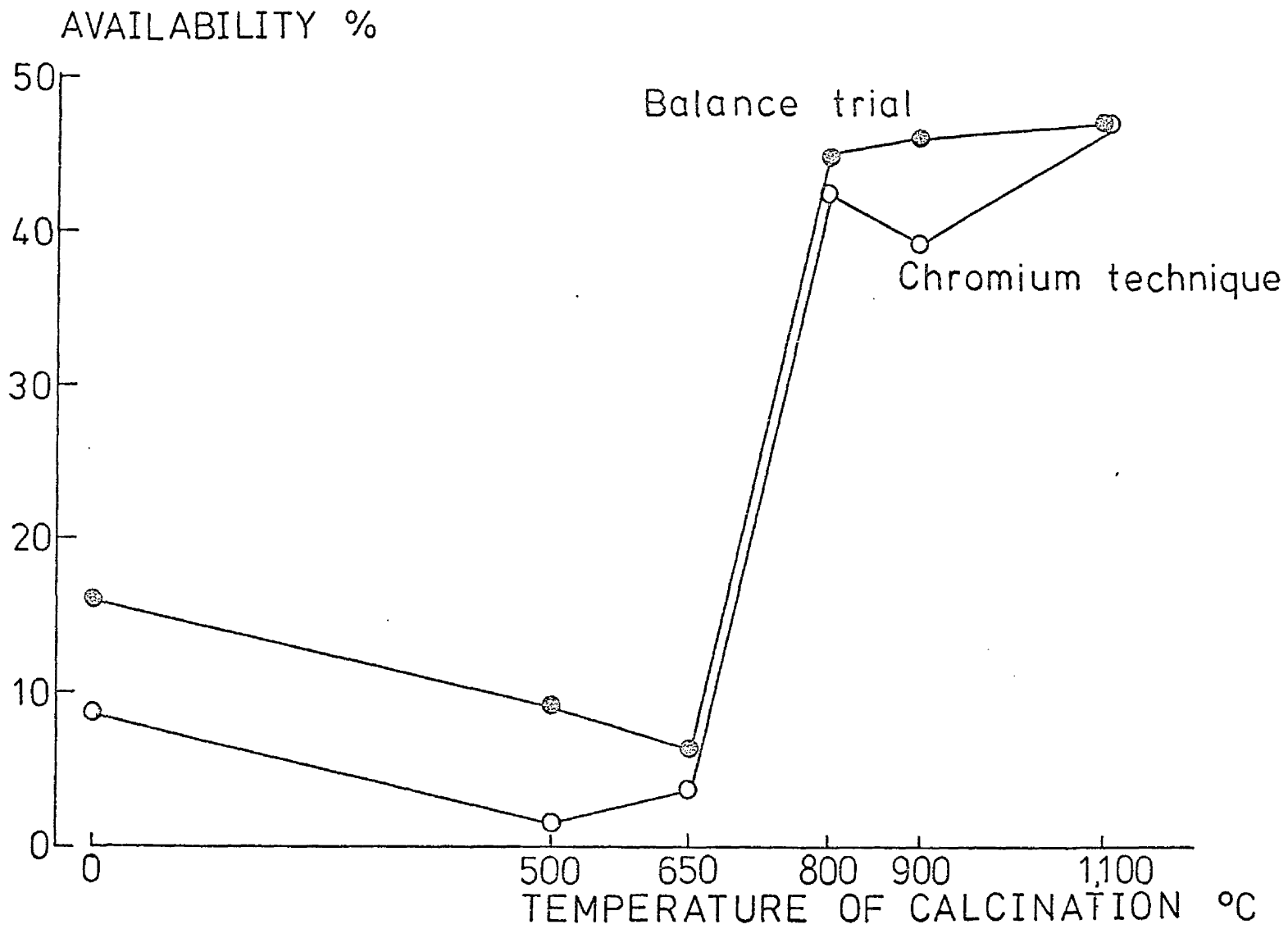
were 8·6, 1·5, 3·7, 42·5, 39·2 and 47·0% respectively and availabilities determined when the grass nuts were given, for D, E and F were 32·3, 49·5 and 37·8% respectively. These are very close to the values obtained in the balance trial, any differences being statistically non-significant, and the two sets of results are compared in Table (i) and Figure (i).

As total faecal collections had been made for the balance trial, the total output of chromium was calculated for a number of the sheep in order to determine the percentage recovery. This was found to be 95 - 100% which confirms that the chromic oxide is indigestible and thus a suitable marker.

Table (i) Availability of supplements used in Experiment 1.2. as determined by the chromium technique.

SUPPLEMENT	Intake Mg/Cr ratio	Availability %	SEM	Balance trial Availability %	SEM
MAIZE DIET					
A, Raw magnesite	8.3083	8.6	11.22	16.0	4.29
B, 500°C C.M.	4.9469	1.5	19.80	9.2	4.29
C, 650°C "	4.1054	3.7	15.01	6.4	4.29
D, 800°C "	3.6256	42.5	9.69	45.0	4.73
E, 900°C "	3.9638	39.2	10.77	46.2	4.73
F, 1,100°C "	3.6650	47.0	11.54	47.2	4.73
GRASS NUTS					
D, 800°C C.M.	3.6256	32.3	8.96	28.4	6.68
E, 900°C "	3.9638	49.5	7.67	43.1	6.68
F, 1,100°C "	3.6650	37.8	14.39	38.4	6.68

Figure (i) A Comparison of availabilities(%) of magnesites calcined at different temperatures (°C) as determined by the balance trial and by the chromium marker technique, for sheep given the maize diet (Expt.1.2.).

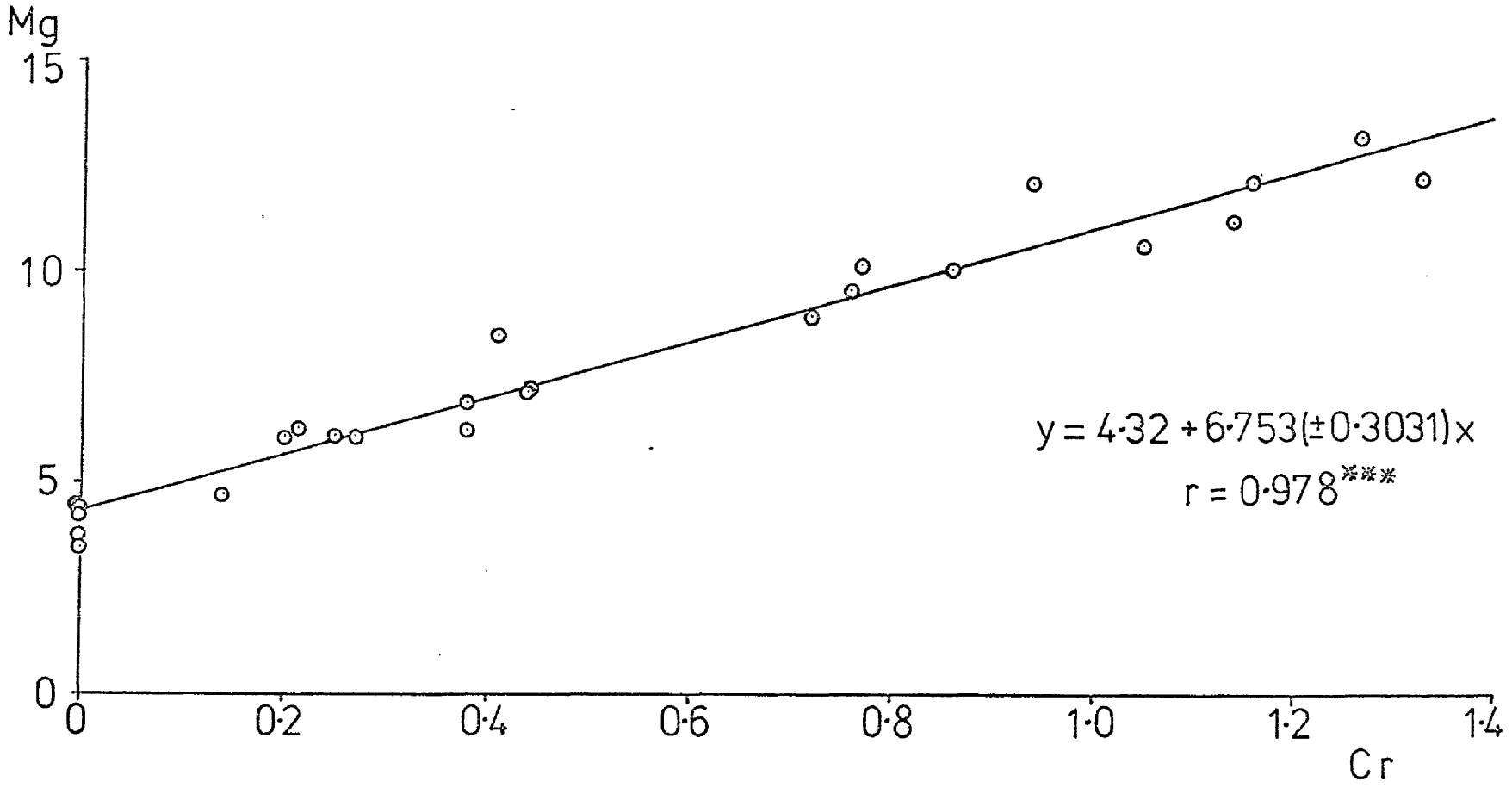

AVAILABILITY %
50
40
30
20
10
0
Balance trial
Chromium technique
0
500
650
800
900
1,100
TEMPERATURE OF CALCINATION °C

Experiment 1.4.

In Experiment 1.4. the magnesium supplements fed to the sheep were four different amounts of Spanish animal feed grade calcined magnesite (i.e. providing 1, 2, 4 and 6 g Mg daily), each with particle size range 150 - 250 μm. Chromic oxide (635·75 g Cr/kg) was added to each in increasing amounts to give a constant supplementary intake ratio of magnesium to chromium (1 g Mg/0·2 g Cr_2O_3, i.e. Mg/Cr = 7·865), and faeces were analysed for both chromium and magnesium.

As the balance trial results could detect no significant change in availability of calcined magnesite with increasing the level of supplementation, the mean faecal magnesium to chromium ratio was determined for all sheep on all treatments together, by regression analysis. Figure (ii) shows the relationship of individual faecal magnesium and faecal chromium concentrations for the twenty-five sheep, and the calculated regression line is shown. The regression of faecal magnesium on chromium is justified (rather than the opposite way around) as chromium being 100% indigestible is therefore the independent variable while the faecal magnesium concentration varies according to the digestibility of the supplement and is therefore the "dependent variable". The high correlation ($P < 0·001$) suggests strongly that the relationship is linear, which in turn confirms the conclusion that availability remains constant at different levels of supplementation. The intercept on the "y" axis gives the mean basal diet output of magnesium (in terms of faecal concentration) and the very highly significant regression coefficient ($P < 0·001$) gives the mean faecal ratio of magnesium derived from the supplement to chromium. From this faecal ratio, 6·753 (SE = 0·3031), and the given intake ratio, 7·865, the availability of the calcined magnesite was calculated as 14·1% (SE = 3·85, calculated directly from SE of faecal ratio). This result from the chromium marker technique compares well, therefore, with the mean result from the

Figure (ii). Regression between faecal magnesium and faecal chromium concentrations (g/kg DM) for the sheep given Spanish calcined magnesite (150-250 μm) (Expt.1.4.).


Mg
15
10
5
0
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
Cr
y = 4·32 + 6·753(±0·3031)x
r = 0·978***

balance data, of 12·6%.

In addition, as total faecal collections had been made in the balance trial, total chromium outputs were calculated and recoveries were estimated to be satisfactory at 95 - 100%.

The two experiments (1.2. and 1.4.) described have therefore demonstrated satisfactorily that the chromium faecal marker technique can be regarded as a reliable method for the determintation of supplementary magnesium availability.

The major advantage of using chromic oxide as a faecal marker is that it removes the laboricus necessity of undertaking total faecal collections. However, a problem associated with its use for the estimation of total faecal output or total dietary digestibility studies is the procurement of faecal samples truly representative of the whole excreta. The existence of a diurnal excretion pattern of chromic oxide in the faeces has been recognised by a number of workers (e.g. Kane, Jacobsen & Moore, 1952; Smith & Reid, 1955; Bloom, Jacobsen, Allen, McGillard & Homeyer, 1957; Putnam et al., 1958; Wilkinson & Prescott, 1970). This may be attributable to a variety of factors including daily dosing pattern, physical nature of the diet, as well as the hypothesis (Kane et al., 1952; Bloom et al., 1957) that the excretion of chromic oxide is regulated by a physiological mechanism. Whatever the cause of the variation in chromic oxide output, it is probable that the undigested supplementary magnesium output is subject to the same variation. It is therefore considered that whereas a single grab sample of faeces might not be representative of the whole excreta, the ratio of magnesium to chromium concentrations will be representative. In addition, care is always taken to administer any chromic oxide intimately mixed with a magnesium supplement to ensure a close association between the two throughout the digesta. Therefore, for the determination

of supplementary magnesium availability, single faecal grab samples taken after one week of receiving the particular supplement are considered sufficient. When a number of supplements are to be compared week by week, grab samples are taken at the same time of day each time in order to minimise any possible error due to the diurnal excretion patterns.

Using this chromium marker technique it has been possible to "screen" many different magnesium supplements and thus obtain extensive availability data. The following three experiments (2.1., 2.2., 2.3.) describe the use of this method for sheep indoors, either group- or individually fed.

Experiment 2.1. A comparison of the availabilities of various magnesium supplements using the chromium marker technique for groups of sheep indoors.

Introduction

Comparative availability data on various magnesium oxides, hydroxide and carbonate (natural magnesite) were investigated, using the chromium faecal marker technique as a "screening" method for groups of sheep indoors.

Materials and Methods

Magnesium supplements

The nine magnesium supplements investigated are given in Table 1. Spanish untreated magnesite (A), particle size range 75 - 500 μm, was the same material as used in Experiment 1.2., obtained from Magnesitas de Rubian S.A. in N.W. Spain. The Spanish calcined magnesite (B) was a sample of the commercial animal feed grade product (Agma FG85) obtained

from a large mixed quantity of several riffled 50 kg sacks, and thus representative of the current (1979) product. It is granular containing particles up to 1·0 mm diameter. The Spanish "Cyclone dust" (TBH powder) (C) is the under-calcined fine dust produced, and discarded, as a by-product from the calcining kiln in N.W. Spain (see Section I "Commercial Magnesium Oxide").

Analar magnesium oxide (British Drug Houses) (D) was included in the trial as it is the purest, chemically active magnesium oxide available. Many workers in the past have used this white powder as a reference standard with which to compare "relative biological availabilities" of other magnesium supplements.

The magnesium hydroxide (E) was obtained from Steetley Minerals Ltd., as in Experiment 1.3. (trade name Lycal 93HS). It is a commercial product, finely powdered (99·1% under 75 μm, 100% under 210 μm), used, for example, as a combustion additive in oil-fired steam raising plants.

Finally, four bags of Greek and Chinese calcined magnesites were obtained from a local agricultural minerals merchant as random samples of the commercial products. Powdered Greek (F) and powdered Chinese (H) products have their major "animal feed" applications for pasture dusting. Both are largely under 75 μm (Chinese powder, 120 mesh, declared up to 125 μm). The Greek granular calcined magnesite (G) ranged from below 75 μm to 2·0 mm particle size. The Chinese granular (J) is declared as 0·3 - 2·0 mm but particle breakdown had produced some smaller fractions in transit. (See Appendix 1 for detailed particle size analysis). It is important to note that the powdered and granular grades from both China and Greece are not necessarily from the same production batches in their respective countries.

Table 1. Expt.2.1. Magnesium Supplements.

	Mg Supplement	gMg/kg
A	Spanish raw magnesite	261.6
B	Spanish granular calcined magnesite	516.2
C	Spanish "cyclone dust"	428.3
D	Analar MgO	599.8
E	Commercial $Mg(OH)_2$	396.0
F	Greek powdered calcined magnesite	510.3
G	Greek granular calcined magnesite	436.6
H	Chinese powdered calcined magnesite	549.2
J	Chinese granular calcined magnesite	499.0

Table 2. Expt.2.1. Diet and magnesium supplements fed to the 3 groups of sheep over the 7 experimental periods.

Diet	Maize diet					Grass nuts	
Period	1	2	3	4	5	6	7
	10 day	1 wk	1 wk	1 wk	1 wk	10 day	1 wk
Group 1 n = 10	O	A	D	O	F	O	C
Group 2 n = 9	O	B	C	O	G	O	H
Group 4 n = 10	O	C	B	O	E	O	J

Experimental design

Twenty-nine one year old wether sheep (Suffolk ♂ x Greyface ♀) were group fed, at maintenance level, about 1 kg/head/day maize cubes (1·5 g Mg/kg DM), as used in the balance trial Experiment 1.2. The sheep were divided at random into three groups (of ten, ten and nine) which were maintained throughout the trial. The experiment was divided into seven periods (numbers 1 - 7). Period 1 lasted 10 days during which no magnesium supplements were fed. During periods 2, 3 and 5, lasting 1 week each, each group received a different magnesium supplement, A, B, C, D, E, F or G (Table 2). A second period without supplementation (period 4) was included with the maize diet. The basal diet was changed in period 6 to 1·2 kg/head/day dried grass nuts (2·4 g Mg/kg DM), as used in experiments 1.1. to 1.4., and in period 7 groups 1, 2 and 3 received supplements C, H and J, respectively (Table 2).

Each magnesium supplement was carefully and thoroughly mixed with a known quantity of chromic oxide (673.3 g Cr/kg) and the Mg/Cr ratio for each is given in Table 3. The mixed Mg/Cr supplement was added to the morning feed, as evenly dispersed as possible, to provide a mean of 2 g Mg/head/day.

Faeces were collected from the sheep at the end of each feeding period, by means of faecal bags affixed by harnesses (as in the previously described balance trial method, Section II A). The collections were made over 18 - 20 hours from 16.00 h on the final day of each period. Total collections were unnecessary for this method and some spillage of faeces from the bags was not critical, however faeces collection via bags was considered better than grab samples for obtaining samples of a reasonable size. The faeces were dried, subsampled and ground for magnesium and chromium analyses.

Table 3. Expt.2.1. Regression data and supplement availabilities.

1) Supplements fed with maize diet

	(a) Mixed supplement Mg/Cr ratio†	Correlation coefficient, r(and significance)	Regression coefficient (b)†	SE of b (and significance)	$\frac{(a)-(b)}{(a)} \times 100\%$ Availability(%)	$\frac{SE\ (b)}{(a)} \times 100\%$ SE(availability)	Significant availability differences
A	3.8853	0.8514***	6.0122	0.9274***	-54.7	23.87	A<B,C,D,E,F, G,H**
B	3.8334	0.9513***	3.2522	0.1756***	15.2	4.58	B<C***; D*; E**
C_1	3.1809	0.9181***	1.8094	0.1301***	43.1	4.09	
D	4.4547	0.9506***	3.0176	0.2323***	32.3	5.21	
E	2.9408	0.9722***	1.9573	0.1112***	33.4	3.78	
F	3.7897	0.9594***	2.8727	0.1990***	24.2	5.25	F<C**
G	3.2424	0.9704***	2.8301	0.1763***	12.7	5.44	G<C***;D*; E**

2) Supplements fed with grass nuts

H	4.0782	0.9538***	3.0626	0.2136***	24.9	5.24	
J	3.7056	0.9344***	5.1558	0.5064***	-39.1	13.67	J<H,C_2***
C_2	3.1809	0.9207***	2.5407	0.2428***	20.1	7.63	C_2<C_1*

† In this Table, and in similar subsequent tables in this thesis, figures are quoted intially to 4 decimal places as these are required for accurate availability calculations.

Results

All the supplements were well consumed throughout the trial. The faecal magnesium concentration was regressed onto the faecal chromium concentration for individuals that had received a particular supplement, including for the same individuals when they received no supplement, and the regression data are shown in Table 3. In each case this gives a very highly significant correlation coefficient ($P<0.001$). Examples of two regression graphs are given in Figures 1 and 2 (one with each basal diet) with the calculated regression lines drawn. The regression coefficients, i.e. the mean faecal ratio of magnesium derived from the supplement to chromium, are also very highly significant, and these are used to calculate mean apparent availabilities of the different supplements (see Table 3). The standard error for an availability result is calculated from the standard error of the regression coefficient expressed as a percentage of the supplement magnesium to chromium ratio. Calculation of both availability and its standard error assumes a constant supplement magnesium to chromium ratio.

Supplement A had significantly lower availability ($P<0.01$) than all other supplements, except J which was similarly low. As supplements to the maize diet C, D, E and F (all powdered) were 43.1, 32.3, 33.4 and 24.2% available, respectively, C being significantly greater than F ($P<0.01$). The granular supplements B and G were much less available, at 15.2% and 12.7%, respectively, both being significantly less than C ($P<0.001$), D ($P<0.05$) and E ($P<0.01$). With grass nuts as the basal diet, J had significantly lower availability ($P<0.001$) than H (-39.1 versus 24.9%) and C had a lower availability on this diet (20.1%) than on the maize diet when it was 43.1% ($P<0.05$).

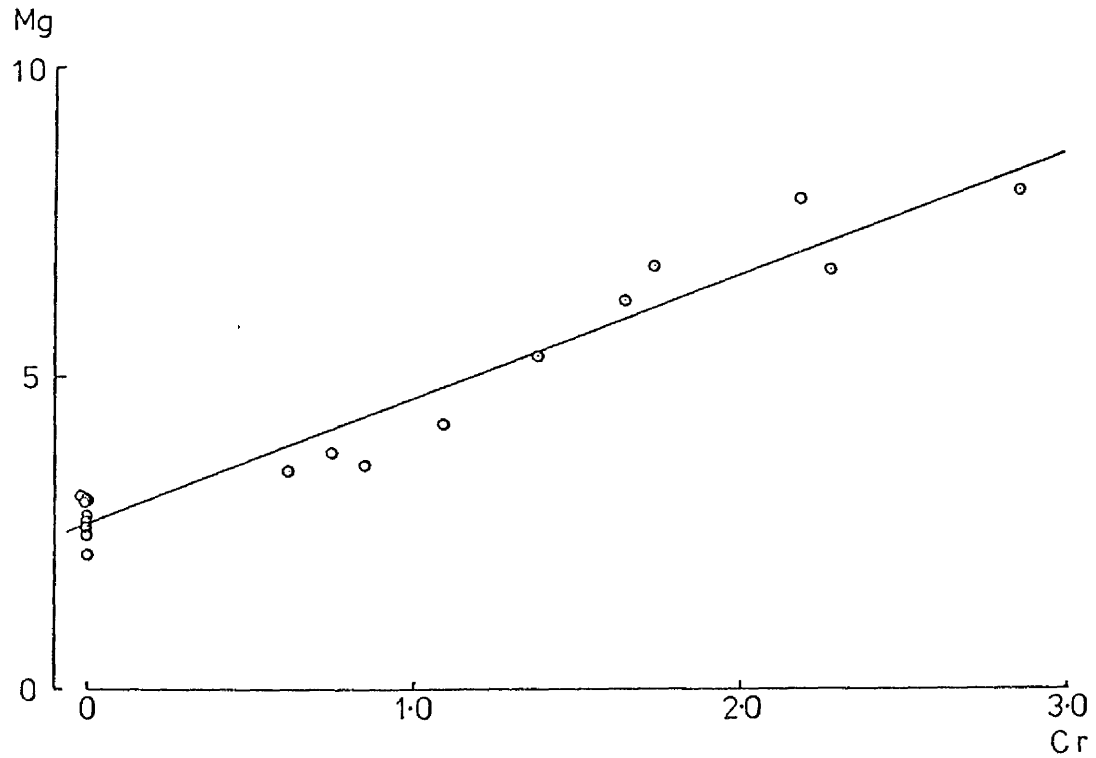
Faecal analysis from period 4, the second period on the maize diet without supplementation, revealed no trace of chromium and gave faecal magnesium concentrations very close to those obtained in the first period.

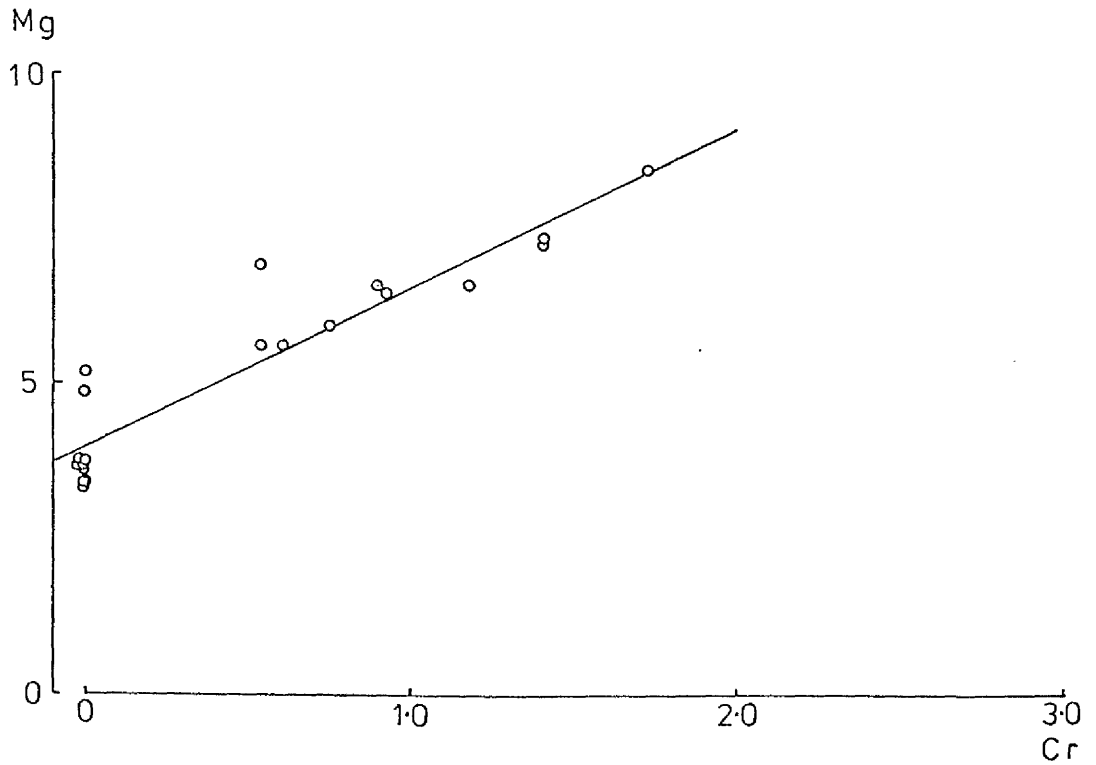
Figure 1. Expt.2.1. Regression between faecal magnesium and faecal chromium concentrations (g/kg DM) for sheep given the maize diet supplemented with magnesium hydroxide (E)

$$y = 2.65 + 1.9573x;\ r = 0.972^{***}$$

Figure 2. Expt.2.1. Regression between faecal magnesium and faecal chromium concentrations (g/kg DM) for sheep given grass nuts supplemented with Cyclone dust (C_2)

$$y = 3.97 + 2.5407x;\ r = 0.921^{***}$$


Mg
10
5
0
0
1·0
2·0
3·0
Cr


Mg
10
5
0
0
1·0
2·0
3·0
Cr

In period 1, mean faecal magnesium concentration for all sheep was 2·71 $\pm$ 0·287 while that in period 4 was 2·69 $\pm$ 0·900 g/kg DM. This therefore indicated that one week was sufficient on a supplementary treatment to eliminate any residual effects of the previous supplement.

Discussion

For sheep given a particular supplementary treatment there is a range of faecal chromium concentrations (g Cr/kg DM) reflecting a range in individual intakes of supplements and/or basal diet. This range of intake aids the calculation of a significant regression line, and the linear relationship observed reinforces the opinion that availability remains constant over different supplementary intakes.

As supplements to the basal diet of maize cubes, the four powdered calcined magnesites were more available (over 24%) than the three granular products (under 15%). Untreated magnesite (magnesium carbonate) was the poorest supplement as expected from the results of experiment 1.2., but the large negative availability (-54.7%) remains anomalous. The two commercial granular animal feed grade calcined magnesites, Spanish and Greek, have similar availabilities, at 15·2 and 12·7% respectively, but the Greek powdered product is nearly twice as available as its granular counterpart, at 24·2% (though just non-significantly). The three most available supplements, cyclone dust, Analar magnesium oxide, and magnesium hydroxide, are not used commercially for animal feed purposes. Indeed, cyclone dust (43·1% available) is presently discarded as a waste product and in this trial it is considerably more available ($P < 0·001$) than the Spanish commercial product itself (15·2% available). Analar magnesium oxide and the relatively pure magnesium hydroxide both have similarly high availabilities of 32·3 and 33·4% respectively.

The two powders studied with the grass nuts diet are again better than the granular supplement, cyclone dust and Chinese calcined magnesite

powder being 20·1 and 24·9% available, respectively, whereas the Chinese granular product (used commercially for animal feeds) appears totally unavailable (-39·1%). It is interesting that the cyclone dust is 43·1% available with the maize diet (which contains 1·5 g Mg/kg DM) but is only half as available ($P<0.05$) with grass nuts (which have a higher magnesium content of 2·4 g/kg DM). This difference in availability of a supplement on these two diets was not (significantly) apparent in a previous trial (Experiment 1.2.). However, within this trial it would evidently be unfair to compare the Chinese products with the other supplements given on the maize diet.

In summary, powdered magnesium products are better than granular supplements in this trial, and availabilities appear greater on the maize diet than on grass nuts.

Experiment 2.2. A comparison of the availabilities of different calcined magnesites using the chromium marker technique for individually fed sheep indoors.

Introduction

The possible value of using the chromium marker technique is apparent from the previous experiment (2.1.) and there is little doubt that the method is useful for the reasonably quick determination of magnesium supplement availabilities for comparative purposes. For the present trial, it was considered advantageous to adapt the method for individually penned sheep in order to facilitate handling and to allow more precise control of individual intakes of both diet and supplements. A low magnesium diet (described in Section III A) was used so that a greater proportion of the total magnesium intake was provided as the supplement; however it must be noted that the requirements of the sheep used would be low (under 1 g Mg/day, A.R.C., 1980) and the basal diet

alone should still satisfy them.

A range of magnesium oxides from different sources are tested in the present trial, including pairs of granular and powdered products from the same or similar sources.

Materials and Methods

Magnesium supplements

Nine different calcined magnesites (C.M.'s) produced by commercial firms, but not all in commercial use as animal feed magnesium supplements, were investigated. These are listed, together with their magnesium contents, in order of use during the trial, in Table 1.

A Swedish company, Boliden, supplied a finely powdered calcined magnesite (B), of unknown precise origin, together with material manufactured by compacting this powder at 60 - 80°C into extremely dense, dust-free, irregular shaped granules (A) ranging mostly from 1·0 - 4·0 mm in size.

A sample of extremely pure (96% MgO) magnesia made from magnesium chloride extracted from sea-water in Ireland (supplied by Wilson Salt Co. Ltd., Belfast) was investigated (C). This again comprised "manufactured" dense granules, spherical in this case, ranging mostly from 0·25 - 2·0 mm diameter. Granulation was effected by treating fine magnesium oxide dust with water in an extruder process.

The powdered and granular commercial "natural" calcined magnesite products from China (D and E) and Greece (F and G) were from the same samples as used in the previous experiment (2.1.). Again it should be noted that the powdered and granular materials are not necessarily different particle size grades of exactly the same product (e.g. in terms of origin and calcining conditions).

Lastly, two particle size fractions from commercial Spanish calcined magnesite were investigated: H, powdered under 75 μm; and

J, coarse granules 500 - 1,000 µm. These fractions have already been investigated in Experiment 1.1., but it must be stressed that the different experiments used distinctly separate samples of Spanish calcined magnesite for fractionation.

Table 1. Expt.2.2. Magnesium supplements.

	Mg Supplement	gMg/kg
A	Swedish granules of C.M. (1·0 - 4·0 mm)	421·0
B	Swedish powdered C.M.	482·3
C	Irish granules of C.M. (0·25 - 2·0 mm)	600·3
D	Chinese powdered C.M.	549·2
E	Chinese granular C.M. (0·3 - 2·0 mm)	499·0
F	Greek powdered C.M.	510·3
G	Greek granular C.M. (0 - 2·0 mm)	445·7
H	Spanish C.M. powder (<75 µm)	506·7
J	Spanish C.M. granules (500 - 1,000 µm)	524·1

Experimental design

Nine approximately one year old wether sheep (Suffolk ♂ x Greyface ♀) were individually penned indoors for the duration of this present trial. They were fed, at roughly maintenance level, 1·5 kg/head/day of a loose mixed low magnesium diet comprising equal proportions of flaked maize, maize dark grains and barley husk siftings, and containing 0·6 Mg/kg DM. (For a detailed appraisal of this diet see Section III A) The basal diet was fed in roughly equal halves, morning and evening. Over nine successive weeks a different calcined magnesite each week was added to the surface of the morning feed. The sheep were numbered one to nine,

and in any one seven-day period each sheep received a different level of magnesium supplementation, ranging from no supplement up to 4·0 g Mg/day (Table 2). In addition, chromic oxide (673·3 g Cr/kg) was added in appropriate quantities to the individually weighed calcined magnesite to give a constant ratio of supplementary magnesium to chromium (1·8567).

On the last day of each seven-day period blood samples were taken at about 16.00 h for plasma magnesium analysis and faeces were collected over approximately 16 hours by means of faecal bags (as described in Section II A). Faeces were dried, ground and subsampled for magnesium and chromium analyses.

Table 2. Expt.2.2. Amounts (g) of Mg and Cr_2O_3 fed daily to sheep numbers 1 - 9 throughout the experiment.

SHEEP	gMg	g Cr_2O_3 @ 673·3 g Cr/kg
1	0	0
2	0.5	0.4
3	1.0	0.8
4	1.5	1.2
5	2.0	1.6
6	2.5	2.0
7	3.0	2.4
8	3.5	2.8
9	4.0	3.2

Constant ratio supplementary Mg : Cr = 1·8567

Results

All the supplements were well consumed throughout the trial. The regression data for faecal magnesium on faecal chromium for each supplement are given in Table 3, together with the calculated apparent availability results (see also Figure 1). The regression coefficients, i.e. the mean faecal ratio of magnesium derived from the supplement to chromium, and the correlation coefficients are both highly significant ($P<0.001$) in all cases, and examples of two such regression graphs are given in Figure 2. There is an even gradation of increased faecal magnesium and chromium concentrations for sheep numbers 1 to 9 reflecting their increased supplementary intakes.

All granular calcined magnesites (A, C, E, G and J) are less available than the powdered materials (B, D, F and H) at various probability levels, but there are no significant differences between the different granular products: The availabilities of A, C, E, G and J are 13·5, 29·1, 20·8, 9·7 and 18·9% respectively. The powdered supplements B, D, F and H have availabilities of 61·0, 49·7, 45·7 and 41·6% respectively, B being significantly greater than H ($P<0.05$) but they are otherwise non-significantly different from one another.

Individual plasma magnesium levels are correlated with individual daily supplementary magnesium intakes for each treatment (Table 4). The correlation is only significant for two of the five granular supplementary treatments, namely J ($P<0.01$) and E ($P<0.05$) but is significant for three of the four powders, namely D, F and H ($P<0.01$). Examples of two such graphs are given in Figures 3 and 4. The regression coefficients are higher for the powdered than for granular supplements, and if all coefficients are correlated with availability the resulting correlation is very highly significant ($r = 0.6277$, $P<0.001$) (Table 4, Figure 5). The mean plasma magnesium concentration for the sheep receiving no supplementary magnesium is 0·86 mmol/l and

Table 3. Expt.2.2. Regression data and supplement availabilities.

	(a)	Regression of faecal Mg on faecal Cr			$\frac{(a)-(b)}{(a)} \times 100\%$	$\frac{SEb}{(a)} \times 100\%$	
	Mixed supplement Mg/Cr ratio	Correlation coefficient, r(and significance)	Regression coefficient b	SE of b (and significance)	Availability %	SE(availability)	Significance
A	1.8567	0.9888***	1.6053	0.0916***	13.5	4.93	A < B,D***; F,H**
B	"	0.9497***	0.7248	0.0903***	61.0	4.86	B > A,E,G***; J**; C,H*
C	"	0.9654***	1.3162	0.1450***	29.1	7.81	C < B*
D	"	0.9757***	0.9333	0.0793***	49.7	4.27	D > A***; E,G,J**
E	"	0.9842***	1.4714	0.1000***	20.8	5.39	E < B***; D,F**; H*
F	"	0.9777***	1.0092	0.0820***	45.7	4.42	F > A,E,G,J**
G	"	0.9782***	1.6762	0.1344***	9.7	7.24	G < B***; D,F**; H*
H	"	0.9644***	1.0836	0.1123***	41.6	6.05	H > A**; E,G,J*; H < B*
J	"	0.9797***	1.5058	1.1163***	18.9	6.29	J < B,D,F**; H*

the mean levels for the eight sheep receiving each supplementary treatment are also given in Table 4.

Figure 1. Exot.2.2. Apparent availabilities (%) of magnesium supplements to the sheep. (Supplement notations in Table 1)

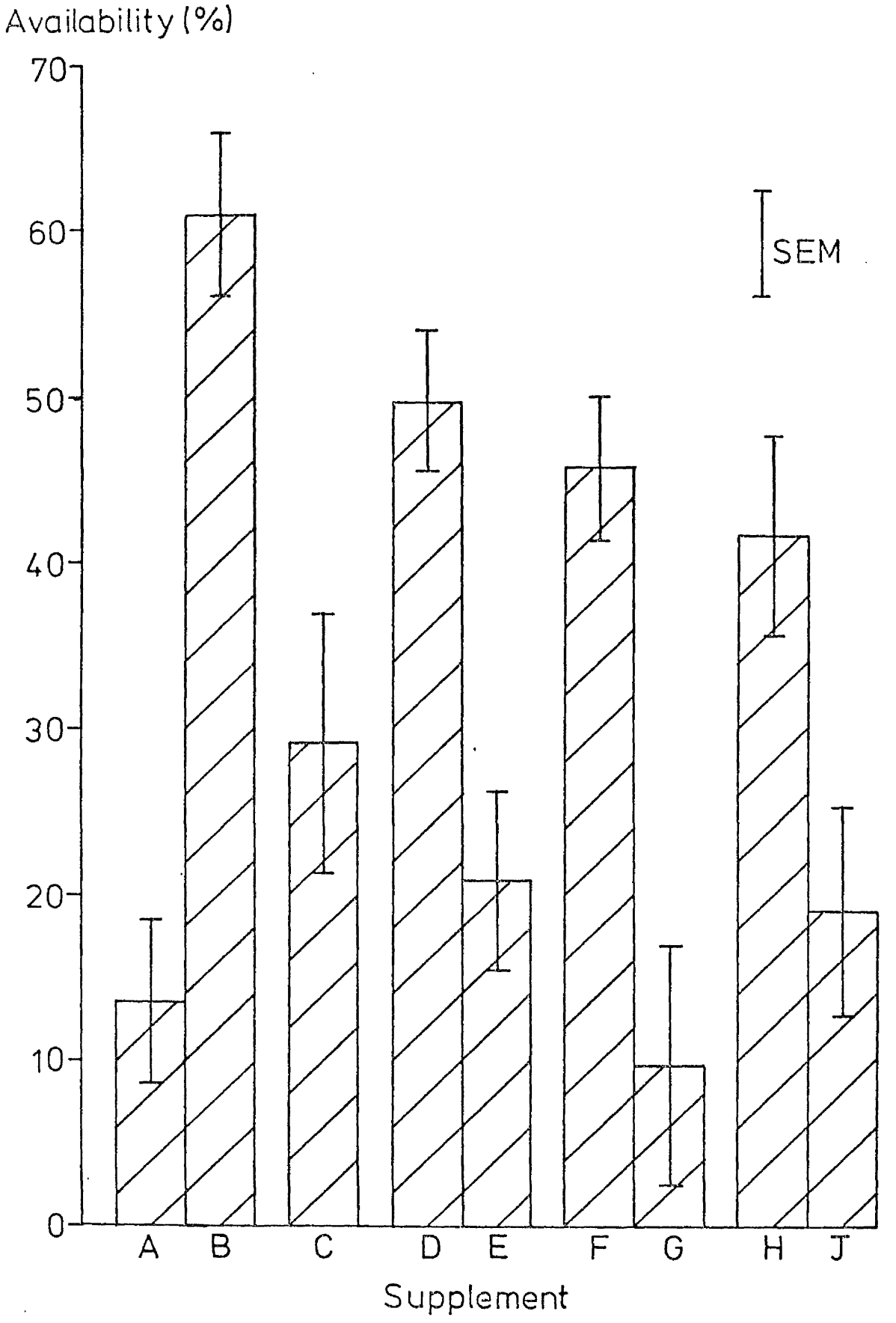

Availability (%)
70
60
50
40
30
20
10
0
SEM
A
B
C
D
E
F
G
H
J
Supplement

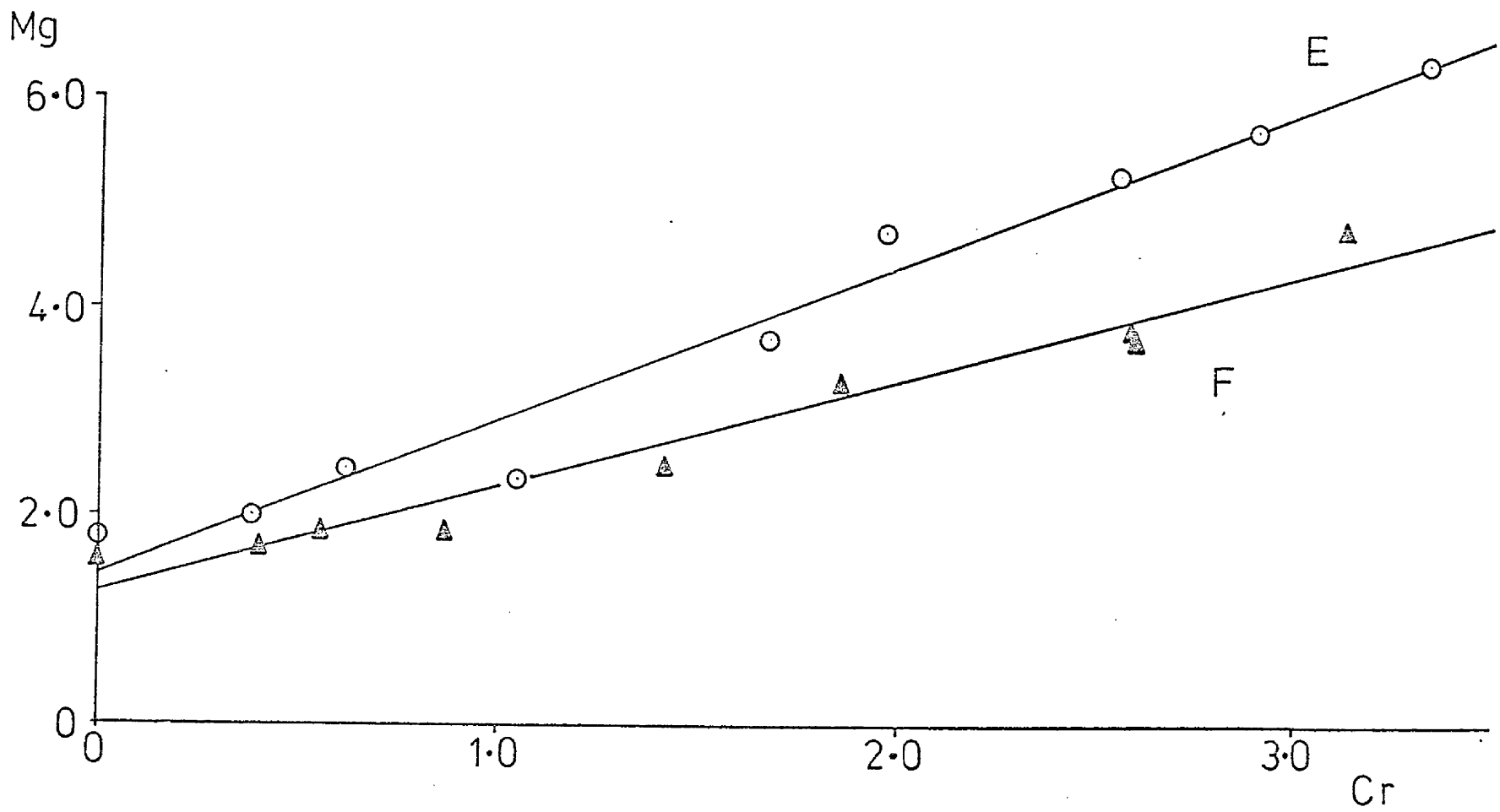
Figure 2. Expt.2.2. Regressions between faecal magnesium and faecal chromium concentrations (g/kg DM) for sheep receiving

○ Chinese granular calcined magnesite (E)

$y = 1.46 + 1.4714x$; $r = 0.984^{***}$

▲ Greek powdered calcined magnesite (F)

$y = 1.28 + 1.0092x$; $r = 0.978^{***}$


Mg
6·0
4·0
2·0
0
0
1·0
2·0
3·0
Cr
E
F

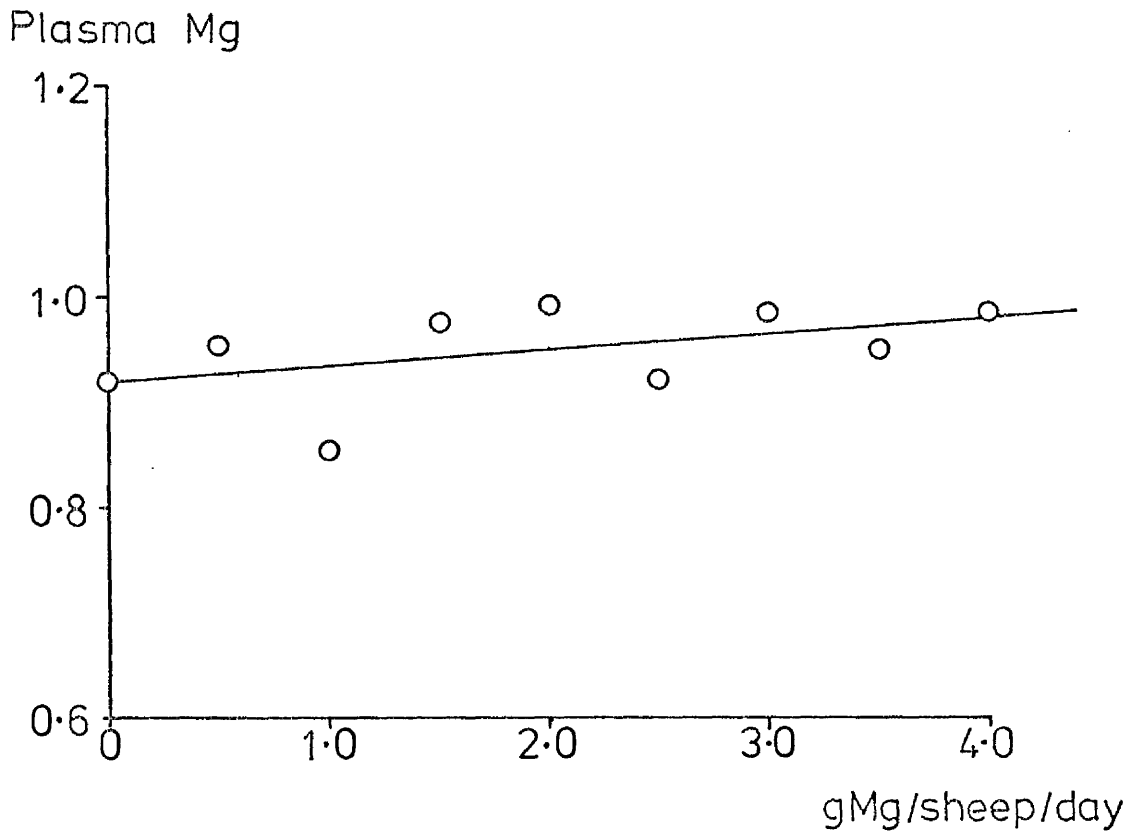
Expt.2.2. Regressions between plasma magnesium concentration (mmol/l) and amount of supplementary magnesium given (gMg/sheep/day):-

Figure 3 Sheep given Swedish calcined magnesite granules (A)

$y = 0.92 + 0.015x$; $r = 0.47$, NS.

Figure 4. Sheep given Greek powdered calcined magnesite (F)

$y = 0.83 + 0.061x$; $r = 0.85^{**}$


Plasma Mg
1·2
1·0
0·8
0·6
0
1·0
2·0
3·0
4·0
gMg/sheep/day

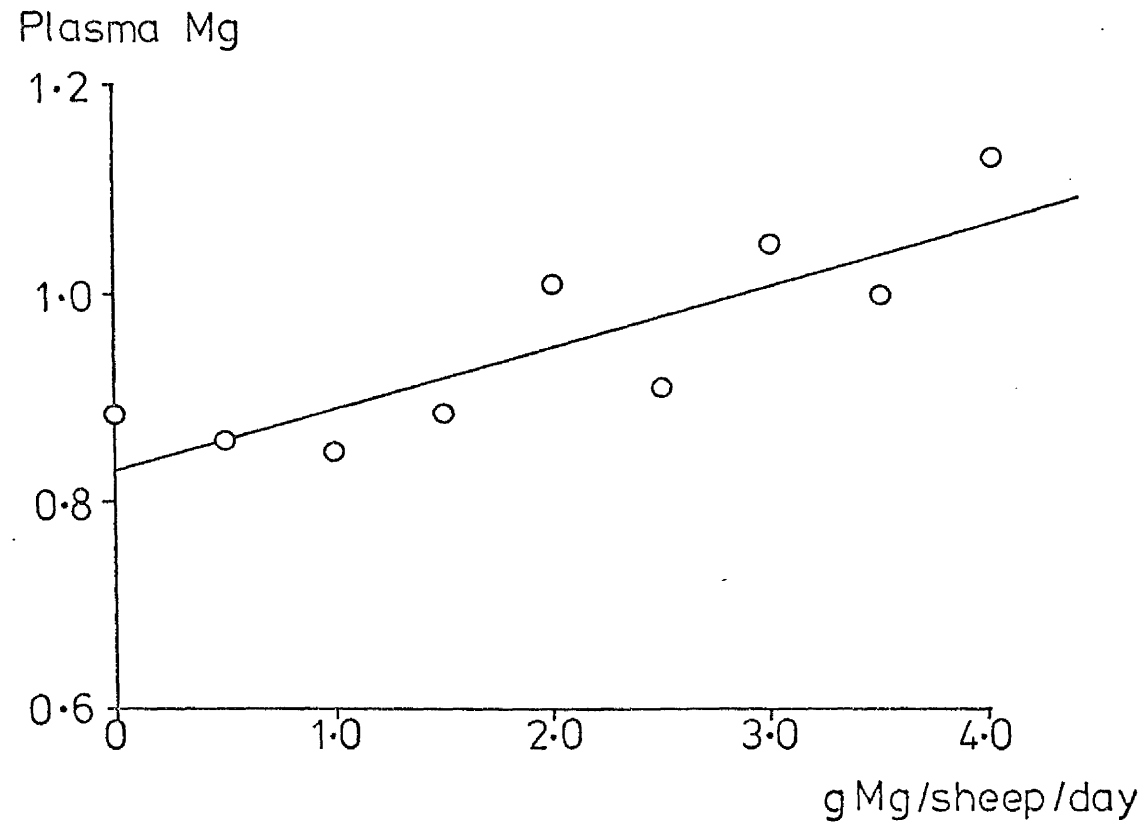

Plasma Mg
1·2
1·0
0·8
0·6
0
1·0
2·0
3·0
4·0
g Mg/sheep/day

Figure 5. Expt.2.2. Correlation between the regression coefficient (b) of plasma magnesium concentration on daily supplementary magnesium intake and supplement availability coefficient.

$$y = 0.0056 + 0.1101x ; \quad r = 0.628***$$

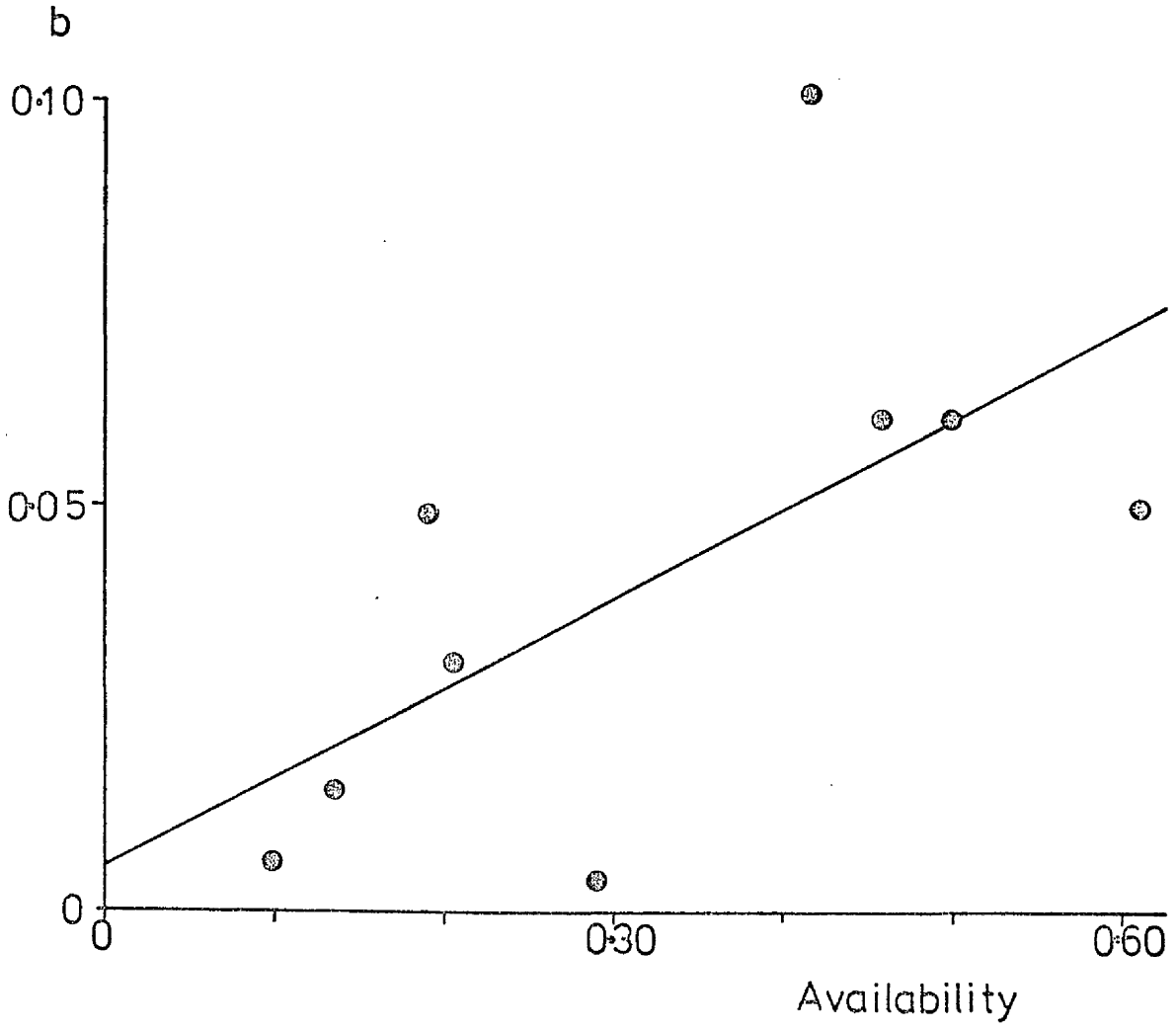

b
0·10
0·05
0
0
0·30
0·60
Availability

Table 4. Expt.2.2. Regression of plasma Mg concentration (mmol/l) on supplementary Mg intake (g Mg/day) for each supplement, correlation with supplement availability, and mean plasma Mg (mmol/l) for sheep receiving each supplement.

	Regression of plasma Mg concentration on daily supplementary Mg intake			
	Correlation coefficient	Regression coefficient (y)	Availability(%) (x)	Mean plasma Mg of supplemented sheep
A	0.467 NS	0.015	13.5	0.95
B	0.592 NS	0.050	61.0	0.99
C	0.106 NS	0.004	29.1	0.85
D	0.818**	0.061	49.7	1.04
E	0.691*	0.031	20.8	0.92
F	0.852**	0.061	45.7	0.96
G	0.090 NS	0.006	9.7	0.90
H	0.893**	0.101	41.6	0.99
J	0.811**	0.049	18.9	0.94

correlation, $r = 0{\cdot}6277^{***}$

$y = 0{\cdot}0056 + 0{\cdot}1101x$

Discussion

The apparent availability data show convincingly that powdered calcined magnesites are generally of higher dietary availability than granular materials. Of the products from each country the granular one invariably had a lower availability than the powdered supplement. It is appreciated, however, that the granular and powdered products were almost certainly not from the same production batches in Greece and China whereas the Swedish and Spanish materials are directly comparable. In

addition the "granular" materials contained varying amounts of some fine material. The mean availability for the five granular supplements was 18·4 $\pm$ 7·42% which was significantly ($P<0·001$) lower than the mean for the four powders at 49·5 $\pm$ 8·35%. The "Swedish" powder (in fact of unknown origin)was the most available, at 61·0%, and this same material compacted into dust-free granules became virtually unavailable, at 13·5%. The Greek granular calcined magnesite had a similar availability, 9·7%, in this present trial as in the previous experiment (2.1.) when it was 12·7%, on a similar diet although higher in magnesium. However, the powdered material was considerably more available in the present study (45·7 v. 24·2%) for reasons unknown. Similarly the Chinese powder was more available in the present study (49·7 v. 24·9%) although a very different diet, grass nuts, was used in experiment 2.1; the Chinese granules were also more available (20·8 v. -39·1%). Both the granular and fine particle size fractions of Spanish calcined magnesite were more available in the present trial than had been previously reported in Experiment 1.1. (<75 µm : 41·6 v. 23·1%; 500 - 1,000 µm : 18·9 v. 2·7%) although again different diets were employed.

The Irish brine magnesia sample had an intermediate availability value, although not significantly different from any of the other granular supplements. It would have been interesting to determine availability of the original powdered calcined magnesite from which the granules were "manufactured."

The size of the increase in plasma magnesium levels in response to feeding increased levels of magnesium as a particular supplement was directly correlated to the availability data ($P<0·001$) i.e. more magnesium was absorbed into the blood from supplements with higher availability, as expected. The correlations were non-significant or less close for the less available granular calcined magnesite but were mainly more significant for the powders, and the regression coefficients were also

greater for the latter. Magnesium in the blood is believed to act as a urine threshold substance, thus preventing any dangerous build-up of excess. However in this trial there was evidently some sustained increase in plasma magnesium concentration where individual sheep were receiving relatively large quantities of available magnesium, i.e. the urine "clearance" was not immediate or complete (and this throws doubt on the validity of the method for determining availability solely on the basis of urinary magnesium excretion; see Section I). The mean blood plasma magnesium concentration for the sheep receiving no supplement was 0·86 mmol/l, which is well within the normal range, and the highest recorded individual plasma magnesium level over all treatments was 1·26 mmol/l which is still well below the hypermagnesaemic level. Thus the plasma magnesium differences observed were all well within the "normal" range for these particular animals, whose magnesium requirements for maintenance in any case were minimal.

The experimental method employed in this experiment whereby sheep were fed individually was considered better than the group-feeding method used in the previous experiment (2.1.) for a number of reasons. Notably it allows precise control over supplementary magnesium intake and amount of basal diet consumed, and this also renders blood data more meaningful. Handling of sheep in individual pens was considerably easier for faeces and blood sample collections, and the method is quick, producing an availability result for a given supplement in just one week with only nine sheep and minimal labour cost. Also, the supplementary magnesium to chromium ratio was standardised over all treatments in this trial so that the calculated regression lines of faecal magnesium on chromium were directly comparable, before actual availability results were calculated.

Thus this experiment has demonstrated the efficiency of the "rapid screening" chromium marker technique for individual sheep in determining the relative availabilities of different calcined magnesites.

It has clearly shown that the powdered calcined magnesites investigated were more available than the granular materials, and this difference was also reflected in the response by the plasma magnesium concentration to increased feeding levels of supplementary magnesium.

Experiment 2.3. Further availability comparisons of magnesium supplements from different sources and of different particle sizes for individually fed sheep indoors.

Introduction

The chromium marker technique is evidently successful as a rapid screening method for supplementary magnesium availability determinations (Experiment 2.2.), whereby individually fed sheep given a low magnesium diet are supplemented with graded levels of magnesium, with chromic oxide as a marker. In the present trial three/four groups of sheep were maintained concurrently to increase the throughput of experimental calcined magnesite samples. As the regressions of faecal magnesium on faecal chromium are invariably well correlated (Experiment 2.2.) the numbers of sheep in each group could be reduced to eight (or even six or seven), but still spanning a similar broad range of supplementary magnesium intakes.

Samples of important calcined magnesites available on the British market are compared, and specific particle size ranges are separated out in order that the effects of source and particle size on availability could be observed separately. In addition, samples of sea-water and brine magnesias, of different particle sizes, are included in the trial to increase the range of sources studied. Availability results for sea-water derived magnesium oxide availability were surprisingly poor in Experiment 1.3. in view of their chemical reactivity, and it was considered necessary to obtain more data for such products.

Table 1. Exot.2.3. Magnesium supplements.

	Magnesium supplement		gMg/kg
A	English sea-water MgO	1·0 - 2·0 mm	577.8
B	" " "	200 - 500 μm	563.5
C	Spanish C.M.	250 - 500 μm	538.4
D	Chinese C.M.	0.5 - 2.0 mm	573.4
E	Greek C.M.	0.5 - 2.0 mm	491.8
F	Spanish C.M.	0.5 - 2.0 mm	507.6
G	Chinese C.M.	75 - 500 μm	543.0
H	Greek C.M.	75 - 500 μm	466.4
J	Spanish C.M.	75 - 500 μm	504.4
K	Chinese C.M.	under 75 μm	533.9
L	Greek C.M.	under 75 μm	463.3
M	Spanish C.M.	under 75 μm	508.0
N	Chinese "granular, 0.3-2.0 mm"	"Whole" commercial C.M.'s	570.1
P	Chinese "gritty, 30 mesh"	"Whole" commercial C.M.'s	543.0
Q	Greek	"Whole" commercial C.M.'s	480.3
R	Spanish	"Whole" commercial C.M.'s	516.2
S	English sea-water MgO powder		573.2
T	English dolomite ($MgCO_3$ $CaCO_3$) powder		125.7
U	American brine MgO "prilled 30", 150 - 500 μm		603.0
W	English sea-water $Mg(OH)_2$ powder		396.1

C.M. = calcined magnesite.

Table 2. Expt.2.3. Particle size (μm) distribution (%) of Spanish, Greek and Chinese calcined magnesites (1980 products).

	<75	75-150	150-250	250-500	500-1,000	1,000-2,000	>2,000
Spanish	15	22	25	19	13	6	-
Greek	8	5	7	12	22	35	11(<3,500)
Chinese "gritty"	14	11	18	46	11	-	-
Chinese "granular"	4	2	2	5	28	45	14(<2,800)

Materials and Methods

Magnesium supplements

A wide range of twenty different magnesium supplements were investigated in this trial (Table 1). New random samples of the current (November 1980) commercial products available in Great Britain were obtained from Spain (granular, "Agma FG85"), Greece (granular, Grecian Magnesite Co., S.A.) and China (two grades, "granular", 0.3-2.0 mm, and "gritty", 30 mesh). Percentage particle size distributions for these four products are given in Table 2 (and Appendix 1). Although similar calcined magnesites from these three countries have been studied earlier (Experiments 2.1. and 2.2.), the samples used in this present trial (hereafter called "1980 products") were obtained separately and may therefore not provide directly comparable results.

Three broad particle size ranges, which together constituted all or most of the products, were separated: namely 0.5-2.0 mm, 75-500 μm and under 75 μm, so that the effects on availability of both particle size and source of material could be studied. The Chinese "granular" and "gritty" products are essentially the same product cut off at 0.3 mm (although some particle breakdown during transit results in the "granular"

grade containing some finer particles). In this experiment the 0.5 - 2.0 mm fraction was obtained from the granular Chinese product, whereas the 75 - 500 μm and under 75 μm fractions were obtained from the "gritty" 30 mesh material. This 75 - 500 μm fraction, however, may be considered representative of both Chinese products.

In the present trial the magnesium supplements studied were "whole" granular Chinese, gritty Chinese, Greek and Spanish calcined magnesites (N, P, Q, R respectively) together with their 0.5 - 2.0 mm fractions (D, E, F for Chinese, Greek and Spanish, respectively) 75 - 500 μm fractions (G, H, J) and under 75 μm fractions (K, L, M).

In addition some English sea-water magnesia products were included (supplied by Steetly Minerals Ltd.). Two particle size ranges of magnesium oxide (A, 1 - 2 mm; B, 200 - 500 μm) were compared, together with one similar particle size fraction of natural Spanish calcined magnesite (C, 250 - 500 μm; from the 1979 product sample as used in Experiment 1.1.). A highly reactive powdered magnesium oxide (Trade name, Lycal 93/12) and powdered commercial magnesium hydroxide (Lycal 93HS) were also obtained (S and W respectively), as well as a sample of finely powdered English dolomite ($MgCO_3$ $CaCO_3$; "Dolofil") (T). Lastly, supplement U was a sample of extremely pure American brine-derived magnesium oxide (trade name "Animag"), prilled into dense granules, largely 150 - 500 μm particle size.

Experimental design

Twenty-four wether sheep (Suffolk ♂ x Greyface ♀), aged approximately 8 - 9 months, were individually penned indoors for the duration of the six-week experiment. They were fed 1.5 kg/head/day of the loose mixed maize-based diet as used in Experiment 2.2., containing 0·6 g Mg/kg DM, given in approximately equal portions morning and afternoon. The sheep were divided at random into three groups of eight for the first four seven-day periods of the experiment. In periods 5 and 6 an additional two

Table 3. Expt.2.3. Groups of sheep and amounts of supplement given/ Sheep/day.

PERIODS 1-4

				Supplement	
Group	(1)	(2)	(3)	gMg/day	g Cr_2O_3/day
Sheep Nos.	1	9	17	0	0
	2	10	18	0.5	0.4
	3	11	19	1.0	0.8
	4	12	20	1.5	1.2
	5	13	21	2.0	1.6
	6	14	22	2.5	2.0
	7	15	23	3.0	2.4
	8	16	24	3.5	2.8

PERIOD 5

Group	(1)	(2)	(3)	(4)		
Sheep Nos.	1	26	25	9	0	0
	2	10	11	21	0.5	0.4
	3	17	12	22	1.0	0.8
	4	18	13	23	2.0	1.6
	5	19	14	24	2.5	2.0
	6	20	15	8	3.0	2.4
	7		16		3.5	2.8

PERIOD 6

Group	(1)	(2)†	(3)	(4)		
Sheep Nos.	1	26	25	9	0	0
	2	10	11	21	0.5	0.4
		17			0.75	0.6
	3	18,20	12	22	1.0	0.8
		19			1.25	1.0
	7			8	1.5	1.2
	4		13	23	2.0	1.6
	5		14	24	2.5	2.0
	6		15		3.0	2.4
			16		3.5	2.8

† It was necessary to maintain low supplementary Mg intakes for sheep in this group due to the lower Mg content of the supplement given and to its fine powdered, and hence bulky, nature.

sheep were included and the twenty-six animals were grouped into four (two groups of seven and two of six sheep). In any one period, each sheep within a group received a different amount of a particular magnesium supplement, including one animal which received no supplement (Table 3), and each group in each period tested a different supplement (Table 4). Supplements were added to the surface of the morning feed and chromic oxide (673·3 g Cr/kg) was added to all individually weighed daily supplements to give a constant magnesium to chromium ratio of 1·8567 (Table 3).

On the last day of each seven-day period blood plasma samples were taken at about 16.00 h, for plasma magnesium analysis, and faeces were collected in faecal bags (as described in Section II A) over a period of about 16 hours. Faeces were dried, ground and subsampled for magnesium and chromium analyses.

Table 4. Expt.2.3. Supplements fed to each group in each experimental period.

Period	1	2	3	4	5	6
Group						
(1)	A	D	G	K	N	S
(2)	B	E	H	L	P	T
(3)	C	F	J	M	Q	U
(4)	-	-	-	-	R	W

Table 5. Exot.2.3. Regression data and supplement availabilities

	(a) Mixed supplement Mg/Cr ratio	Regression of faecal Mg on faecal Cr: Correlation coefficient, r(and significance)	Regression coefficient b	SE of b (and significance)	$\frac{(a)-(b)}{(a)} \times 100\%$ Availability %	$\frac{SEb}{(a)} \times 100\%$ SE(availability)	Significance
A†	1.8567	0.9658***	1.5981	0.1827***	13.9	9.84	A<K**
B	"	0.9843***	1.6478	0.1204***	11.3	6.48	B<N,R*; K**
C	"	0.9580***	1.6288	0.1991***	12.3	10.72	C<K**
D	"	0.9933***	1.4711	0.0698***	20.8	3.76	D<R*; K**
E	"	0.9694***	1.8498	0.1911***	0.4	10.29	E<F,M,N,P,R*; K**
F	"	0.9852***	1.3648	0.0970***	27.4	5.22	F<K*
G	"	0.9794***	1.3641	0.1147***	26.5	6.18	G<K*
H	"	0.9692***	1.8049	0.1872***	2.8	10.08	H<N,P,R*; K**
J	"	0.9874***	1.5186	0.0993***	18.2	5.35	J<R*; K**
K	"	0.9702***	0.9945	0.1014***	46.4	5.46	
L	"	0.9911***	1.6214	0.0881***	14.3	4.74	L<N*; R**; K***
M	"	0.9718***	1.3183	0.1306***	29.0	7.03	
N	"	0.9873***	1.3085	0.0943***	29.5	5.08	N<K*
P	"	0.9622***	1.2620	0.1785***	32.0	9.61	
Q	"	0.9825***	1.3937	0.1171***	24.9	6.31	Q<K*
R	"	0.9790***	1.1636	0.1211***	37.3	6.52	
S	"	0.9634***	1.4318	0.1778***	22.9	9.58	
T	"	0.9171**	1.5282	0.1104***	17.7	5.95	T<R*; K**
U	"	0.9858***	1.3820	0.1055***	25.6	5.68	U<K*
W	"	0.9818***	1.3460	0.1302***	27.5	7.01	
						Mean SE=7.04	

† See Table 1

Results

The supplements were well consumed by all the sheep during the trial. Regression data for faecal magnesium on faecal chromium concentration for each supplementary treatment were calculated and are given in Table 5. Correlation coeffcients were high in each case and availabilities were calculated from the regression coefficients in the usual way (Experiments 2.1., 2.2.).

Apparent availability results are also presented in Figure 1. Of the "whole" commercial products, Spanish (R, 37.3%) is more available than Chinese gritty (P, 32.0%), Chinese granular (N, 29.5%) then Greek (Q, 24.9%), but these differences are not statistically significant. Of the 0.5 - 2.0 mm particle size ranges, Greek (E, 0·4%) is less available than Spanish (F, 27·4%) ($P<0.05$) and Chinese (D) is also more available at 20·8%. E and D are both less available than R ("whole" Spanish) at 37·3% ($P<0.05$), and all are considerably less than K (Chinese <75 µm, 46.4%) ($P<0.05$, 0·01). E (Greek, 0·5 - 2·0 mm) has the lowest availability of all supplements investigated. Of the 75 - 500 µm range, G, H and J (Chinese, Greek and Spanish) are 26·5%, 2·8% and 18·2% available, respectively, but the differences again are not quite statistically significant due to the relatively large standard errors. The Greek under 75 µm fraction (L, 14·3%) is significantly less available than the same Chinese fraction (K, 46·4%) ($P<0.001$) and the Spanish equivalent is intermediate (M, 29·0%).

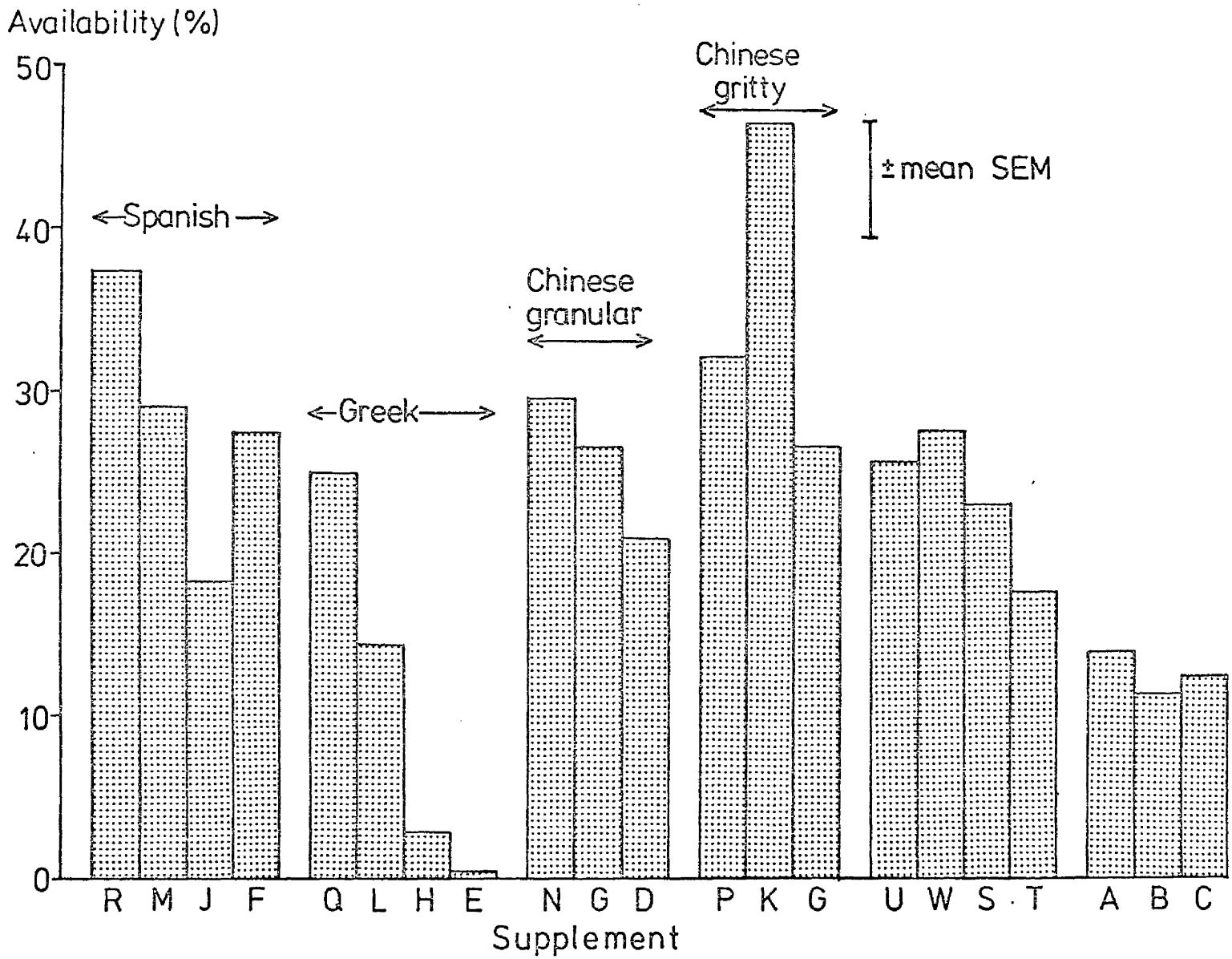
The English magnesium oxide powder (S, 22·9%) is more available than the two larger particle size fractions, between which there is little difference in availability (A, 13·9%; B, 11·3%). The comparable fraction to B, of natural magnesia, was also similarly available at 12·3% (C). Magnesium hydroxide (W) was 27·5% available, and dolomite was 17·7% (T) which was significantly less than K ($P<0.01$). American magnesium oxide (U) had similar availability to the English products S

Figure 1. Expt.2.3. Apparent availabilities (%) of magnesium supplements. Supplement notations are given in Table 1. For the four commercial products, availabilities are grouped in order:-

Spanish and Greek: "whole", <75, 75-500, 500-2,000 μm

Chinese granular: "whole", 75-500, 500-2,000 μm

Chinese gritty: "whole", <75, 75-500 μm


Availability (%)
50
40
30
20
10
0
Chinese gritty
± mean SEM
←Spanish→
Chinese granular
←Greek→
R M J F Q L H E N G D P K G U W S T A B C
Supplement

and W, at 25·6%.

The mean plasma magnesium concentration for sheep over all groups and periods receiving no supplementary magnesium was 0·82 mmol/l. All supplementary treatments except T raised the plasma magnesium level and the mean concentrations for sheep receiving different supplements are given in Table 6. Each sheep received a different amount of a given supplement and the plasma magnesium concentration was regressed on supplementary magnesium intake. The resulting correlation and regression coefficients for each treatment are given in Table 6. The regression coefficients, i.e. the slope of the increase in plasma magnesium content with increasing magnesium intake, did not correlate well with supplement availability ($r = 0.1649$, $b = 0.0007$). Similarly the mean plasma magnesium concentrations for each supplementary treatment did not correlate with availability ($r = -0.0247$, $b = -0.0001$).

Discussion

For the Greek and Chinese calcined magnesites there is a marked increase in availability with decreasing particle size, but this is surprisingly not the case in Experiment 2.3. for the Spanish product (see Figure 1). The availability of the Spanish coarse fraction (0·5 - 2·0 mm) is relatively high and the availability of the "whole" Spanish product appears high in relation to that of each of the constituent particle size ranges. The three ranges studied indeed constitute the whole of the Spanish and Chinese "gritty" products but 11% of the Greek product is over 2·0 mm, as is 14% of the Chinese granular product (see Table 2). The availability of the whole Greek product is also high in relation to its separate particle size availabilities, and the unaccounted 11% over 2·0 mm would actually be expected to have an even lower availability than the smaller particles studied. The Greek and Spanish "whole" product results are therefore

anomalous in relation to the individual availabilities of their different particle size ranges, but the availabilities of the whole Chinese products do relate more closely with those of the constituent particle size ranges (comparing whole gritty with under 75 μm and 75 - 500 μm, and whole granular with 75 - 500 μm and 0·5 - 2·0 mm).

Table 6. Expt.2.3. Mean plasma magnesium concentrations (mmol/l) and regression data for plasma magnesium on daily supplementary magnesium intake (g Mg/day).

Supplement	Mean plasma Mg concentration	Regression of plasma Mg concentration on daily supplementary Mg intake: Correlation coefficient r	Regression coefficient (b)
A	0.95	0.6302 NS	0.0262
B	1.04	0.8843**	0.1196
C	0.99	0.8567**	0.0581
D	0.93	0.0351 NS	0.0017
E	0.97	0.7011 NS	0.0526
F	0.99	0.9299***	0.0527
G	1.08	0.9327**	0.0827
H	1.05	0.6804 NS	0.0707
J	1.04	0.9160**	0.1012
K	1.04	0.9191**	0.1039
L	1.06	0.8684**	0.0977
M	1.02	0.9477***	0.0873
N	0.91	0.3865 NS	0.0279
P	0.98	0.6721 NS	0.0557
Q	0.94	0.1403 NS	0.0059
R	0.97	0.9232**	0.1011
S	0.97	0.7636*	0.0850
T†	0.82	-0.3997 NS	-0.0613
U	0.97	0.7848*	0.0510
W	0.98	0.9620**	0.1183

† Note that intakes (g Mg/day) of supplement T were considerably lower than those of other supplements (see Table 2).

The Chinese powdered fraction, under 75 μm, is the most available calcined magnesite in Experiment 2.3. (46·4%). It is considerably more available than either of the whole Chinese products (29·5% and 32·0%) and the equivalent fractions of the Spanish (29·0%) and Greek (14·3%) products. It is also more available than the powdered English sea water-derived, chemically reactive magnesium oxide (22·9%) and magnesium hydroxide (27·5%). The larger particles of sea-water oxide are less available than the powder, at 13·9% and 11·3% for 1 - 2 mm and 200 - 500 μm respectively, and the equivalent 250 - 500 μm Spanish (natural magnesia) fraction has similar availability, at 12·3%. This availability of 12·3% is low in comparison with the 1980 Spanish material results (F, M, J and R) but agrees fairly well with the result obtained in Experiment 1.1. for the same fraction (16·8%). The American brine-derived magnesium oxide, 150 - 500 μm particle size, appears more available (25·6%) than the equivalent English sea-water product (200 - 500 μm, 11·3%). Dolomite ($MgCO_3.Ca\ CO_3$) is 17·7% available which is perhaps high when compared with the virtual unavailability reported for magnesite ($MgCO_3$) (Experiments 1.2., 2.1.), but the carbonate is finely powdered in this trial thus possibly rendering it more available.

The Chinese fraction under 75 μm, and the granular and gritty products, as well as the Spanish whole product, all had availabilities greater than the mean value of 29·4% (for a range of feedingstuffs) quoted by the A.R.C.(1980), and could therefore be considered "good". The Greek fractions, English granular fractions and the Spanish fraction 250 - 500 μm (as used in Experiment 1.1., 1979 product) had availabilities below 17·0%, the lower decile value quoted by the A.R.C. (1980), and may therefore be considered "poor". However most supplements fell into the intermediate range 17·0 - 29·4% and could be regarded as "moderate".

Overall, of the calcined magnesites available commercially for animal feed purposes investigated here, the Greek product appears inferior to the Spanish and Chinese granular products, which were similar to each other, but the Chinese "gritty" product appears the best due to its smaller particle size. However, it must be appreciated that the random samples of these products used in this trial may not be truly representative of calcined magnesites from these particular countries and thus the relative merits of a particular country of origin should not be stated categorically from these results. For example, although the Spanish calcined magnesite is known to come from one particular quarry and calcining plant, this was not known for the Greek and Chinese products. However, it can certainly be concluded from the present trial that the source of a calcined magnesite can be important as well as particle size in determining availability. In addition the availabilities of sea-water and brine-derived magnesium oxides do not appear as high as their chemical reactivities would suggest.

In this trial mean plasma magnesium concentrations appeared virtually unrelated to supplement availability. However the increases in plasma magnesium with increased supplementary magnesium intake (i.e. regression coefficients, Table 6) were greater and more significant for all powdered magnesium oxides and hydroxide. However, for the granular fractions (0·5 - 2·0 mm), this relationship was only significant for the Spanish material and the regression coefficients were considerably lower than for the corresponding powders. The regression coefficients were of intermediate values for the medium (75 - 500 μm) particle size range. Overall, however, there was no correlation between these regression coefficients and supplement availability, unlike the findings of Experiment 2.2. Thus the blood data in this experiment were of little use in determining the relative merits of the various magnesium supplements.

SECTION III

ATTEMPTS TO INDUCE HYPOMAGNESAEMIA AND ITS ALLEVIATION BY DIETARY MAGNESIUM SUPPLEMENTS

Many workers have tried to produce hypomagnesaemia in experimental animals for a number of reasons, notably to study magnesium metabolism, absorption, pathogenesis of the condition, treatment and preventitive measures. It is well known that starvation or underfeeding and exposed environments can rapidly produce lowered serum magnesium concentrations in sheep and cattle (e.g. Christian & Williams, 1960; Herd, 1966; Lomba, Chauvaux & Bienfet, 1972). Other workers have successfully induced hypomagnesaemia by chemical treatments, e.g. adrenaline infusion in ewes (Rayssiguier, 1977), but dosing with urea or ammonium acetate failed to lower serum magnesium concentrations in wether sheep (Christian & Williams, 1960; Wilson, 1963). Dietary additions of lipids have in some cases resulted in decreased serum magnesium levels (Wind et al., 1966; Wilson et al., 1969) whereas other workers have reported no such effect (Grace & Body, 1979). However, the feeding of low magnesium diets has invariably induced some degree of hypomagnesaemia in appropriate ruminants (especially if lactating), although the "natural" cause of the condition is not always a simple dietary magnesium deficiency.

A. Low Magnesium Diets

Herbage containing less than 2 g Mg/kg DM is considered to be magnesium-deficient for adult ruminants (see Section I.3.). However, the Agricultural Research Council (A.R.C. 1980) quote magnesium requirements as dietary concentrations (assuming metabolisability of the dietary energy, q, to be 0·6) for pregnant or lactating ewes as 1·0 - 1·7 g Mg/kg DM, for growing wether sheep as 0·8 - 1·5, for pregnant or lactating cows as 1·4 - 2·1, and for growing beef cattle as 1·0 - 2·1 g Mg/kg DM. Thus a "low magnesium" diet depends on the production requirements of particular animals but may generally be assumed to contain under 1 g Mg/kg DM.

Natural feedstuffs generally contain sufficient magnesium and much attention has centred on the use of artificial foods which are markedly magnesium-deficient. However, a high proportion of purified starchy foods in the diet results in digestive disturbances which may lead to scouring, loss of appetite, and consequent decreased production. In addition artificial foods may be unacceptable to the animal. Normal roughages are moderately rich in magnesium and research workers at the National Institute for Research in Dairying have reported that paper pulp, shredded and treated with molasses or invert sugar, and dried, is a satisfactory low magnesium roughage for dairy cows (Rook & Campling, 1959) although some individual cows found it relatively unacceptable. They have achieved dietary magnesium concentrations as low as 0·1 - 0·2 g Mg/kg DM (Storry & Rook, 1963) using paper pulp with maize gluten, or paper pulp with wheat straw and flaked maize or a maize-based concentrate mix (Rook, 1961; Rook, 1963; Rook, Campling & Johnson, 1964). By feeding such diets to dairy cows they have reported marked decreases in serum magnesium, with urinary magnesium excretion dropping to zero, and with clinical hypomagnesaemic tetany occurring in some cases.

Tomas and Potter (1976a) used tissue paper with starch, casein, and molasses as a low magnesium diet (0·2 - 0·3 g/kg DM) for sheep, though it was not always readily consumed, and Suttle and Field (1969) employed oat hulls together with starch and sugar as a semi-purified diet for ewes (0·5 g Mg/kg DM).

Maize products have been widely used as they are generally low in magnesium e.g. maize cobs 0·7, maize gluten meal 0·5 (N.R.C., 1971), flaked maize c.1·0 g Mg/kg (S.A.C., 1978). However it has been reported that the inclusion of flaked maize in a diet increases the efficiency of dietary magnesium utilisation (Rook, 1961; Rook & Campling, 1962). Gerken and Fontenot (1967) and O'Kelley and Fontenot (1969) devised diets based on maize cobs, ground shelled maize and maize gluten meal

for steers (0·9 - 1·2 g Mg/kg DM), and McAleese and Forbes (1959) used maize cobs, sugar and soya protein for lambs (0·2 g Mg/kg DM). Maize starch has also been successfully employed in a number of diets, for example (i) with wheat straw for lactating ewes, 0·1 - 0·3 g Mg/kg DM (L'Estrange & Axford, 1964); (ii) with glucose monohydrate and cellulose for wethers, c.0·5 g Mg/kg DM (Ammerman et al. 1972; Chicco et al., 1972); (iii) with ground, over-ripe Timothy hay, in a wet mash for wethers (House & Mayland, 1976a); (iv) with washed straw and cellulose powder, 0·04 - 0·06 g Mg/kg DM (Strachan & Rook, 1975; Allsop & Rook, 1979).

Most of the diets mentioned include a magnesium-free mineral/trace element/vitamin mix, and possibly additional synthetic protein (such as casein), non-protein nitrogen (such as urea), fats (such as maize oil or soya oil) and sugars. Thus whether semi-purified or completely artificial, these diets aim to be well balanced nutritionally in all respects except for the magnesium deficiency.

Experiment 3.1. An assessment of the ability of two experimental low magnesium diets to induce hypomagnesaemia in ewes, and its treatment with two grades of calcined magnesite of different particle size.

Introduction

The present experiment describes two low magnesium diets which were fed to lactating ewes in an attempt to reduce their plasma magnesium concentrations in order to investigate the relative efficacies of different calcined magnesites in restoring plasma magnesium. In addition to an assessment of their ability to induce hypomagnesaemia, it was important to ascertain the acceptability of the diets to the sheep, as reasonable food intakes must be maintained in order to maintain levels of production (i.e. milk yield in this trial).

Table 1. Expt.3.1. Experimental Diets.

(i)	(ii)
1 kg barley husk siftings	0·5 kg barley straw
1 kg sugar	1 kg sugar
60 g urea	
40 g dicalcium phosphate	
20 g salt	
10 g limestone	
6 g vitamins/trace element mix	

Experimental

Part (i)

In a preliminary 21-day trial, five lactating Finn Dorset ewes, weighing about 60 kg and each with twin lambs aged 6-8 weeks, were individually penned indoors. They were offered 2 kg/head/day of diet (i), which comprised a mixture in equal proportions of sugar (sucrose) and barley husk siftings, together with urea and appropriate minerals and vitamins (Table 1). This provided daily about 17 MJ metabolisable energy (ME) and 180 g digestible crude protein (DCP) which was considered adequate (A.R.C., 1980), but provided only about 0·8 g magnesium which was considerably below the A.R.C. (1980) recommended dietary allowance of about 2 g/day. Initially, small quantities of crushed barley were mixed with the diet to aid acceptance by the sheep. Blood samples were taken every 3 days for plasma magnesium and calcium analysis.

Part (ii)

An additional eighteen lactating ewes, mainly Greyface, again weighing about 60 kg and each with twin lambs aged about 8 weeks, were individually penned alongside the original five. The 23 sheep were then

offered 1 kg/day of sugar (plus urea, vitamins and minerals) and 0·5 kg/day barley straw (diet (ii), Table 1). This provided 19 MJ ME and 180 g DCP daily, but only about 0·35 g magnesium which was considerably less than the A.R.C. (1980) recommended daily allowance. The sheep were divided into three groups for the 11-day trial, in a randomised block design, and the three experimental treatments were:

Group (a) no supplementary magnesium (7 sheep).

Group (b) 0·200 g Mg/day as Spanish calcined magnesite 500 - 1,000 μm (8 sheep).

Group (c) 0·200 g Mg/day as Spanish calcined magnesite 0 - 150 μm (8 sheep).

The diet was given as 0·5 kg sugar mix at approximately 07.30 h and 0·5 kg sugar mix plus the 0.5 kg straw at about 16.00 h, and initially the supplements were added to the surface of the morning feed. However from day 4 onwards, when some ewes failed to consume all of their allocated sugar ration, all supplements were drenched into the sheep, at the morning feed, in a minimal quantity of water. The sheep were bled at the start of the experiment on day 7, and finally on day 11, each time at about 10.00 h.

Table 2. Expt.3.1. Mean plasma magnesium and calcium concentrations (mmol/l) of the five sheep in Part (i) of the experiment.

Day	0	3	6	9	12	15	18	21
Mg	1.73	1.05	0.75	0.76	0.84	0.82	0.84	0.77
SEM	0.181	0.115	0.029	0.061	0.078	0.053	0.078	0.060
Ca	2.00	2.08	2.68	2.42	2.45	2.56	2.49	2.36
SEM	0.126	0.148	0.078	0.029	0.018	0.034	0.043	0.044

Results

Part (i)

Table 2 shows the mean plasma magnesium and calcium levels for the five ewes during the preliminary 21-day trial. There was a large fall in magnesium concentration during the first 6 days (1·73 to 0·75 mmol/l), with a simultaneous rise in calcium concentration (2·00 to 2·68 mmol/l). The initial mean magnesium concentration of 1·73 mmol/l was exceptionally high, the "normal" range being about 0·74 - 1·43 (see Section I) as the sheep, it was revealed, had previously been fed magnesium-rich cattle cobs (17·8 g Mg/kg DM). After 6 days the plasma magnesium values did not decrease further, remaining between 0·75 - 0·84 mmol/l, still within the normal range. After the first week the ewes started to leave food residues and the average intake was down to about 1·8 kg/day.

Part (ii)

The mean plasma magnesium and calcium concentrations are given in Table 3. Plasma magnesium concentrations dropped in all groups over the 11 days but this fall was greatest for group (a) (nil supplement) from 0.88 to 0·59 mmol/l, intermediate for group (b) (coarse supplement, 500 - 1,000 μm calcined magnesite) from 0·77 to 0·63 mmol/l, and least for group (c) (fine supplement 0 - 150 μm) from 0·84 to 0·77 mmol/l. Plasma calcium concentrations rose slightly as the magnesium fell but there were no significant differences between different treatments, values remaining in the fully normal range 2·24 - 2·58 mmol/l. Several sheep failed to consume all of their sugar ration, intakes falling in some cases to 0·5 kg/day, though all sheep readily consumed their total straw allocation.

Table 3. Expt.3.1. Mean plasma magnesium and calcium concentrations (mmol/l) for the groups of sheep in Part (ii) of the experiment.

Magnesium

Treatment	(a)	(b)	(c)	SEM	Significance
	Nil	Coarse MgO	Fine MgO		
Day 0	0.88	0.77	0.84	0.064	NS
Day 7	0.55	0.67	0.76	0.111	NS
Day 11	0.59	0.63	0.77	0.068	NS
Drop from day 0 to 11	-0.33	-0.14	-0.08	0.061	(a)>(b)(c)*

Calcium

Treatment	(a)	(b)	(c)	SEM
Day 0	2.35	2.32	2.30	0.043
Day 7	2.38	2.39	2.24	0.038
Day 11	2.53	2.53	2.58	0.034

Discussion

Diet (i), which comprised a sugar/barley siftings mix, was difficult to mix evenly and was not always readily consumed by all sheep. In addition there was little change in plasma magnesium concentrations in sheep receiving this diet from six to twenty-one days, values remaining well within the normal range, and thus the diet failed to induce hypomagnesaemia.

Diet (ii), which comprised sugar and barley straw, was more successful in producing sub-normal plasma magnesium concentrations within just a few days (as shown by the unsupplemented group of sheep, (a)), but no cases of clinical tetany were observed. A similar diet consisting of barley straw, sugar and casein, providing 0·20 g Mg/day, has previously been reported to have induced hypomagnesaemia and even one case of clinical

tetany in non-pregnant, non-lactating ewes (Ritchie, Hemingway, Inglis & Peacock, 1962). However in the present trial, as the sugar ration on the whole was poorly consumed, it was probable that the fall in plasma magnesium concentrations was also due to serious underfeeding, and the ewes noticeably lost condition during the trial. This was acceptable for a short-term experiment but it is evident that this particular diet would be unsatisfactory for use over long periods for productive animals, maintaining a realistic level of production. The total diet was neither very fibrous nor bulky as the straw allocation had to be minimised to maintain low magnesium intake, and this probably contributed to the decline in appetite.

However, whether the total diet was consumed or not, the ewes became hypomagnesaemic and coarse particles of calcined magnesite (500-1,000 μm particle size) failed to maintain plasma magnesium concentrations, whereas fine particles (0 - 150 μm) did maintain plasma magnesium within the normal range. The only statistically significant difference ($P<0.05$) was that both supplementary treatments resulted in a lower drop in plasma magnesium from day 0 to day 11 than the zero supplement treatment. From Table 3 there appears to be a definite trend for fine particles to be better than coarse particles of calcined magnesite in raising plasma magnesium concentration.

Experiment 3.2. A brief assessment of a low magnesium diet based on maize constituents.

Introduction

The experimental low magnesium diets reported earlier (Experiment 3.1.) proved unsatisfactory and the aim now was still to devise a realistic diet to support ewes over long periods during pregnancy and lactation. It was considered preferable to use entirely natural

feedingstuffs, despite claims that this increases dietary magnesium availability (Rook et al., 1964), as these would be generally more acceptable to the animal and should produce a more "natural" rumen fermentation. Maize products are known to be low in magnesium and an experimental diet consisting of flaked maize, maize dark grains and barley husk siftings was investigated as each of these components was also readily obtainable. The present experiment describes a preliminary feeding trial with non-productive ewes to observe the acceptability of such a diet and to assess the feasibility of using it on a larger scale. Maize dark grains have a strong flavour and could be unpalatable if included in large proportions in a diet, whereas flaked maize is generally palatable. Barley siftings are included to supply roughage but the rate of inclusion may be critical to avoid scouring, without unduly raising the magnesium content of the diet.

Experimental

Eight non-pregnant, non-lactating Greyface ewes were individually penned indoors for the 25-day trial and were offered, in roughly equal portions morning and afternoon, a loose-mixed low magnesium diet. The three major dietary constituents were flaked maize, maize dark grains (from Girvan Distillery) and barley husk siftings (from the Highland Distilleries Company Ltd., Morayshire) (see Table 1). A mix in the ratio 3 : 3 : 2 (respectively) was fed at 800 g/sheep/day for 5 days, and then a 3 : 3 : 1 mix at 700 g/sheep/day for 13 days. The sheep were then divided so that four received 500 g/day while the four others were fed 1400 g/day (of the 3 : 3 : 1 mix) for 7 days until the end of the experiment.

Observations were made on the acceptability of the diet, and all ewes were bled on days 0, 7, 12, 18 and 25 of the experiment, for plasma magnesium and calcium determinations.

Table 1. Expt.3.2.

(a) Analysis of dietary components (g/kg DM)

	Flaked maize	Maize dark grains	Barley siftings
Dry matter (g/kg FM)	839.9	881.6	850.5
Crude Protein	94.2	357.4	44.7
Crude Fibre	23.2	150.9	357.0
Ether Extract	13.1	56.7	7.7
Ash	8.2	20.2	85.4
Mg	0.558	0.566	0.659

(b) Dietary ratios of constituents

(i) Flaked maize : dark grains : siftings = 3 : 3 : 2 } plus 1% limestone 1% salt

(ii) " " " 3 : 3 : 1 }

(c) Approximate composition of total diets (per kg DM)

	MJ ME	g CP	g Mg
(i)	11	180	0.59
(ii)	12	200	0.58

Results

The diet, at each mix ratio and level of feeding, was readily consumed by all sheep throughout the trial. There was no obvious scouring due to lack of roughage when barley siftings were included in the diet at the lower rate (c.14% in 3 : 3 : 1 mix) although the faeces were sometimes noticeably soft.

The blood data are given in Table 2. Plasma magnesium decreased from an initial mean of 0·80 mmol/l to 0·66 mmol/l in 7 days and was still at 0·65 mmol/l after 12 days. It increased slightly to 0·75 mmol/l

on the eighteenth day, when the ewes were divided, and the plasma magnesium of those receiving 1400 g diet/day increased to 0·82 while that of the sheep receiving only 500 g/day decreased to 0·65 mmol/l.

Plasma calcium concentrations varied slightly in parallel with the variations in plasma magnesium, but values remained between 2·35 and 2·64 mmol/l which is well within the normal range (e.g. Allsop, 1947, quotes that values under 8·4 mg %, i.e. 2·1 mmol/l, may be considered hypocalcaemic).

Table 2. Expt.3.2. Mean plasma magnesium and calcium concentrations (mmol/l).

	mean plasma Mg ± S.D.		mean plasma Ca ± S.D.	
day 0	0.80	0.133	2.64	0.184
day 5	Change in diet mix			
day 7	0.66	0.188	2.39	0.163
day 12	0.65	0.103	2.35	0.072
day 18	0.75	0.129	2.58	0.115
	Change in feeding levels			
	0.5 kg/day	1.4 kg/day	0.5 kg/day	1.4 kg/day
day 25	0.65±0.148	0.82±0.049	2.41±0.099	2.52±0.111

Discussion

The loose mixed diet composed of flaked maize, maize dark grains and barley siftings is evidently quite acceptable and palatable to sheep and would therefore seem to be very promising for longer-term feeding trials. Plasma magnesium levels of the non-productive sheep in this trial decreased only slightly, but the initial mean of 0·8 mmol/l was fairly low ("normal" range c.0·74 - 1·32 mmol/l, see Section I). It

was therefore considered likely that highly productive sheep such as ewes at peak lactation with twin lambs, with a heavy magnesium demand, might well develop hypomagnesaemia, and possibly clinical tetany on such a diet. In addition, the three constituents admixed relatively easily to give a uniform mixture which did not separate out with storage. Thus the diet would be practical for the production of large quantities to maintain appropriate numbers of sheep over long periods.

Experiment 3.3. An attempt to induce hypomagnesaemia in ewes given a low magnesium diet during pregnancy and lactation, and its alleviation by various dietary magnesium supplements.

Introduction

The low magnesium dietary constituents used in Experiment 3.2. appeared to be totally acceptable to sheep and together could provide a well balanced diet with respect to energy and protein. The present experiment used a diet containing equal proprtions of the three constituents, flaked maize, maize dark grains and barley siftings, fed to ewes throughout the latter part of gestation and for nine weeks of lactation. It was intended to induce hypomagnesaemia in the lactating ewes and to then study the relative efficacies of different dietary inorganic magnesium supplements in raising plasma magnesium concentrations. The diet was in this trial severely deficient in magnesium in relation to the high magnesium requirement of a productive lowland ewe, especially in view of the fact that the diet was otherwise balanced to produce and maintain high levels of production (i.e. normal lamb birth weights and milk yields). Thus the likelihood of producing hypomagnesaemia in the ewes was greater than in the previous preliminary trial (Experiment 3.2.). The use of entirely natural feedingstuffs

(maize and barley derivatives) should result in a "normal" rumen fermentation pattern, and hence the results obtained for supplementary magnesium should relate well to the practical situation. In addition to blood data, availability data on each supplement was obtained using the chromium faecal marker technique (described in Section II B).

Ritchie and Hemingway (1963) reported that when grazing lactating ewes were drenched daily with up to 4 g magnesium as magnesium oxide, plasma magnesium concentrations were not raised, as measured by samples taken 24 hours after administration of the supplement. However, a transient elevation was detected, peaking at 4 hours after drenching, which was not sustained for the full 24 hour period between drenches. In the present experiment, therefore, with lactating ewes indoors, it was decided to take blood samples serially during a 24-hour period in addition to samples taken regularly at 24 hours after administration of supplementary magnesium.

Materials and Methods

Magnesium supplements

The seven magnesium supplements used in this trial are listed, along with their magnesium contents, in Table 1. Three particle size grades of Spanish calcined magnesite (trade name Agma FG85) were separated out: "fine", under 75 µm; "medium", 150 - 250 µm; and "coarse", 500 - 1,000 µm (supplements F, A and C respectively). In addition a sample of the "medium" calcined magnesite was subjected to a temperature of 1300°C for 2 hours in a small furnace in order to "dead-burn" the material and supposedly render it less reactive (supplement B). (This supplement was dark reddish-brown in colour and had an extremely low L.O.I. of 0·03%). The waste product from the Spanish calcination plant, "cyclone dust" (TBH powder) was included in the trial (see Section I 4) as supplement T. Lastly two further magnesium salts were investigated, to compare with

the oxides, namely magnesium phosphate (a mixture of particle sizes with 80% 250 - 1,000 μm, supplied by Boliden, Sweden) and finely powdered magnesium hydroxide (trade name Lycal 93HS, Steetley Minerals Ltd.), supplements P and L, respectively.

Table 1. Exot.3.3. Magnesium supplements.

	Supplement	g Mg/kg
F	Fine Spanish Calcined magnesite (under 75 μm)	506.7
C	Coarse Spanish calcined magnesite (500 - 1,000 μm)	524.1
T	Spanish cyclone dust (powder)	428.3
P	Magnesium phosphate	210.0
L	Magnesium hydroxide (powder)	376.9
A	Medium Spanish calcined magnesite (150 - 250 μm)	527.1
B	Dead-burnt medium Spanish Calcined magnesite	531.8

Experimental design

Thirty-six pregnant ewes (mainly Greyface) with mean liveweight 70 kg were housed from about 5 weeks before lambing and were group-fed 1·5 kg/head/day of the experimental low magnesium diet, given in two equal portions at 07.00 h and 16.00 h. The diet was a loose mix of flaked maize, maize dark grains and barley siftings in equal proportions (mean analyses given in Table 2), with 1% limestone, 1% dicalcium phosphate, 1% salt and 0·3% vitamin/trace element mix added, and containing about 11 MJ ME, 165 g CP and 0·59 g Mg/kg DM. All the sheep were bled at weekly intervals until lambing.

Table 2. Expt.3.3. Analysis of dietary components (g/kg DM).

	Flaked maize	Maize dark grains	Barley siftings
Dry matter (g/kg FM)	839.9	881.6	850.5
Crude Protein	94.2	357.4	44.7
Crude Fibre	23.2	150.9	357.0
Ether Extract	13.1	56.7	7.7
Ash	8.2	20.2	85.4
Mg	0.558	0.566	0.659

At lambing, each ewe was individually penned and bled at 1, 4, 7 and 10 days post-parturition. At between 1 - 2 weeks post-parturition, when a drop in blood magnesium had been established the ewes were divided, according to their previous blood magnesium patterns and to whether they had twins or singles, into three similar groups of twelve†, and magnesium supplementation of the basal ration (still given at 1·5 kg/sheep/day) commenced. Details of the supplements fed to each group in each experimental period are given in Table 3. In the first period of supplementation each ewe received 0·5 g Mg day but thereafter received 1 g Mg daily as a given supplement. Supplements were added to the surface of the morning feed at 07.30 h, and throughout the five periods of supplementation the ewes were bled every three days at 08.00 h. On one day in each period, the ewes were bled serially several times over 24 hours, and the times of day at which these samples were taken are given

†The number per group varied between nine and twelve throughout the trial as some ewes lambed later than others and therefore started magnesium supplementation later, and some other ewes had to be withdrawn from the experiment before its completion.

in Table 4. All blood samples were analysed for plasma magnesium and calcium concentrations.

At the start of the third period of supplementation, about 5 weeks after lambing, the quantity of basal diet fed per ewe was increased to 2 kg/day (given in equal protions morning and afternoon), in order to maintain ewe milk production and lamb growth rates. The amount of magnesium supplied daily by the basal diet alone is given in Table 3 along with the Agricultural Research Council (1980) recommended daily requirements (and allowances) of magnesium for this class of stock.

Table 3. Exot.3.3. Supplements given to the three groups of ewes in the five experimental periods.

Period of supplementation	1	2	3	4	5
Duration of period (days)	15	9	9	9	9
g Mg/ewe/day from supplement	0.5	1.0	1.0	1.0	1.0
Group 1	†O	O	F	P	O
Group 2	F	F	O	L	A
Group 3	C	C	T	O	B
g Mg/ewe/day from basal diet	0.76	0.76	1.02	1.02	1.02
Daily requirement (and dietary allowance), g Mg. A.R.C. 1980 ††	1.34(2.32) - 1.92(3.32)				

†O = No supplement (controls)

††Requirement (and allowance) prior to lambing 1.00(1.72) — 1.13(1.90) g Mg/day

Table 4. Exot.3.3. Pattern of serial bleeding times.

Period	Day	Times of bleeding (h)								
1	15		Feed & supplement	08.00		12.00			Feed alone	
2	6	07.00		08.00	10.00	12.00	14.00	16.00		
3	6			08.00	10.00	12.00		16.00		20.00
4	9			08.00	10.00	12.00		16.00		20.00
5	9			08.00	10.00	12.00		16.00		20.00

Chromic oxide was added to each supplement as an indigestible faecal marker, in the ratio 0·400 g chromic oxide (673·3 g Cr/kg) to 1·0 g magnesium. Faeces "grab" samples were taken from each ewe, after one week on a given supplement, and were dried, ground and analysed for magnesium and chromium, to provide availability data.

All lambs were weighed at birth and again at approximately 6 weeks.

Results

Blood data : (i) Plasma magnesium concentrations

1. Changes before the start of supplementation (Table 5, Figure 1).

The mean plasma magnesium concentration decreased from 0·82 to 0·67 mmol/l in one week on the experimental diet, and then gradually increased over the next four weeks back to 0·82 mmol/l just before lambing. Immediately after lambing, the plasma magnesium concentration dropped to 0.51 mmol/l in one week before beginning to rise again by two weeks post-lambing (0·55 mmol/l) when dietary magnesium supplementation commenced.

Table 5. Expt.3.3. Mean plasma magnesium concentrations (mmol/l) of all ewes during gestation and after lambing up to the start of supplementation.

Weeks on Experimental Diet		Mg	SEM
Before lambing	0	0.82	0.021
	1	0.67	0.028
	2	0.72	0.028
	3	0.75	0.023
	4	0.79	0.023
	5	0.82	0.020
After lambing	1 day	0.65	0.029
	4 day	0.57	0.024
	7 day	0.51	0.024
	10 day	0.51	0.025
(Day 0 supplementation)	14 day	0.55	0.026

2. Changes after the start of supplementation

a) Blood samples taken 24 h after administration of supplements (Table 6, Figure 1).

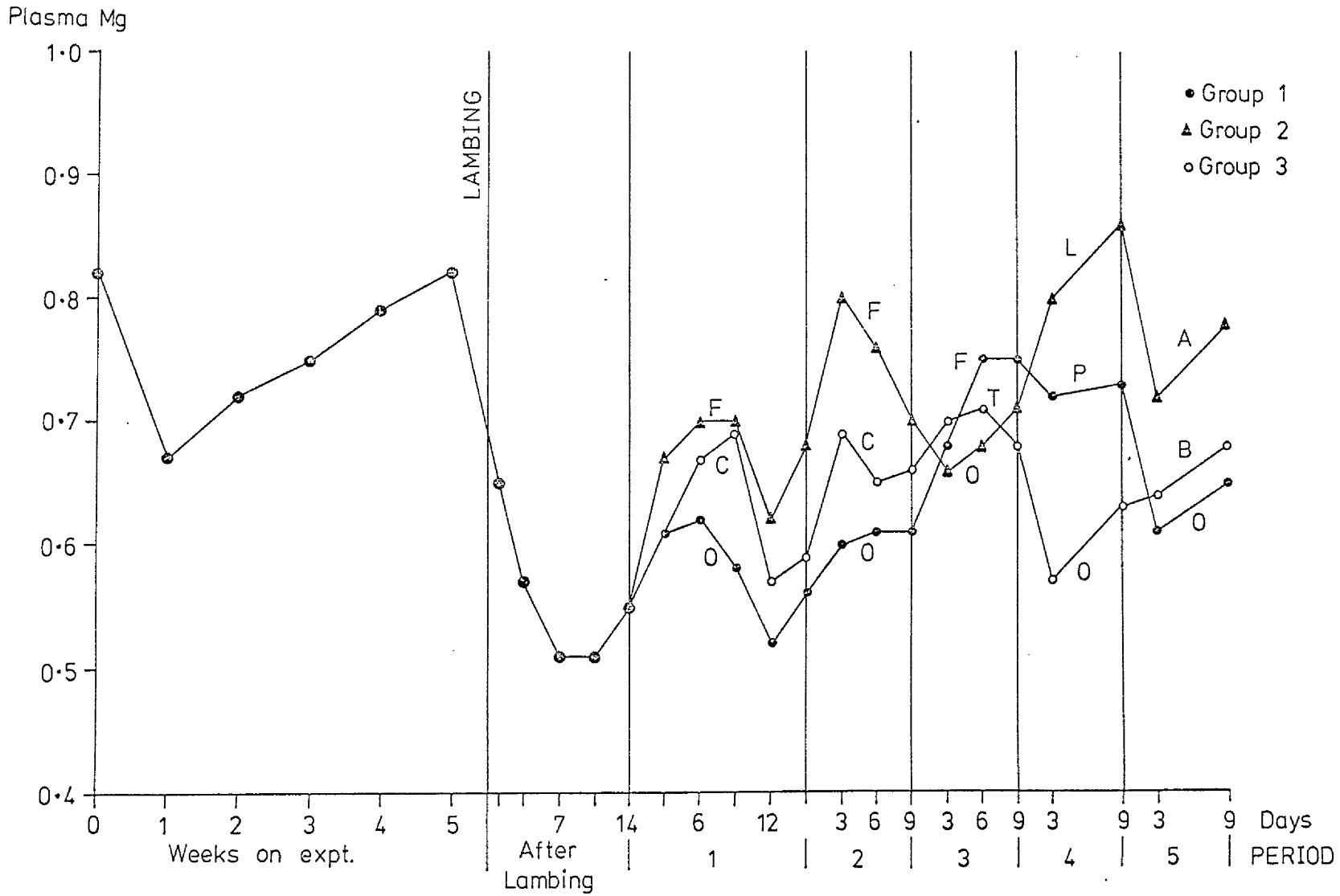
In Period 1, when only 0·5 g Mg/ewe/day was given, differences between the mean plasma magnesium concentrations for the three groups were small. Only after fifteen days did the group receiving fine Spanish calcined magnesite (C.M.) have a significantly higher plasma magnesium value ($P<0·05$) than the control group, while the coarse C.M. group remained low. In Period 2, when the same groups were receiving the same supplements as in Period 1 at 1·0 g Mg/ewe/day, differences in mean plasma magnesium concentrations were slightly greater, with that of the group given fine C.M. (F) being higher than that of the control (unsupplemented) group after 3($P<0·01$) and 6($P<0·05$) days, but the differences were no longer significant after 9 days. In Period 3, there

Table 6. Expt.3.3. Mean plasma Mg levels (mmol/l) after the start of supplementation (Blood samples taken 08.00 h). See Table 1 for key to treatment notations.

Group	1 Mg	1 SEM	2 Mg	2 SEM	3 Mg	3 SEM	Significance
Period 1	O		F		C		
Day 3	0.61	0.044	0.67	0.050	0.61	0.043	NS
6	0.62	0.043	0.70	0.058	0.67	0.039	NS
9	0.58	0.047	0.70	0.043	0.69	0.047	NS
12	0.52	0.045	0.62	0.052	0.57	0.041	NS
15	0.56	0.034	0.68	0.039	0.59	0.039	F>O*
Period 2	O		F		C		
3	0.60	0.035	0.80	0.041	0.69	0.038	F>O**
6	0.61	0.047	0.76	0.037	0.65	0.045	F>O*
9	0.61	0.046	0.70	0.027	0.66	0.039	NS
Period 3	F		O		T		
3	0.68	0.039	0.66	0.042	0.70	0.035	NS
6	0.75	0.034	0.68	0.043	0.71	0.044	NS
9	0.75	0.029	0.71	0.048	0.68	0.041	NS
Period 4	P		L		O		
3	0.72	0.042	0.80	0.047	0.57	0.041	L>O**, P>O*
9	0.73	0.043	0.86	0.039	0.63	0.047	L>O**
Period 5	O		A		B		
3	0.61	0.047	0.72	0.031	0.64	0.036	NS
9	0.65	0.055	0.78	0.038	0.68	0.038	NS

Figure 1. Expt.3.3. Mean plasma magnesium concentrations (mmol/l) of all ewes until the start of supplementation, and of the three groups of ewes receiving different supplements in Periods 1 to 5†. (Blood samples taken at 08.00 h)

†See Tables 1 and 3.


Plasma Mg
1·0
0·9
0·8
0·7
0·6
0·5
0·4
LAMBING
Group 1
Group 2
Group 3
F
C
O
F
C
O
F
T
O
L
P
O
A
B
O
0 1 2 3 4 5 7 14 6 12 3 6 9 3 6 9 3 9 3 9 Days
Weeks on expt.
After Lambing
1
2
3
4
5
PERIOD

were no significant differences between control, cylcone dust (T) and fine C.M. (F) treatments, and similarly in Period 5, between controls, medium C.M. (A) and "dead-burnt" medium C.M. (B). In Period 4, however, the group receiving magnesium hydroxide (L) had a higher mean plasma magnesium concentration than the control group ($P<0.01$) after 3 and 9 days, and the magnesium phosphate (P) supplemented group also had a higher value than the controls ($P<0.05$) on day 3.

Over the duration of the experiment after the start of supplementation, there was a general tendency for plasma magnesium levels to increase gradually, but values remained largely below, or very slightly above, the lower limit of the normal range. The control group (1) in Periods 1 and 2 had never received dietary magnesium supplementation and the mean plasma magnesium concentration increased gradually from 0·51 mmol/l at 1 week post-lambing to 0·62 mmol/l at about 3 weeks, thereafter remaining between 0·52 and 0·61 mmol/l until the end of Period 2. At the start of Period 3 the basal ratio allowance was increased, so that the basal magnesium intake was raised from 0·76 to 1·02 g/ewe/day, and indeed the mean plasma magnesium value of the new control group (2) did remain higher in this Period. A different group acted as control in each of Periods 3, 4 and 5, but the mean plasma magnesium concentration in each case fell within three days of the withdrawal of supplementary magnesium.

b) Serial blood samples taken over 24 h in each Period (Table 7, Figure, 2)

Period 1. Mean plasma magnesium concentration was raised at 12.00 h for all groups, and for the groups receiving fine C.M. (F) and coarse C.M. (C) this rise was significant ($P<0.05$), being 0·15 and 0·11 mmol/l, respectively. Thus, although only the fine C.M. group had a higher plasma magnesium value than the controls at 08.00 h ($P<0.05$), both the fine C.M. ($P<0.01$) and coarse C.M. ($P<0.05$) groups were shown to be higher at 12.00 h.

Table 7. Expt.3.3. Mean plasma magnesium concentrations (mmol/l) of ewes taken serially over one day in each period of supplementation.

PERIOD 1 (Day 15)

Group	08.00h	SEM	12.00h	SEM	Significance of 08.00 v. 12.00h comparison
(1) O	0.56	0.034	0.66	0.037	NS
(2) F	0.68	0.039	0.83	0.036	12>8*
(3) C	0.59	0.039	0.70	0.034	12>8*
Significance (supplements comparison)	F>O*		F>O**, C*		

PERIOD 2 (Day 6)

Group	07.00h	08.00	SEM	10.00	12.00	SEM	14.00	16.00	Significance of 08.00 v. 12.00 comparison
(1) C	0.63	0.60	0.049	0.69	0.71	0.042	0.66	0.64	NS
(2) F	0.78	0.79	0.026	0.96	1.01	0.033	0.97	0.93	12>8**
(3) C	0.67	0.67	0.047	0.80	0.86	0.039	0.81	0.80	12>8*
Significance (supplements comparison)		F>O*			F>O***, C* C>O*				

PERIOD 3 (Day 6)

Group	08.00h	SEM	10.00	12.00	SEM	16.00	20.00	Significance of 08.00 v. 12.00 comparison
(1) F	0.75	0.034	0.95	1.00	0.031	0.93	0.90	12>8***
(2) O	0.68	0.043	0.79	0.84	0.036	0.76	0.79	12>8*
(3) T	0.71	0.044	0.90	0.91	0.035	0.83	0.82	12>8**
Significance (supplements comparison)	NS			F>O**				

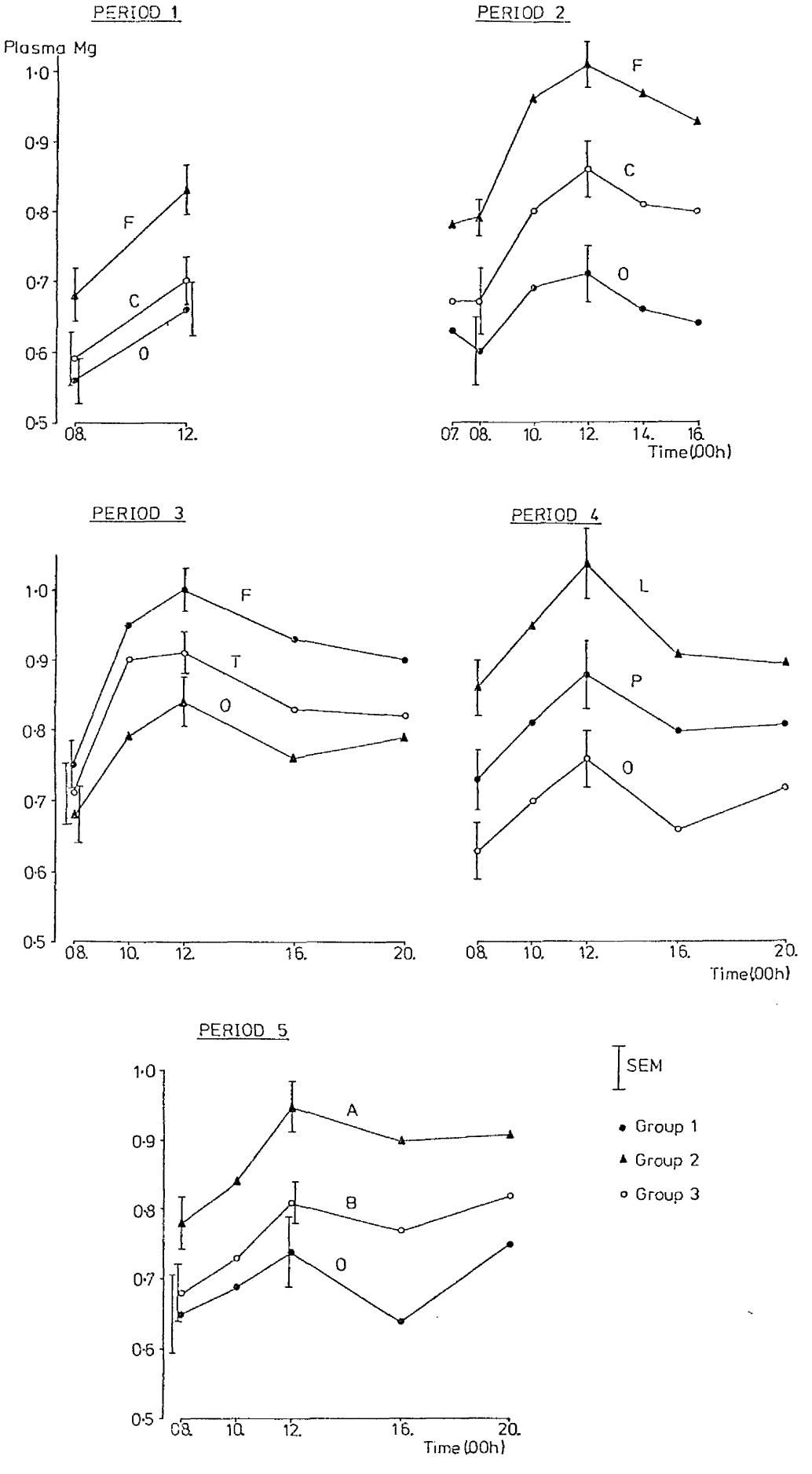
PERIOD 4 (Day 9)

Group	08.00h	SEM	10.00	12.00	SEM	16.00	20.00	Significance of 08.00 v. 12.00 comparison
(1) P	0.73	0.043	0.81	0.88	0.047	0.80	0.81	12>8*
(2) L	0.86	0.039	0.95	1.04	0.052	0.91	0.90	12>8*
(3) O	0.63	0.047	0.70	0.76	0.040	0.66	0.72	12>8*
Significance (supplements comparison)	L>O**			L>O***, P*				

PERIOD 5 (Day 9)

Group	08.00h	SEM	10.00	12.00	SEM	16.00	20.00	Significance of 08.00 v. 12.00 compairson
(1) O	0.65	0.055	0.69	0.74	0.049	0.64	0.75	NS
(2) A	0.78	0.038	0.84	0.95	0.036	0.90	0.91	12>8**
(3) B	0.68	0.038	0.73	0.81	0.032	0.77	0.82	12>8*
Significance (supplements comparison)	NS			A>O**, B*				

Figure 2. Expt.3.3. Mean plasma magnesium concentrations (mmol/l) of the groups of ewes from blood samples taken serially during one day in each Period. (Note: supplemented feed given at 07.30 h and unsupplemented feed 16.00 h).


PERIOD 1
Plasma Mg
1·0
0·9
0·8
0·7
0·6
0·5
F
C
O
08.
12.
PERIOD 2
F
C
O
07.
08.
10.
12.
14.
16.
Time(00h)
PERIOD 3
F
T
O
08.
10.
12.
16.
20.
PERIOD 4
L
P
O
08.
10.
12.
16.
20.
Time(00h)
PERIOD 5
A
B
O
08.
10.
12.
16.
20.
Time (00h)
SEM
Group 1
Group 2
Group 3

Period 2. All groups showed negligible change in plasma magnesium concentration immediately after feeding and administration of supplements (i.e. 07·00 v. 08·00 h) but all showed an increase at 12.00 h, the rise being significant for the fine C.M. (F) group (up by 0·23 mmol/l ($P<0·001$)) and for the coarse C.M. (C) group (0·19 mmol/l ($P<0·05$)). At 08.00 h only the fine C.M. treatment was better than the controls ($P<0·05$), but at 12.00 h fine C.M. was shown to be better than both the controls ($P<0·001$) and coarse C.M. ($P<0·05$), and coarse C.M. was better than the control group ($P<0·05$). Plasma magnesium values in all groups decreased gradually after 12.00 h but were not quite as low as the 08.00 h levels by 16.00 h when the ewes received their next (unsupplemented) feed.

Period 3. All groups had higher plasma magnesium concentrations at 12.00 h than at 08.00 h, the fine C.M. (F) group being up by 0·25 mmol/l ($P<0·001$), the Cyclone dust (T) group up by 0·20 mmol/l ($P<0·01$), and the control group up 0·16 mmol/l ($P<0·05$). Differences between the groups were not significant at 08.00 h but were much greater at 12.00 h when the fine C.M. group had a higher plasma magnesium value (by 0·16 mmol/l) than the controls ($P<0·01$), and that of the Cyclone dust group was non-significantly higher than the controls (by 0·07 mmol/l). Plasma magnesium values fell after 12.00 h but were not quite down to the 08.00 h levels by 20.00 h. The mean plasma magnesium concentration of the control group increased slightly from 0·76 to 0·79 mmol/l from 16.00 h to 20.00 h in response to the unsupplemented feed given at 16.00 h.

Period 4. The mean plasma magnesium concentration of all groups was higher at 12.00 h than at 08.00 h ($P<0·05$), for the controls by 0·13, for the magnesium phosphate (P) supplemented group by 0·15, and for the magnesium hydroxide (L) supplemented group by 0·17 mmol/l. At 08.00 h the only significant difference was between the hydroxide group (at

0.86 mmol/l) and the controls (0·63 mmol/l) ($P<0·01$), but at 12.00 h this difference was greater ($P<0·001$) and the plasma magnesium value of the hydroxide group was also higher than that of the phosphate group ($P<0·05$). Again, mean plasma magnesium concentrations were still raised at 20.00 h (as compared with 08.00 h), but the gradual decline from 12.00 h appears to be interrupted by the unsupplemented feed given at 16.00 h.

Period 5. Each group had a higher plasma magnesium value at 12.00 h than at 08.00 h. For the control group this rise of 0.09 mmol/l was not significant, but it was significant for the medium C.M. (A) group, up by 0·17 mmol/l ($P<0·01$), and for the "dead-burnt" medium C.M. (B) group, up by 0·13 mmol/l ($P<0·05$). Thus, although no differences were significant between the groups at 08.00 h, at 12.00 h the medium C.M. supplemented group had a significantly higher plasma magnesium concentration than both the controls ($P<0·01$) and the dead-burnt C.M. group ($P<0·05$). The decrease in plasma magnesium levels after 12.00 h were again interrupted, for all groups, by the unsupplemented feed given at 16.00 h, and levels were still slightly raised at 20.00 h.

c) Blood samples taken at 12.00 hours (Table 8)

As treatment differences in mean plasma magnesium concentrations were most marked at 12.00 h, these values have been compared over all periods in Table 8, where they are listed in order of merit. Values for the zero supplement treatment are combined from each period, and values for the fine C.M. treatment (F) are meaned over Periods 2 and 3. All treatments except coarse C.M. at 0·5 g Mg/day (C(0·5)) significantly raised plasma magnesium concentrations above the zero level of 0·74 mmol/l. Magnesium hydroxide (L) and fine Spanish C.M. (F) produced the highest plasma magnesium levels at 1·04 and 1·00 mmol/l, respectively. Medium Spanish C.M. (A) treatment was slightly lower at 0·95 mmol/l, but coarse Spanish C.M. (C) was considerably lower, at 0·86 mmol/l

($C < F,L, P < 0{\cdot}001$; $C < A, P < 0{\cdot}05$). Fine and coarse C.M.s given at only 0·5 g Mg daily resulted in significantly lower plasma magnesium concentrations of 0·83 and 0·70 mmol/l respectively, than the same supplements given at 1·0 g Mg daily ($P < 0{\cdot}001$). Dead-burnt Spanish C.M. (B) produced a relatively poor blood magnesium response, 0·81 mmol/l ($B > 0$, $P < 0{\cdot}05$), while Spanish cyclone dust resulted in a mean plasma concentration of 0·91 mmol/l and magnesium phosphate produced an intermediate level of 0·88 mmol/l.

Table 8. Expt.3.3. Mean plasma concentration at 12.00 h (mmol/l) for sheep given each treatment (1·0 g Mg/day unless shown differently).

Supplement	Mean plasma Mg	SEM	Significance
L	1.04	0.029	L > A*; T**; P,C,F(0·5),B,0,C(0·5)***
F	1.00	0.022	F > T**; P,C,F(0·5),B,0,C(0·5)***
A	0.95	0.028	A > C*, F(0·5)*; B,0,C(0·5)***
T	0.91	0.026	T > F(0·5),B*; 0,C(0·5)***
P	0.88	0.028	P > 0,C(0·5)***
C	0.86	0.026	C > 0,C(0·5)***
F (0·5gMg)	0.83	0.025	F(0·5) > 0**; C(0·5)***
B	0.81	0.028	B > 0*
Control(0)	0.74	0.011	
C (0·5gMg)	0.70	0.025	

(ii) Plasma calcium concentrations (Figure 3, Table 9)

The mean plasma calcium concentrations for all groups, irrespective of their supplementary treatments, remained well within the normal range throughout the experiment, the lowest mean value observed being 2·25 mmol/l and the highest 2·71 mmol/l. There was a slight rise from 2·32 mmol/l at the start of the experiment to 2·53 mmol/l before lambing, a fall to 2·31

one day after lambing, a steady increase in the next two weeks to around 2·6 - 2·7, then a steady decrease in the following two weeks back to around 2·3 mmol/l. This characteristic trend after lambing was observed and reported by Ritchie (1971). Thereafter plasma calcium levels remained around 2·5 mmol/l in control sheep during Periods 2 and 3, and 2·35 mmol/l in control sheep during Periods 4 and 5. All groups followed the same trend and different magnesium supplements did not differentially affect plasma calcium concentrations as measured both by blood samples taken 24 hours after magnesium supplementation, and by samples taken serially throughout the day.

Faeces Availability data (Table 10, Figure 4)

Faecal magnesium was regressed onto faecal chromium concentration for individual sheep receiving a given supplementary treatment, including data for the control (unsupplemented) sheep in the regression, and in each case this gave a highly significant correlation coefficient. For each magnesium supplement (with associated chromium) given, the highly significant regression coefficient gives the faecal ratio of magnesium derived from the supplement to chromium, from which the apparent magnesium availability can be calculated (as described in Section II B).

In each period, pairs of supplements were compared. In Period 1, fine C.M. was more available (45·9%) than coarse C.M.(28·1%) when fed at 0·5 g Mg/day, and again in Period 2 when fed at 1·0 g Mg/day, with fine C.M. at 36·6% and coarse C.M. at 23·0% (although these differences were not quite statistically significant). In Period 3 fine C.M. was slightly (non-significantly) better than cyclone dust (46·6% and 39·9% respectively), and in Period 4 magnesium hydroxide and magnesium phosphate had similar apparent availabilities (43·8 and 47·6% respectively). It was shown in Period 5 that subjecting medium C.M. to 1300°C for 2 hours decreased its apparent availability from 49·6% to 27·5% ($P < 0·05$).

Table 9. Expt.3.3. Mean plasma calcium concentrations (mmol/l) of ewes taken serially over one day in each Period.

Time of day (h)		07.00	08.00	10.00	12.00	14.00	16.00	20.00
Period 1								
Group (1)	O	-	2.29	-	2.46	-	-	-
(2)	F	-	2.32	-	2.45	-	-	-
(3)	C	-	2.35	-	2.41	-	-	-
Period 2								
(1)	O	2.44	2.45	2.50	2.51	2.44	2.45	-
(2)	F	2.44	2.45	2.48	2.50	2.45	2.45	-
(3)	C	2.48	2.53	2.51	2.60	2.58	2.51	-
Period 3								
(1)	F	-	2.62	2.53	2.54	-	2.60	2.43
(2)	O	-	2.53	2.49	2.61	-	2.56	2.43
(3)	T	-	2.51	2.52	2.54	-	2.53	2.44
Period 4								
(1)	P	-	2.35	2.39	2.42	-	2.42	2.36
(2)	L	-	2.31	2.29	2.36	-	2.32	2.32
(3)	O	-	2.32	2.34	2.39	-	2.39	2.30
Period 5								
(1)	O	-	2.38	2.38	2.43	-	2.36	2.36
(2)	A	-	2.31	2.29	2.32	-	2.32	2.29
(3)	B	-	2.32	2.33	2.41	-	2.32	2.34

Figure 3. Expt.3.3. Mean plasma calcium concentrations (mmol/l) of all ewes until the start of magnesium supplementation, and of the three groups of ewes in Periods 1 to 5 (Blood samples taken at 08.00 h).

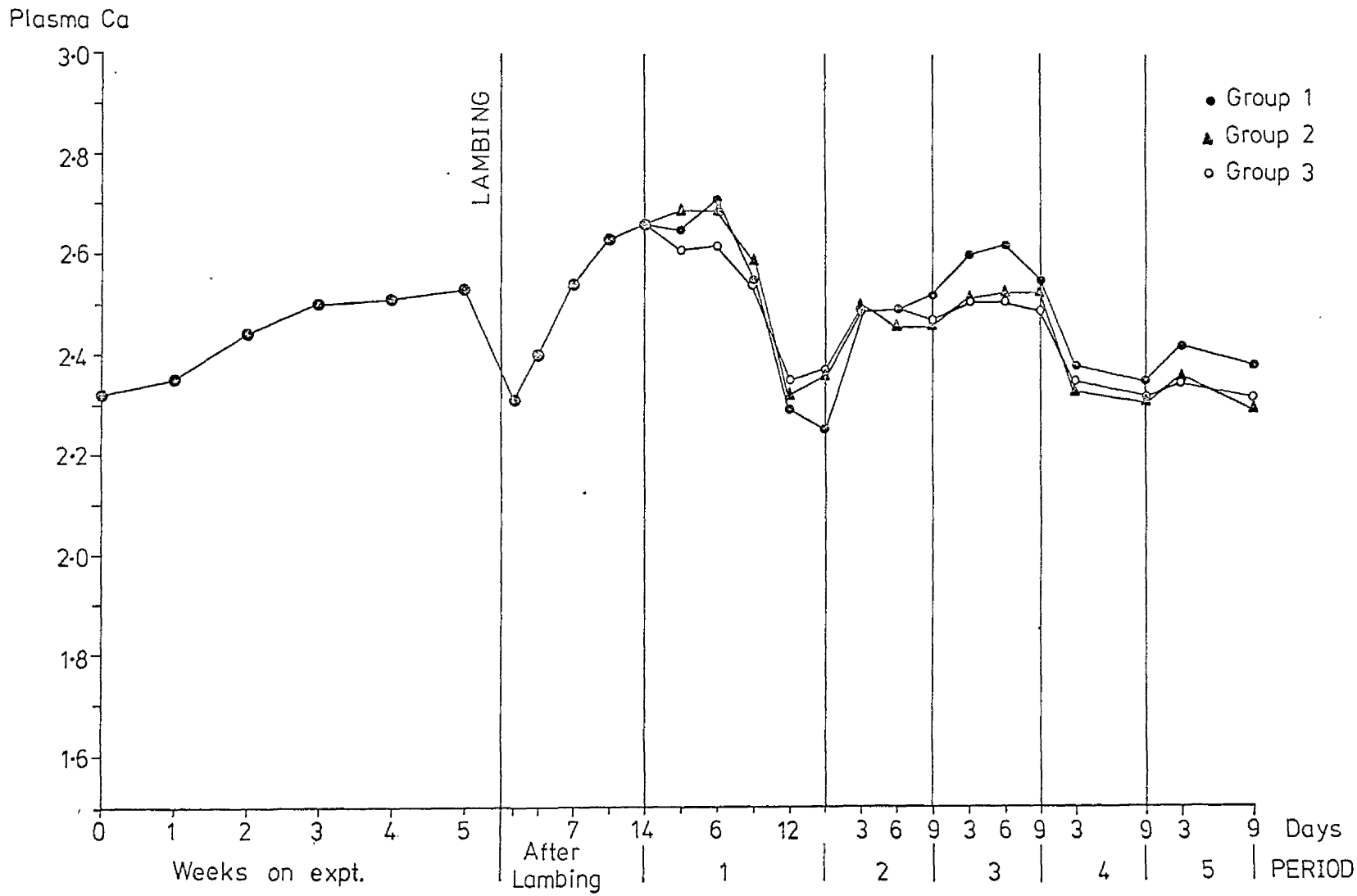
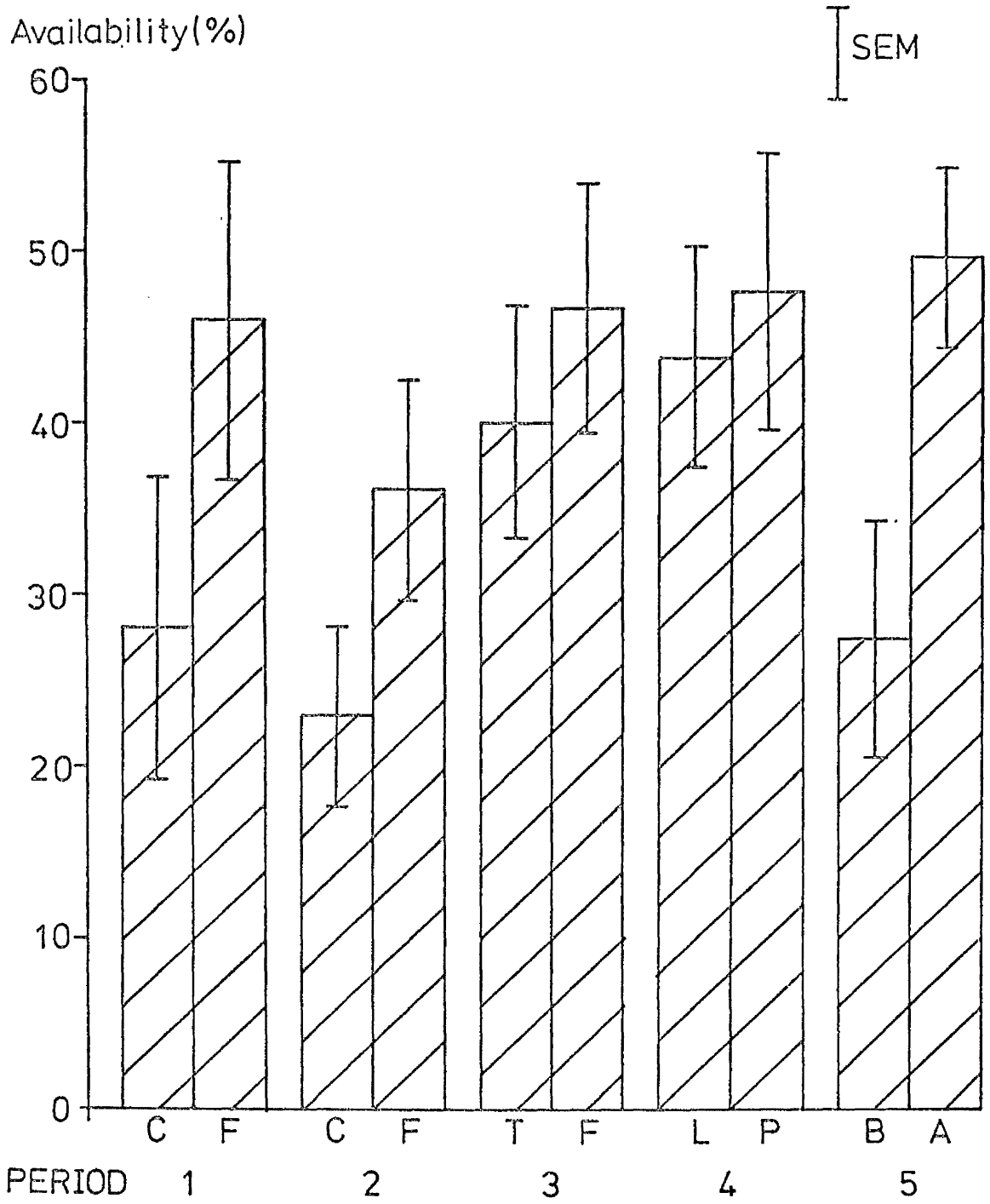

Plasma Ca
3·0
2·8
2·6
2·4
2·2
2·0
1·8
1·6
LAMBING
Group 1
Group 2
Group 3
0 1 2 3 4 5 7 14 6 12 3 6 9 3 6 9 3 9 3 9 Days
Weeks on expt.
After Lambing
1
2
3
4
5
PERIOD

Table 10. Expt.3.3. Faecal magnesium and chromium regression data and apparent availabilities.

Period	† Supplement	(a) Supplement Mg/Cr ratio	Regression of faecal Mg on faecal Cr: Correlation coefficient (and significance)	(b) Regression coefficient	SEb (and significance)	Apparent availability %	SE of availability	Significance
1	C	3.7133	0.7861***	2.6073	0.3251***	28.1	8.76	NS
	F	"	0.6187**	2.0079	0.3452***	45.9	9.30	
2	C	"	0.9505***	2.6577	0.1964***	23.0	5.29	NS
	F	"	0.9123***	2.3530	0.2423***	36.6	6.53	
3	T	"	0.8880***	2.2316	0.2690***	39.9	7.25	NS
	F	"	0.8656***	1.9848	0.2493***	46.6	6.71	
4	L	"	0.8975***	2.0850	0.2375***	43.8	6.40	NS
	P	"	0.8314***	1.9442	0.2981***	47.6	8.03	
5	A	"	0.9141***	1.8722	0.1941***	49.6	5.23	A>B*
	B	"	0.9276***	2.6906	0.2529***	27.5	6.81	

† See Table 1.

Figure 4. Exot.3.3. Apparent availabilities (%) of magnesium supplements in each Period. (Supplement notations in Table 1).


Availability(%)
SEM
60
50
40
30
20
10
0
C F C F T F L P B A
PERIOD 1 2 3 4 5

The availabilities of fine and coarse C.M.'s given at 0·5 g Mg/day do not differ significantly from the same supplements given at 1·0 g Mg/day. The mean availability for fine C.M. over Periods 1, 2 and 3 was 41·4% (SEM = 4·51) and that of coarse C.M. over Periods 1 and 2 was significantly lower ($P<0·01$) at 23·5% (SEM = 4·98).

Lamb data

Average lamb birth weights were 4·2 kg for twins and 4·6 kg for singles, and the lambing percentage was 160% for the 36 ewes. Average growth rates up to 6 weeks were 0·17 kg/day for twins and 0·29 kg/day for singles.

Discussion

The ewes maintained reasonable condition, with an average 1·6 lambs/ewe, throughout the experiment and lamb growth rates were good, indicating on average good milk production by the ewes. This therefore reflected the ability of the "natural" low magnesium diet to maintain realistic production levels.

The A.R.C. (1980) daily requirements for a 75 kg ewe in the last month of pregnancy are 1·00 - 1·13 g Mg, with recommended dietary allowance 1·72 - 1·90 g Mg/day, whereas in the present experiment 70 kg ewes were receiving only 0·76 g Mg/day. However, after the initial fall in mean plasma magnesium concentration during the first week when given the low magnesium diet, plasma magnesium could still increase gradually up to 0·82 mmol/l just before lambing, despite the apparent shortfall in magnesium intake. The A.R.C. (1980) recommend that a 75 kg lowland ewe yielding 2 kg milk daily should be allowed 3·32 g Mg/day (requirement 1·92 g Mg) whereas all ewes in the present trial received only 0·76 g Mg/day for up to the first two weeks of lactation. Not surprisingly, the mean plasma magnesium concentration dropped during this period, and throughout the rest of the trial the control groups (different in each

supplementary period) remained hypomagnesaemic (i.e. mean plasma magnesium remained below the normal range). However, there were no cases of clinical hypomagnesaemic tetany in spite of some individual ewes having extremely low plasma magnesium concentrations; for example, one ewe had a plasma magnesium concentration of only 0·17 mmol/l four days after lambing (with associated calcium level 2·20 mmol/l), and seven ewes had values below 0·40 mmol/l. It was observed, however, that these ewes consistently had the lowest plasma magnesium concentrations within their respective groups, even when showing considerable response to dietary magnesium supplementation, indicating perhaps that their individual "normal" plasma magnesium concentrations were low. It was also perhaps significant that the plasma calcium concentrations of these ewes remained entirely normal, above 2·0 mmol/l, as it has been suggested that a concomitant hypocalcaemia precedes the onset of clinical hypomagnesaemic tetany (Hemingway & Ritchie, 1965).

In Period 1, ewes were supplemented with 0·5 magnesium/day, raising their total daily magnesium intake to 1·26 g. This was still short of the A.R.C. (1980) recommended allowance, but the likelihood of detecting differences between supplementary magnesium sources with different availabilities was considered to be greater if given as a minimal quantity of magnesium. However, differences between the group mean plasma magnesium concentrations remained small as seen in blood samples taken 24 hours after administration of supplements. Thus the level of supplementation was raised to 1 g Mg/ewe/day for the rest of the experiment, giving a total daily magnesium intake of 1·76 g in Period 2, and 2·02 g from Period 3 onwards (when the basal diet intake was increased). Periods 3, 4 and 5 covered the second to third months of lactation when magnesium requirements of the ewe would start to decline (A.R.C., 1980, recommended daily allowance for ewes yielding 1 kg milk/day is 2·32 g, with requirement 1·34 g). However, even at a

supplementation rate of 1 g Mg/day, few differences between supplements could be detected by the plasma magnesium concentrations 24 hours after administration, and many supplements appeared to have no effect at all. It then became clear that a magnesium supplement given once daily had only a transient effect on plasma magnesium concentration, in all cases the peak concentration being observed at four hours after the administration of the supplement. This therefore provides a similar observation with ewes indoors to that of Ritchie & Hemingway (1963) with ewes at grass. There was no immediate response to magnesium supplements, as shown in Period 2 of this trial, but the pattern of rise and fall of plasma magnesium concentration during the day was similar for all treatments, including, to a lesser extent, the control groups. Plasma magnesium levels steadily decreased after the 12.00 hour peak, interrupted slightly by the unsupplemented afternoon feed, presumably back to roughly the original 08.00 hour level. This has very important implications for the practice of administration of magnesium supplements for the prevention of hypomagnesaemic tetany. Many casualties occur during the night, and indeed if supplementary magnesium is given once daily in a morning feed, animals would have their lowest plasma magnesium concentrations at night, when environmental stress factors, especially cold temperatures, can come into play. Thus, in practice, it may be more relevant to administer magnesium supplements more than once daily, and indeed frequency rather than quantity of magnesium supplementation may be the critical factor. Furthermore this pattern of blood response, seen only for a few hours after administration of magnesium supplements, indicates rapid absorption (but only of a small proportion of that administered) from the rumen. This is perhaps important in view of the current belief that the rumen is a major site for magnesium absorption (see Section I 1), and subsequent experiments in this research thesis report in vivo assessments of rumen digestibility

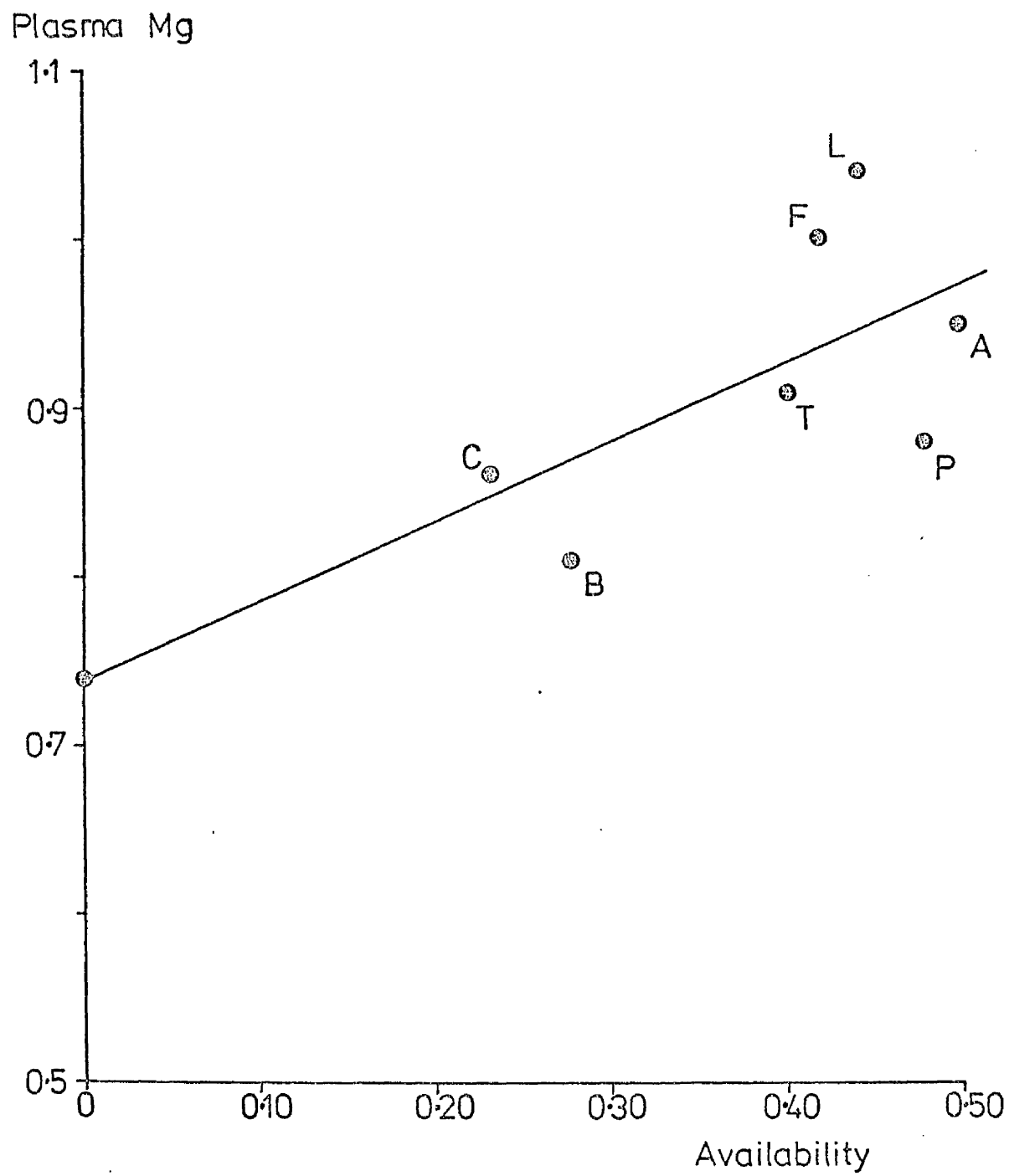
using a nylon bag technique (see Section V A).

In the present experiment, the size of the transient rise in plasma magnesium concentration resulting from administration of different supplements varied so that differences between supplements were more evident at 12.00 hours whereas they could pass undetected at 08.00 hours. By comparing 12.00 h blood samples within each experimental period, it was shown that fine particles of calcined magnesite (< 75 μm) were better than coarse granules (500 - 1,000 μm) at two levels of supplementation, fine calcined magnesite was better than "cyclone dust", magnesium hydroxide was better than the phosphate, and medium grade calcined magnesite (150 - 250 μm) was better than dead-burnt medium calcined magnesite. Comparing between Periods it could be seen, in addition, that the medium (150 - 250 μm) calcined magnesite raised plasma magnesium only slightly less than fine (< 75 μm), but considerably more than coarse (500 - 1,000 μm) calcined magnesite. Coarse calcined magnesite given at 1 g Mg/day and fine calcined magnesite given at only 0·5 g Mg/day raised plasma magnesium to similar levels (0·83 - 0·86 mmol/l) indicating that the latter is about twice as effective. Powdered magnesium hydroxide and the fine calcined magnesite were similar, but the undercalcined cyclone dust was slightly poorer. Magnesium phosphate raised plasma magnesium to an intermediate extent, greater than coarse and dead-burnt calcined magnesites, but less than the three powdered and the medium grade supplements.

The concurrent data on apparent availability of the magnesium supplements generally reinforced the conclusions drawn from the blood magnesium data. Comparing the pairs of supplements within Periods, the one with the higher availability raised plasma magnesium to the greater extent, except in Period 4. In this Period the blood results clearly suggested magnesium hydroxide to be better than phosphate, but, anomalously, their apparent availabilities were similar. Availability values were generally

Figure 5. Expt.3.3. Correlation between the mean plasma magnesium concentrations (mmol/l) for ewes given each supplementary treatment and the supplementary magnesium apparent availability coefficient.

$$y = 0.74 + 0.47x \; ; \quad r = 0.799^{*}$$


Plasma Mg
1·1
0·9
0·7
0·5
L
F
A
T
P
C
B
0
0·10
0·20
0·30
0·40
0·50
Availability

high, at 37 - 50% for all supplements except the coarse and the dead-burnt calcined magnesites (23 - 27%). This might be due to the nature of the basal diet as Rook (1961), for example, observed increased magnesium utilisation when flaked maize was included in diets. However, over the whole experiment, mean plasma magnesium concentrations (at 12.00 hours) for different supplements fed at 1 g Mg/day were well correlated with the supplement availability (see Figure 5), giving a correlation coefficient of 0·799 ($P<0·05$) (A mean availability of 41·6%, from Periods 2 and 3, was used for fine calcined magnesite, F).

Thus this trial demonstrates a useful experimental method for comparing dietary magnesium supplements whereby apparent availability and blood magnesium data are obtained for hypomagnesaemic ewes given a "natural" low magnesium diet. In addition, it shows that magnesium supplements given just once daily have only a transient effect on plasma magnesium concentration and therefore leave the animal virtually "unprotected" over much of the day.

Experiment 3.4. The induction of hypomagnesaemia in lactating ewes and its alleviation by various dietary magnesium supplements.

Introduction

The success of the low magnesium diet based on natural feedingstuffs, as used in Experiment 3.3., is apparent with respect to (a) its ability to sustain a realistic level of production in pregnant and lactating ewes, while (b) resulting in significantly lowered mean plasma magnesium concentrations during lactation. This therefore provided a useful experimental situation, with productive hypomagnesaemic ewes, in which to assess the relative efficacies of different dietary magnesium supplements, in terms of plasma magnesium response and apparent

availability. The present experiment was therefore modelled on Experiment 3.3. in order to study a further range of different magnesium supplements.

Mean plasma magnesium concentrations did not fall significantly until after lambing in the previous trial and so the ewes in the present experiment were not introduced to the low magnesium diet until late gestation. The previous trial also showed that differences between plasma magnesium concentrations resulting from different supplementary treatments were greatest at 12.00 hours (4 hours after administration of supplements). Thus the labour-cost of the experimental method was considerably reduced as supplements were fed daily for one week with the ewes being bled just once, at 12.00 hours, on the last day.

A range of different magnesium supplements for which apparent availability data has already been obtained, using wether sheep indoors (Section II), was chosen for investigation, and the present trial provides both blood data and availability results using lactating hypomagnesaemic ewes.

Materials and Methods

Magnesium supplements

The fifteen magnesium supplements investigated in this trial are listed in Table 1. Supplements a, b and c were Spanish magnesite rock calcined in a laboratory furnace at 650°, 800° and 1,100°C, respectively, as used in the balance trial Experiment 1.2. All were in the particle size range 75 - 500 μm, and a sample of commercial Spanish calcined magnesite (Agma FG85) of this same particle fraction was included for comparison (supplement e). Supplement d was "medium" Spanish calcined magnesite, i.e. 150 - 250 μm, subjected to "dead-burning" at 1300°C for 2 hours, as used in the previous Experiment 3.3. but included here to complete the temperature series.

Table 1. Expt.3.4. Magnesium supplements.

	Supplement	gMg/kg
a	Spanish magnesite calcined at 650°C, 75 - 500 µm	298.2
b	" " " 800°C, 75 - 500 µm	460.8
c	" " " 1,100°C, 75 - 500 µm	498.0
d	" " " 1,300°C, 150 - 250 µm	531.8
e	Spanish calcined magnesite, 75 - 500 µm	504.4
f	Greek calcined magnesite, under 75 µm	463.3
g	" " " , 0.5 - 2.0 mm	491.8
h	Chinese calcined magnesite, under 75 µm	533.9
j	" " " , 0.5 - 2.0 mm	573.4
k	English sea-water MgO powder	573.2
l	English dolomite powder ($MgCO_3.CaCO_3$)	125.7
m	American brine MgO "prilled 30"	603.0
n	Spanish calcined magnesite, 0.5 - 2.0 mm	507.6
p	Analar MgO powder	603.0
q	High energy feedblock containing $MgO/MgPO_4$	77.51

Random samples of Greek and Chinese commercial products were included, as used in the availability trial with wether sheep, Experiment 2.3. Two particle fractions of each were used, "fine" and "coarse", under 75 µm and 0.5 - 2.0 mm, respectively (Greek, supplements f and g; Chinese, supplements h and j, respectively). In addition a coarse, 0.5 - 2.0 mm, Spanish fraction (supplement n) was included (as used in Experiment 2.3, from Agma FG85 November 1980). Detailed particle size analysis of these commercial products can be seen in Appendix 1.

Samples of English sea-water derived magnesium oxide powder ("Lycal 93/12"), English dolomite powder ("Dolofil"), and American brine-

derived magnesium oxide ("Animag, prilled 30") (supplements k, l, and m, respectively) were as used in the earlier availability trial Experiment 2.3., and analar magnesium oxide (supplement p) was included as in the availability Experiment 2.1. Lastly samples from a high-energy molasses-based feed block ("Crystalyx", Pfizer) containing 7·7% magnesium (supplement q) was included, as used in field trials with cattle (Section IV B). The magnesium content was estimated to be 87% from fine Spanish calcined magnesite (Agma powder), 6% from magnesium phosphate, and 7% from the rest of the feedblock constituents.

Experimental design

Thirty-six Greyface ewes were housed and were group-fed 1·5 kg/head/day of the low magnesium diet as described in Experiment 3.3., from about 3 weeks prior to lambing. Blood samples were taken initially and again after 2 weeks on the experimental diet.

Each ewe was individually penned once she had lambed, and at between 1 - 2 weeks after lambing, the ewes were divided into groups of similar initial mean plasma magnesium concentration and magnesium supplementation of the basal ration commenced. Details of the supplements fed to each group during each emperimental period are given in Table 2. The number of ewes per group varied from 8 to 9 and the number of groups varied from 4 to 5 due to the later lambers being included later in the experiment and some ewes having to be discarded. The basal diet, at 1·5 kg/ewe/day, was given in approximately equal halves at 07.30 h and 16.00 h, and all supplements, giving 1·0 g magnesium/ewe/day, were added to the morning feed. There were five periods of supplementation, each lasting seven days, and at the start of Period 4 the basal ration was increased to 2 kg/ewe/day.

Table 2. Expt.3.4. Supplements given to the groups of 8 or 9 lactating ewes during each experimental period.

Period of Supplementation	1	2	3	4	5
Group (1)	0	0	f	k	n
(2)	b	d	0	-	-
(3)	a	c	h	0	q
(4)	-	e	g	l	0
(5)	-	-	j	m	p

Blood samples were taken at the start of Period 1 and on the last day (seventh day of supplementation) at 08.00 h and again at 12.00 h. Thereafter the ewes were bled just once at 12.00 h on the last day of each seven-day supplementary period. All blood samples were analysed for plasma magnesium and calcium concentrations.

Chromic oxide was added to all supplements at the rate 0·500 g chromic oxide (673·2 g Cr/kg) to 1·0 g magnesium, and grab samples of faeces were taken from each ewe during the morning of the last day of each period. Faeces were dried and ground for subsequent magnesium and chromium analyses.

Results

Blood data

The initial mean plasma magnesium and calcium concentrations of all the ewes were 0·75 and 2·36 mmol/l, respectively, with little change after two weeks on the experimental diet, at 0·78 and 2·25 mmol/l, respectively. Plasma magnesium concentrations dropped immediately after lambing to 0·67 mmol/l at the start of the dietary magnesium supplementation (1 - 2 weeks post-parturition) with an associated mean calcium concentration 2·40 mmol/l.

The mean plasma magnesium and calcium concentrations for each group

in each period are given in Table 3. Initially, plasma magnesium results have been compared within each Period. In Period 1, both 650°C and 800°C calcined magnesites (a and b) failed to raise significantly the mean plasma magnesium concentration as measured by blood samples taken at 08.00 hours, however both significantly raised plasma magnesium above the zero level of 0·67 ($P<0·001$), at 0·96 and 0·95 mmol/l, respectively, at 12.00 hours. Again in Period 2, all three supplementary treatments raised plasma magnesium to a similar extent: 1,100°C calcined magnesite (C.M.) and commercial 75 - 500 μm Spanish C.M. ($P<0·001$), and 1,300°C C.M. ($P<0·01$), at 0·90, 0·89, and 0·80 mmol/l, respectively (c, e and d), compared with a "zero level" of 0·61 mmol/l. In Period 3, however, only the fine (under 75 μm) fractions of Greek and Chinese C.M. (f and h) raised plasma magnesium significantly above the zero level of 0·6 mmol/l, at 0·94 and 0·98 mmol/l, respectively ($P<0·001$). These two supplements were both significantly better than the corresponding two coarse fractions, with Greek 0·5 - 2·0 mm (g) at 0·78 ($g<f,h$, $P<0·05$) and Chinese 0·5 - 2·0 mm (j) at 0·74 mmol/l ($j<f$, $P<0·05$; $j<h$, $P<0·01$).

English sea-water magnesium oxide (k) raised plasma magnesium to the greatest extent in Period 4, at 0·92 mmol/l, compared with "zero" at 0·58 ($P<0·001$) dolomite (l) at 0·64 ($P<0·001$) and American brine magnesia (m) at 0·71 ($P<0·01$). Dolomite did not significantly raise magnesium above that of zero supplement level, and American magnesia raised it only slightly ($P<0·05$). In Period 5, all supplements raised the plasma magnesium concentration over the zero supplemented level of 0·64 mmol/l, with Spanish C.M. 0·5 - 2.0 mm (n) at 0·86 ($P<0·001$), analar magnesium oxide (p) at 0·85 and the "feedblock" (q) at 0·84 mmol/l ($P<0·01$).

Mean plasma calcium values remained between 2·37 and 2·58 mmol/l throughout and were unaffected by different magnesium supplementary treatments (see Table 3).

Table 3. Expt.3.4. Mean plasma magnesium and calcium concentrations (mmol/l) at 12.00 hours for all supplementary magnesium treatments in each period (and at 08.00 hours in Period 1)

PERIOD	TREATMENT	Mean plasma Mg	SEM †	Significance	Mean plasma Ca	SEM ††	Significance
1	0	0.66	0.049		2.41	0.044	
08.00h	a	0.74	0.045	NS	2.37	0.032	NS
	b	0.75	0.049		2.42	0.030	
	0	0.67	0.057	a,b>0***	2.52	0.055	
12.00h	a	0.96	0.054	12.00>08.00h,	2.49	0.039	NS
	b	0.95	0.057	a** b*	2.58	0.063	
2	0	0.61	0.048		2.46	0.028	
	c	0.90	0.048	c,e>0***	2.49	0.020	NS
	d	0.80	0.048	d>0**	2.53	0.039	
	e	0.89	0.048		2.46	0.037	
3	0	0.60	0.071		2.51	0.076	
	f	0.94	0.060	f,h>0***	2.44	0.061	
	g	0.78	0.056	h>g*, j**	2.48	0.024	NS
	h	0.98	0.056	f>g, j*	2.50	0.023	
	j	0.74	0.056		2.51	0.031	
4	0	0.58	0.046		2.50	0.024	
	k	0.92	0.046	k>m**	2.49	0.034	NS
	l	0.64	0.046	k>l,0***	2.55	0.019	
	m	0.71	0.046	m>0*	2.48	0.027	
5	0	0.64	0.042		2.50	0.038	
	n	0.86	0.042	n>0***	2.45	0.040	NS
	p	0.85	0.042	p,q>0**	2.45	0.038	
	q	0.84	0.042		2.47	0.030	

† Analysis of variance within each period. †† Simple "t" test.

Table 4. Expt.3.4. Mean plasma magnesium concentrations (mmol/l) for the whole experiment (overall analysis of variance)

	Supplement	Mean Plasma Mg	(EMS= 0.0200) SEM	Significance
h	Chinese < 75 µm	0.98	0.050	h>q,d*; g**; j,m,l,o***
a	650°C C.M. 75-500 µm	0.96	0.050	a>d,g*; j**; m,l,o***
b	800°C C.M. 75-500 µm	0.95	0.053	b>d,g*; j,m**; l,o***
f	Greek < 75 µm	0.94	0.053	f>g*; j,m**, l,o***
k	English MgO powder	0.92	0.047	k>g,j*; m**; l,o***
c	1,100°C C.M. 75-500 µm	0.90	0.050	c>j*; m**; l,o***
e	Spanish C.M. 75-500 µm	0.89	0.050	e>j,m*; l,o***
n	Spanish C.M. 0·5-2·0 mm	0.86	0.047	n>m*; l,o***
p	Analar MgO	0.85	0.047	p>m*; l**; o***
q	Feedblock Mg	0.84†	0.047	q>l**; o***
d	1,300°C C.M. 150-250 µm	0.80	0.050	d>l*; o**
g	Greek 0·5-2·0 mm	0.78	0.050	g>l*; o**
j	Chinese 0·5-2·0 mm	0.74	0.050	j>o*
m	American MgO	0.71	0.047	
l	Dolomite	0.64	0.047	
o	No supplement	0.62	0.023	

† After analysis it was discovered that in fact only 0·77 g Mg/day was given as supplement "q".

An overall analysis of variance was carried out in order to compare supplements between different Periods (Table 4). The mean value for zero supplement level is taken over all five experimental Periods. The supplements are listed in Table 4 in order of decreasing merit, and any statistically significant differences are given.

Availability results

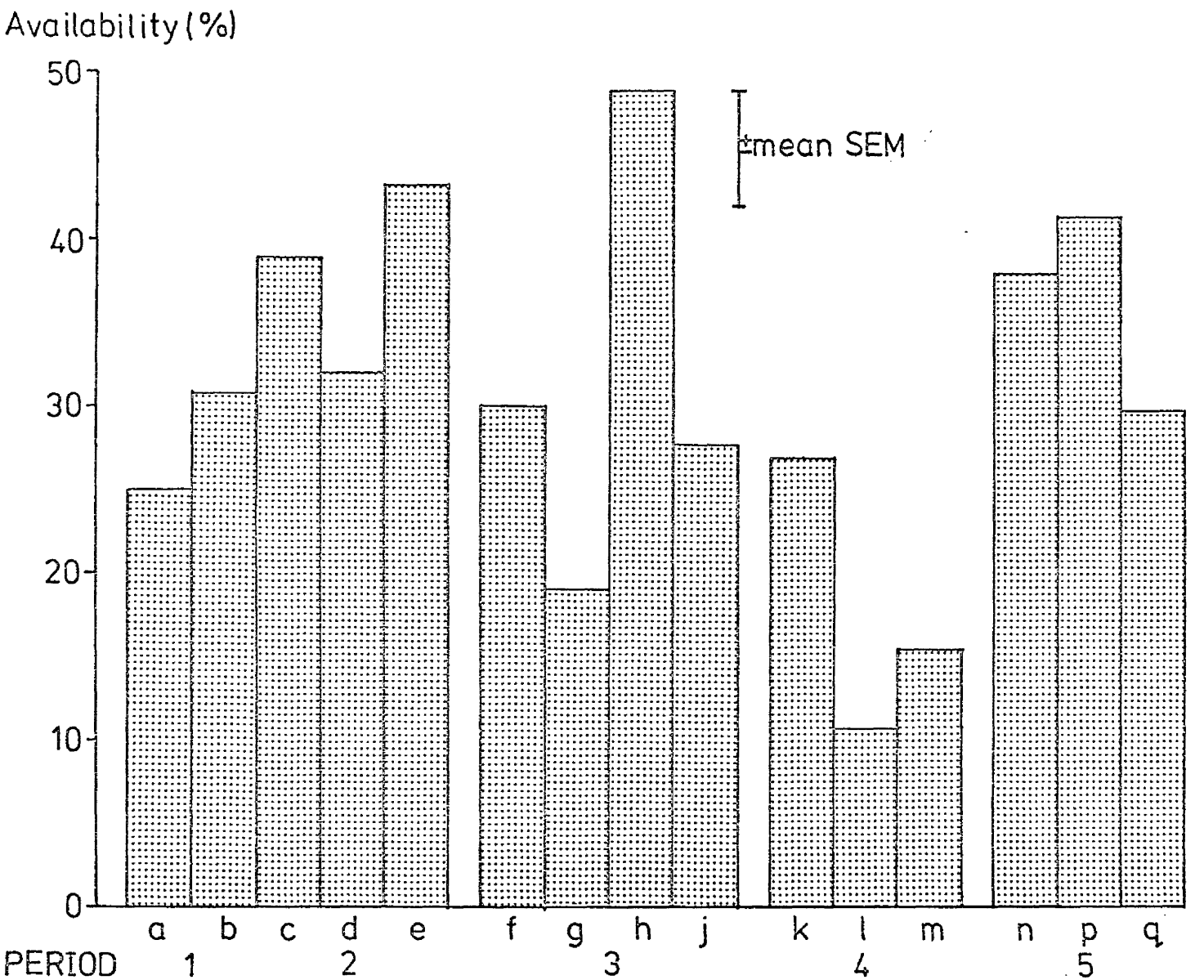
Faecal magnesium and chromium regression data are given in Table 5. For each magnesium supplement given there are very highly significant correlation coefficients and regression coefficients. Apparent availabilities are calculated using the latter (see Section II B) and are given in Table 5, and Figure 1.

The lowest availabilities were for dolomite (l, 10·7%) and American magnesia (m, 15·4%). Coarse (0·5 - 2.0 mm) fractions of Chinese and Greek C.M. (j, 27·7% and g, 19·0%, respectively) were less available than the fine (under 75 μm) fractions (h, 48·9%, $P<0·05$, and f, 30·0%, non-significant, respectively). Coarse Spanish C.M. was more available (n, 38·0%) than the Greek ($P<0·05$) or Chinese, but was similar to that of commercial Spanish 75 - 500 μm (e, 43·2%). Magnesites calcined at 650°, 800°, 1,100° and 1,300°C (a,b,c and d) had availabilities of 25·0, 30·8, 38·9% and 32·0%, respectively (with no significant differences). Analar magnesium oxide (p) and English sea-water magnesium oxide (k) had availabilities of 41·5 and 27·0% respectively, and the availability of the feedblock magnesium was 29·8%.

Table 5. Expt.3.4. Faecal magnesium and chromium regression data and apparent availabilities.

Period	Supplement	Supplement Mg/Cr ratio	Regression of faecal Mg on faecal Cr: Correlation coefficient (and significance)	Regression of faecal Mg on faecal Cr: Regression coefficient (b)	Regression of faecal Mg on faecal Cr: SEb (and significance)	Apparent availability %	SE of availability	Significance
1	a	2.9706	0.9174***	2.2278	0.2680***	25.0	9.02	
	b	"	0.8889***	2.0550	0.3057***	30.8	10.29	
2	c	"	0.9247***	1.8141	0.1432***	38.9	4.82	c >m*;g**;l***
	d	"	0.9747***	2.0210	0.1198***	32.0	4.03	d >g*;l**
	e	"	0.9506***	1.6876	0.1423***	43.2	4.79	e >m**;g,l***
3	f	"	0.8999***	2.0805	0.3198***	30.0	10.73	
	g	"	0.9826***	2.4060	0.1371***	19.0	4.62	
	h	"	0.9361***	1.5176	0.1720***	48.9	5.79	h>a,j,k,q*; m**;g,l***
	j	"	0.9636***	2.1487	0.1798***	27.7	6.05	j>l*
4	k	"	0.9105***	2.1682	0.2462***	27.0	8.29	
	l	"	0.9736***	2.6524	0.1553***	10.7	5.23	
	m	"	0.9307***	2.5143	0.2470***	15.4	8.31	
5	n	"	0.9279***	1.8407	0.1850***	38.0	6.23	n >g,m*;l**
	p	"	0.8963***	1.7367	0.2148***	41.5	7.23	p > g,m*;l**
	q	2.3026	0.9441***	1.5827	0.1476***	29.8	6.41	q >l*

Figure 1. Exot.3.4. Apparent availabilities (%) of magnesium supplements (Supplement notations in Table 1).


Availability (%)
50
40
30
20
10
0
± mean SEM
a b c d e f g h j k l m n p q
PERIOD 1 2 3 4 5

Discussion

As in the previous trial (Experiment 3.3.), the ewes maintained reasonable body condition and the lambs, at 1·71 per ewe, grew well, indicating on average good milk production by the ewes. The diet was therefore shown to maintain production as well as inducing hypomagnesaemia. The mean plasma magnesium concentration dropped rapidly after lambing and thereafter remained relatively constant at 0·62 (0·58 - 0·67) mmol/l for all control groups. There were two fatalities from hypomagnesaemic tetany during the trial at the end of Period 2, i.e. about 3 weeks post-parturition. Both ewes were in the control group and had therefore received no magnesium supplement since the start of the experiment before lambing. In addition, both ewes had twin lambs and consequently high magnesium demands. Both showed inappetance just one or two days prior to death, and both had extremely low plasma magnesium concentrations on the day before: (i) 0·14 and (ii) 0·12 mmol/l. Both ewes also had concomitant hypocalcaemia: (i) 1·80 and (ii) 1·28 mmol/l. This contrasts with the previous trial (Experiment 3.3.) in which some ewes had low magnesium values associated with normal calcium, e.g. 0·17 mmol/l Mg; 2·2 mmol/l Ca, and no cases of hypomagnesaemic tetany occurred. The present findings therefore agree with those of Hemingway & Ritchie (1965) that ruminants appear able to experience severe hypomagnesaemia for long periods without signs of tetany whereas the majority of cases of clinical tetany exhibit a combined hypocalcaemia and hypomagnesaemia. One of the affected ewes ((i)) in the present trial received a treatment of subcutaneous magnesium sulphate and calcium borogluconate at the onset of convulsions, but failed to respond. The second ewe (ii) had received one daily supplement, i.e. 1 g Mg, as the fine Greek (under 75 μm) calcined magnesite some seven hours prior to her death. This indicates that for a severely hypomagnesaemic and hypocalcaemic ewe, a single treatment with this particular supplement

did not raise plasma magnesium and calcium sufficiently to prevent tetany, even though it proved to be one of the better magnesium supplements, with respect to mean plasma magnesium levels, over the experiment as a whole.

It was established in Period 1 that plasma magnesium concentrations were considerably higher at 12.00 hours than at 08.00 hours, as in the previous trial (Expt.3.3.), and thereafter blood samples were taken only at 12.00 h on the appropriate days in order to detect differences between supplementary treatments. The control, unsupplemented groups of ewes, although different in each one-week period, had a relatively constant mean plasma magnesium concentration (0·62 mmol/l) throughout. Thus all supplementary treatments were directly comparable over the whole experiment. However, the 12.00 hour "zero level" in the previous trial was greater ($P < 0.001$), at 0·74 mmol/l and so actual magnesium values could not be compared directly between the two experiments. It is possible that the higher lambing percentage in the present experiment (and consequent higher magnesium requirements by ewes for milk production) might explain the greater degree of hypomagnesaemia in the flock.

The powdered (under 75 μm) fractions of Chinese and Greek commercial calcined magnesites were good, in terms of the plasma magnesium response, and of the two highly (chemically) reactive powdered magnesium oxides, the English sea-water MgO was better than the Analar product. Dolomite ($MgCO_3.CaCO_3$) was poor, despite being finely powdered, but the feedblock magnesium (87% fine MgO, 7% magnesium phosphate) was good even although only 0·77 g Mg/ewe/day had been given. The coarse granular fractions of Greek and Chinese products were poor but the same fraction of Spanish calcined magnesite was better, indicating the importance of source on the availability of a material. Interestingly the "medium" fraction of Spanish calcined magnesite (75 - 500 μm) was not significantly better than the coarse granules in this trial.

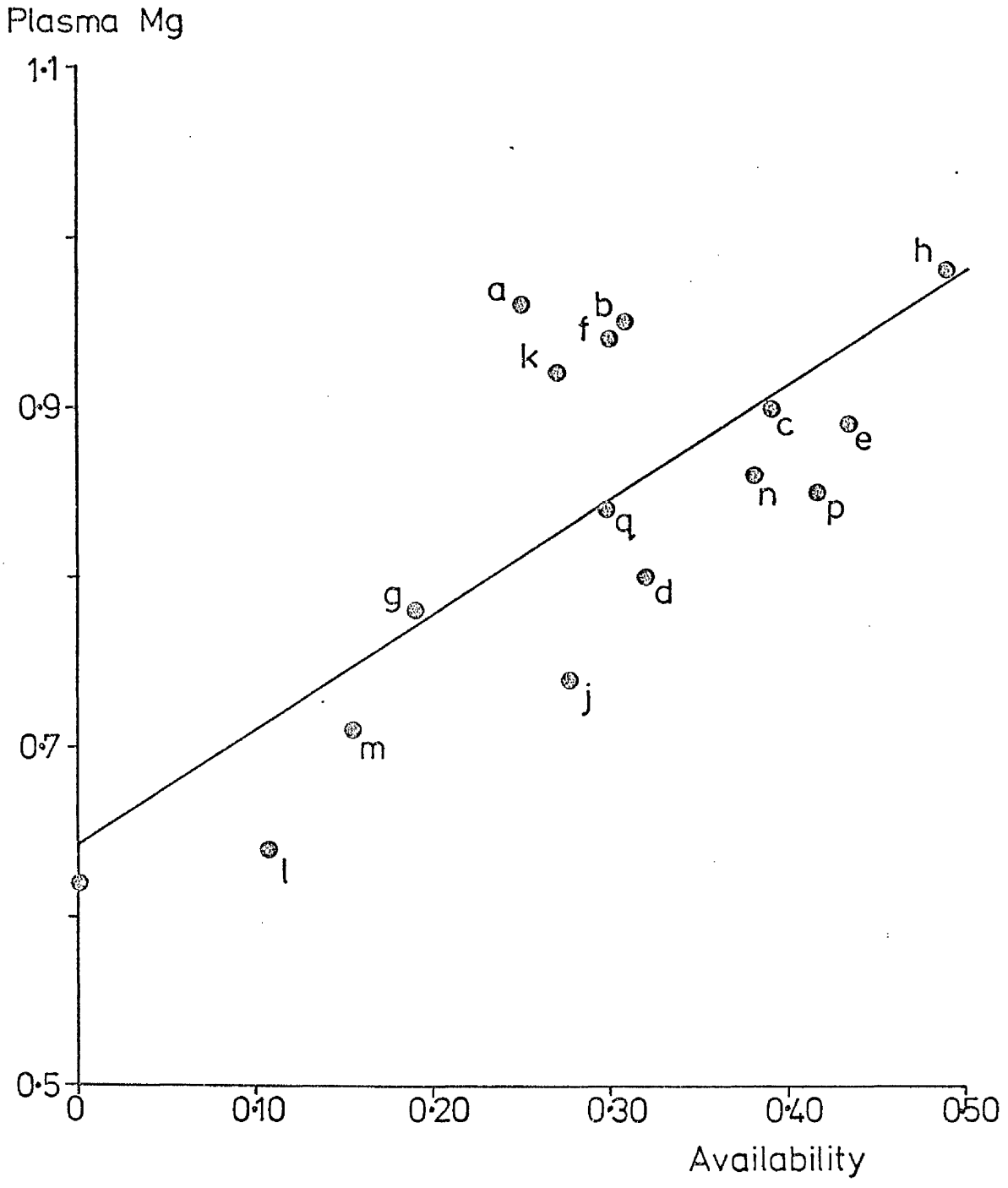
Of the magnesites calcined at different temperatures, the 650°C product raised plasma magnesium concentrations to a surprising extent as it is only partly calcined ($MgCO_3$.MgO) and the magnesium carbonate in the dolomite was extremely poor. However it could be that the magnesium in the oxide is very rapidly available while that in the carbonate remains virtually unavailable. Thus at the supplementation rate used, the 650°C product could release sufficient magnesium from its oxide fraction alone to raise plasma magnesium by several units whereas its overall apparent availability from faecal data remained fairly poor (25·0%). The 800°C product was similarly effective, the 1,100°C product slightly less, and the 1,300°C product considerably less, in terms of plasma magnesium response, thus indicating the detrimental effect on calcined magnesite of excessively high temperatures.

The extremely pure American magnesia, virtually 100% MgO, failed to raise plasma magnesium to any significant extent, and in addition it had a low apparent availability (15·4%). The poor nature of this product may be attributable to the very dense granules (150 - 500 μm diameter) of which it is composed which might be only slowly soluble in the reticulo-rumen and gut contents.

Apparent availability differences between supplements were largely reflected in differences in plasma magnesium response. Availabilities did not differ significantly for the temperature treated magnesites, but were significantly greater for fine calcined magnesites than for coarse fractions (Greek and Chinese). As the blood data suggest, the Spanish coarse fraction was more available than the corresponding Greek and Chinese fractions, and was similar to the medium Spanish fraction (75 - 500 μm). Dolomite and American magnesium oxide were both poorly available, thus agreeing with their poor plasma magnesium responses. Overall, mean plasma magnesium concentration for each supplementary treatment correlates well, as expected, with the supplement apparent availability ($r = 0{\cdot}7672$, $P < 0{\cdot}001$), as shown in Figure 2.

Figure 2. Expt.3.4. Correlation between the mean plasma magnesium concentrations (mmol/l) for ewes given each supplementary treatment and the supplementary magnesium apparent availability coefficient.

$$y = 0.64 + 0.68x \; ; \quad r = 0.767^{***}$$

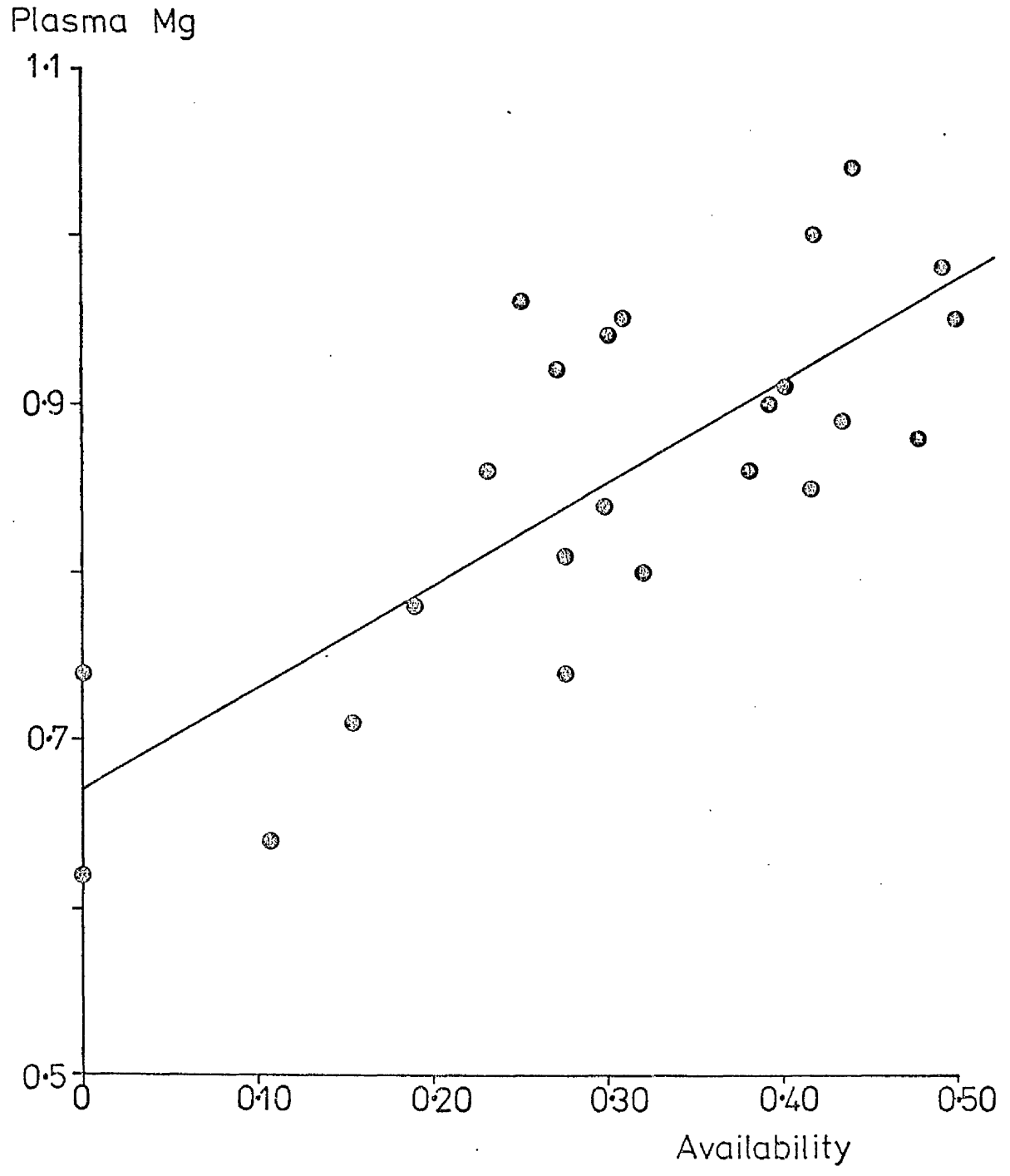

Plasma Mg
1·1
0·9
0·7
0·5
0
0·10
0·20
0·30
0·40
0·50
Availability
a
b
f
k
h
c
e
n
p
q
d
g
j
m
l

This experiment has therefore successfully used the technique developed in the previous trial (3.3.) whereby the relative efficacies of dietary magnesium supplements given to hypomagnesaemic lactating ewes may be assessed in terms of apparent availability and plasma magnesium response. Fine calcined magnesites appear better than coarse fractions, and differences are apparent between products from different sources. Dolomite appeared to be an extremely poor magnesium source and high temperature treatment is detrimental to calcined magnesite. Relatively pure magnesium oxides are good, but less efficacious than might be expected from their high chemical reactivity, especially as the English sea-water and Analar oxides are also finely powdered. The American brine magnesia is, however, very poorly available in spite of its pure nature. Thus some useful comparisons between magnesium supplements have been detected.

In Figure 3, the correlations between mean plasma magnesium concentration and supplement availability from the results of both Experiments 3.3. and 3.4. are combined, and the resultant correlation is highly significant with $r = 0{\cdot}774$ ($P < 0{\cdot}001$). This emphasises the usefulness of the experimental method employed for assessing the relative efficacies of different dietary magnesium supplements. In addition the good agreement between blood responses and apparent availabilities, as calculated by the chromium marker technique, provides further evidence for the validity of this method for availability determination.

Figure 3. Expt.3.4. Correlation between the mean plasma magnesium concentrations (mmol/l) for ewes in both Expts.3.3. and 3.4. given each supplementary treatment and the supplementary magnesium apparent availability coefficient.

$$y = 0.67 + 0.61x \; ; \quad r = 0.774^{***}$$


Plasma Mg
1·1
0·9
0·7
0·5
0
0·10
0·20
0·30
0·40
0·50
Availability

B. Low Phosphorus Diet

Experiment 4.1. An attempt to induce hypomagnesaemia by giving a low phosphorus diet to ewes during pregnancy and lactation.

Introduction

It had previously been observed (Fishwick, personal communication) that some beef cows given a low phosphorus diet during late pregnancy and lactation developed low blood magnesium levels. Five out of nine cows that had received 12 g P/day throughout (approximately half of the A.R.C., 1980, requirement) were hypomagnesaemic at calving and for the following six weeks, with plasma magnesium concentrations between 0·22 to 0·64 mmol/l. However only two of the nine control animals, given 28 g P/day, were notably hypomagnesaemic at 6 weeks after calving (plasma magnesium 0·37 and 0·59 mmol/l). The present experiment describes a feeding trial in which ewes were given a phosphorus-deficient diet during pregnancy and lactation in an attempt to induce some measure of hypomagnesaemia.

Dietary phosphorus deficiency in the adult ruminant can produce osteomalacia in which some resorption of bone ash occurs. Bone ash contains approximately 360 g Ca/kg, 170 g P/kg and 7 g Mg/kg. The calcium and phosphorus are closely combined so that both elements are withdrawn simultaneously during resorption, whether under calcium or phosphorus deficient conditions. Chronic phosphorus deficiency is therefore characterised by a reduced bone ash content. Benzie et al. (1959) reported a 40% loss of total skeletal ash in ewes given 1·0 - 1·5 g P/day during pregnancy and lactation, compared with only 19% loss for ewes given 4·0 - 4·5 g P/day. However, Fishwick et al. (1977) found no significant depletion in rib bone ash content of phosphorus depleted cows, although the radiographic density of their tail bones was reduced. The magnesium in bone is not normally considered to be a readily mobile

reserve of available magnesium in the adult ruminant body. During bone resorption, however, some magnesium will inevitably be released and excreted from the body. Thus by dietary phosphorus inadequacy it may be possible to deplete the body of a proportion of its magnesium, whether bound or available, and perhaps induce hypomagnesaemia in spite of an apparently adequate dietary magnesium intake.

Materials and Methods

Thirty-three aged draft Scottish Blackface ewes were mated at pasture and brought indoors when about 2 - 4 weeks pregnant. They were divided at random into two groups which were maintained throughout pregnancy and six weeks of lactation. One group of twenty-five ewes received a low phosphorus diet (LP) and the eight control animals were given a similar, but phosphorus-supplemented diet (HP). The mean compositions of the two major dietary components, dried molassed sugar beet pulp and barley husk siftings, are given in Table 1.

Table 1. Expt.4.1. Composition (g/kg DM) of dried molassed sugar beet pulp and barley siftings.

	Dried Molassed Sugar Beet Pulp	Barley Siftings
Dry matter (g/kg FM)	839.3	839.5
Crude protein	109.8	39.4
Crude fibre	142.2	352.2
Ether extract	2.8	7.7
Ash	75.1	78.2
Phosphorus	0.64	0.72
Magnesium	1.69	0.74
Calcium	4.67	1.61

Until the last week of pregnancy, both diets consisted of a 1 : 1 mix of sugar beet pulp and siftings, with 1% urea, 1% salt and 0·3% vitamin/trace element mix added. The HP diet also contained 2% dicalcium phosphate and the LP diet 1·26% ground limestone (in order to balance the additional calcium). From the last week of pregnancy until six weeks of lactation both diets comprised a 2 : 1 mix of sugar beet pulp and siftings with 2% urea and other additions as before. Feeding levels were increased appropriately to supply energy and protein requirements during pregnancy and lactation. Daily amounts of phosphorus, magnesium and calcium received by each ewe are given in Table 2, together with the A.R.C. (1980) recommended requirements.

The sheep were group fed in two approximately equal amounts, morning and afternoon. Blood samples were taken every 2 weeks prior to lambing, then at 1 day post-parturition and weekly for six weeks of lactation. The ewes were then put out to grass and were bled after 3, 7 and 14 days.

Results

Nine ewes in the LP group lost their lambs, largely because of low birthweights and/or poor milk supply, and the growth rates of the lambs born to the LP ewes were also reduced. Mean blood data are given only for the remaining sixteen LP ewes, with an average 1·31 lambs/ewe, throughout the trial. The eight control HP ewes had an average 1·37 lambs/ewe. The mean plasma phosphorus, magnesium and calcium concentrations for both groups are given in Table 3 and Figure 1.

Mean plasma phosphorus concentration decreased steadily for the LP ewes from 1·93 mmol/l to 0·55 mmol/l in the first 10 weeks, and thereafter remained at around 0·4 mmol/l until transfer to pasture after 6 weeks of lactation. It then increased rapidly to 2·45 mmol/l in 3 days, decreasing to 1·83 mmol/l by 2 weeks at grass.

Table 2. Expt.4.1. Daily quantities of diet, Mg, Ca and P given/ewe and A.R.C. (1980) recommended requirements during pregnancy and lactation.

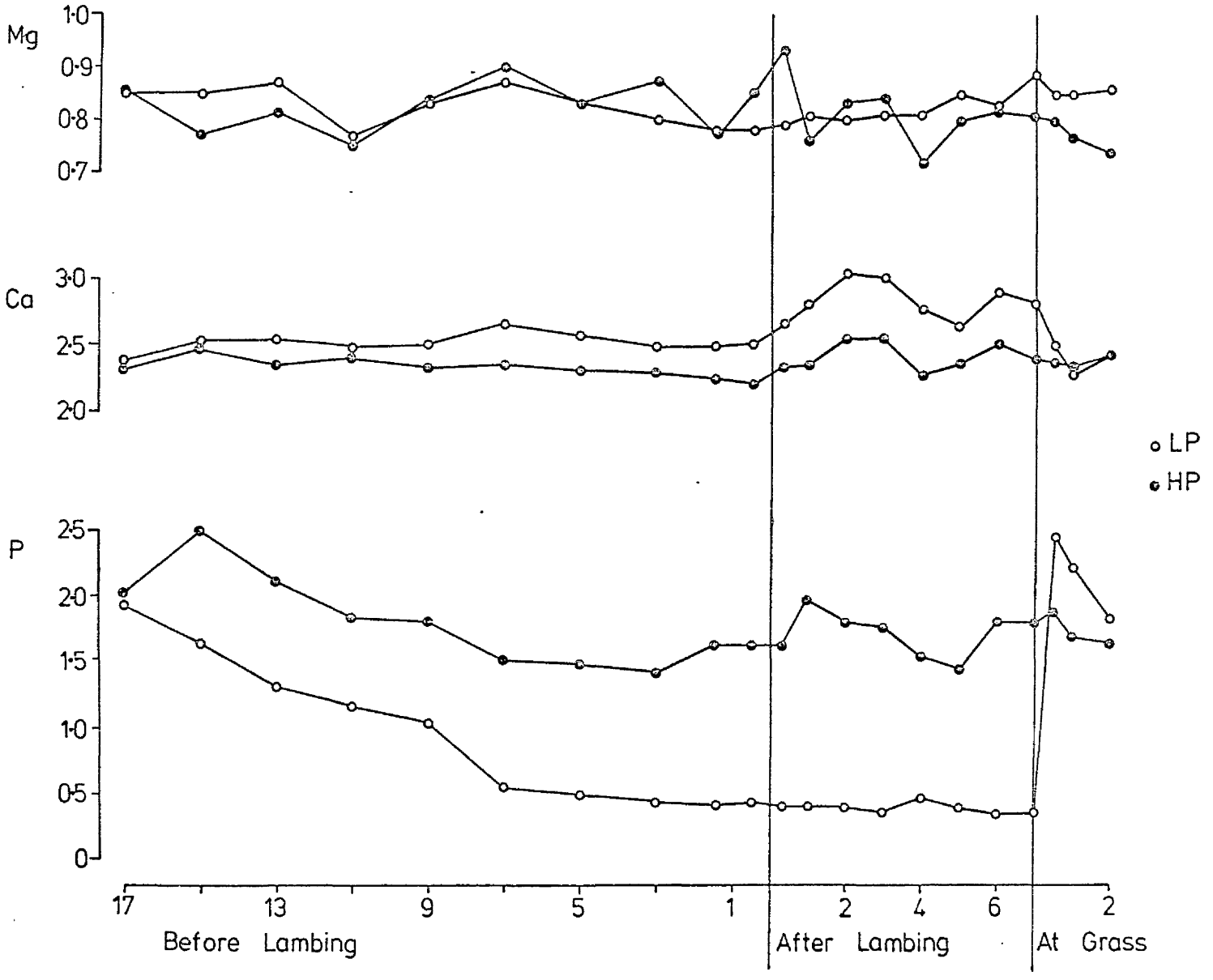
						A.R.C. (1980) daily requirements		
Week of pregnancy	Diet kg FM/day	gMg/day	gCa/day	LP gP/day	HP gP/day	gMg/day (allowance)	gCa/day†	gP/day†
4	0.9(1 : 1)	0.9	6.1	0.5	3.3	-	-	-
7	1.0	1.0	6.8	0.6	3.7	0.95	1.4	1.3
14	1.2	1.2	8.2	0.7	4.4	1.13	1.8	1.7
16	1.5	1.5	10.2	0.85	5.5	1.35	2.7	2.0
20	1.5(2 : 1)	1.8	10.8	0.85	5.5	1.57	3.4	2.2
Lactation	2.0	2.4	14.4	1.1	7.3	1.95-2.95	3.7-6.0	3.4-5.6

† Note that Ca and P requirements were greater in the A.R.C. (1965) 1st Edition which was consulted when this experiment was designed.

Table 3. Expt.4.1. Mean plasma concentrations (mmol/l) of phosphorus, magnesium and calcium

	PHOSPHORUS				MAGNESIUM				CALCIUM			
Weeks before Lambing	LP	S.D.	HP	S.D.	LP	S.D.	HP	S.D.	LP	S.D.	HP	S.D.
17	1.93	0.405	2.02	0.497	0.85	0.135	0.86	0.060	2.38	0.270	2.32	0.188
15	1.64	0.338	2.49	0.446	0.85	0.131	0.77	0.089	2.54	0.123	2.47	0.181
13	1.31	0.297	2.10	0.425	0.87	0.134	0.81	0.137	2.54	0.152	2.35	0.120
11	1.17	0.400	1.83	0.246	0.77	0.167	0.75	0.103	2.48	0.135	2.40	0.183
9	1.04	0.386	1.80	0.754	0.83	0.086	0.79	0.087	2.50	0.205	2.33	0.136
7	0.55	0.288	1.51	0.303	0.87	0.143	0.90	0.142	2.65	0.166	2.35	0.151
5	0.49	0.212	1.48	0.647	0.83	0.146	0.83	0.149	2.57	0.131	2.31	0.247
3	0.44	0.123	1.42	0.359	0.80	0.110	0.87	0.063	2.48	0.206	2.29	0.259
1½	0.42	0.087	1.63	0.400	0.78	0.098	0.77	0.078	2.49	0.198	2.25	0.186
½	0.43	0.171	1.62	0.270	0.78	0.131	0.85	0.182	2.51	0.415	2.21	0.240
After Lambing												
1 day	0.41	0.135	1.62	0.216	0.79	0.113	0.93	0.136	2.65	0.366	2.33	0.119
1 Wk.	0.41	0.126	1.97	0.448	0.81	0.130	0.76	0.121	2.81	0.318	2.35	0.151
2	0.40	0.159	1.79	0.459	0.80	0.113	0.83	0.118	3.04	0.426	2.55	0.327
3	0.36	0.129	1.75	0.496	0.81	0.143	0.84	0.134	3.01	0.441	2.55	0.215
4	0.47	0.243	1.54	0.427	0.81	0.158	0.72	0.087	2.77	0.415	2.28	0.207
5	0.39	0.140	1.44	0.327	0.85	0.125	0.80	0.111	2.65	0.295	2.37	0.185
6	0.35	0.152	1.80	0.437	0.83	0.165	0.82	0.115	2.90	0.512	2.51	0.338
Out to grass												
0 day	0.36	0.126	1.79	0.374	0.89	0.172	0.81	0.140	2.82	0.469	2.40	0.438
3 day	2.45	0.774	1.88	0.602	0.85	0.187	0.80	0.168	2.50	0.249	2.38	0.277
7 day	2.22	0.553	1.69	0.445	0.85	0.164	0.77	0.138	2.29	0.223	2.34	0.194
14 day	1.83	0.443	1.64	0.329	0.81	0.148	0.74	0.092	2.43	0.176	2.42	0.221

Figure 1. Expt.4.1. Mean plasma magnesium, calcium and phosphorus concentrations (mmol/l) for LP and HP ewes throughout the experiment.


Mg
1·0
0·9
0·8
0·7
Ca
3·0
2·5
2·0
P
2·5
2·0
1·5
1·0
0·5
0
o LP
• HP
17
13
9
5
1
2
4
6
2
Before Lambing
After Lambing
At Grass

Mean plasma phosphorus for the HP group increased initially from 2·02 to 2·49 mmol/l, then decreased slightly to around 1·4 - 1·6 mmol/l prior to lambing. After lambing, it was variable but remained (in the normal range) between 1·4 - 2·0 mmol/l and did not alter much at transfer to grass.

Mean plasma calcium concentrations remained slightly higher for the LP group than the HP group throughout pregnancy and lactation, being around 2·5 and 2·3 mmol/l, respectively, just prior to lambing, 3·04 and 2·55 mmol/l 2 - 3 weeks after, and 2·90 and 2·51 mmol/l at 6 weeks after lambing. At turnout to grass plasma calcium dropped for the LP group to the same level as the HP group, at 2·3 - 2·4 mmol/l.

Mean plasma magnesium concentrations for both groups remained relatively steady throughout, at between 0·72 amd 0·93 mmol/l, with no significant treatment differences and with little change at the turnout to grass.

Appetite declined in the LP group from about 7 weeks prior to lambing when plasma phosphorus had fallen to below 0·55 mmol/l. The LP ewes then consumed an average of only 1 kg fresh matter daily until they lambed, and 1·0 - 1·2 kg in lactation. The HP ewes fully consumed the amount offered (Table 2) which was limited to 2 kg/head/day after lambing.

Discussion

The low phosphorus diet effectively reduced plasma phosphorus levels in pregnant ewes and values remained low throughout lactation. Appetite was depressed in these ewes after 10 weeks on the diet, when mean plasma phosphorus dropped below 0·55 mmol/l, and this is a well-documented symptom of phosphorus deficiency (e.g. A.R.C. 1980). There was consequently considerable loss in production by these ewes (reported elsewhere). They were consuming only 50 - 60% of their daily ration, but their dietary mineral requirements (as given in Table 2) were

presumably also lowered by the decreased level of production. However, in spite of this under nutrition, mean plasma magnesium changed very little in both HP and LP groups throughout pregnancy, lactation and subsequent turnout to grass. Plasma calcium levels were generally higher for the LP ewes than for the controls especially during lactation. This may be attributed to some bone resorption due to the phosphorus deficiency releasing calcium into the blood (and the amounts of magnesium released were too small to raise plasma magnesium significantly). At turnout to grass, both calcium and phosphorus levels were unaltered in the HP group but in the LP group plasma phosphorus rose rapidly, with a simultaneous drop in plasma calcium. Thus recovery to normal plasma phosphorus levels was rapid (3 days) after the long period of phosphorus depletion (c.23 weeks).

It was evident from this trial that the low phosphorus diet given to ewes in pregnancy and lactation was not a successful method of inducing hypomagnesaemia. Mean plasma magnesium remained within the normal range throughout and any individual ewes with slightly sub-normal values during the trial had such values at the start of the experiment.

In subsequent work (not reported in this thesis) these studies have been extended to compare the A.R.C. (1980) requirements for phosphorus with those suggested by the A.R.C. (1965). It has been confirmed that the reduced A.R.C. (1980) requirements are fully adequate under these experimental conditions to maintain normal blood phosphorus concentrations and voluntary feed intake.

SECTION IV

MAGNESIUM SUPPLEMENTS GIVEN TO ANIMALS IN OUTDOOR SITUATIONS

The indigestible faecal marker technique for availability assessments, using chromic oxide incorporated with dietary magnesium supplements (Section II B), has an important application for use with livestock at grass. Hypomagnesaemia in adult ruminants occurs almost exclusively at pasture and it is therefore important to conduct availability investigations in this natural situation. There are, however, no published reports on supplementary magnesium availability using animals at grass. In addition, availability results obtained indoors using animals given concentrate diets may not necessarily be assumed to reflect the outdoor situation. Complete balance trials are impractical for animals at pasture as total faeces collections are virtually impossible to obtain and it is difficult to assess individual total intakes of grass. However, using the indigestible marker technique single faecal "grab" samples are sufficient to carry out regression analysis of faecal magnesium on faecal chromium concentrations for availability calculation, and, in addition, knowledge of precise individual food intakes (i.e. grass) is not required. The supplement is given with a constant ratio of magnesium to chromium and so precise individual intakes are not critical. In practice, a range of supplementary intakes gives a range of faecal magnesium and chromium concentrations and therefore gives a more convincing regression line. Large numbers of animals can be used in natural farm situations and the effects of the variations in individual grass intakes are levelled out in the regression analysis. In addition, the magnesium supplement (with associated chromium) can be presented in various ways and formulations as used in practice thus providing, in addition to availability assessments, data on the variation in individual intakes in each situation. Kendall (1977) has shown that a single rectal grab sample of faeces gives a very good indication of relative feedblock intake within a group of animals, as assessed by the range of faecal chromium

concentrations. Thus apparent availabilities of magnesium supplements to animals at grass can be obtained and, simultaneously, information can be accumulated to define the optimum mode of presentation that ensures relatively uniform intakes within a group. In addition, blood samples can be taken which allow an assessment of the general magnesium status of a herd in a particular situation as well as giving individual blood magnesium concentrations which may be compared with the individual supplementary magnesium intakes. Cattle have been used in the following experiments in preference to sheep as grab samples of faeces are generally easier to obtain from the former and they are quieter to handle outdoors.

The following field experiments divide into three main sections:

A. Group feeding beef cows from troughs. (The supplement is either included in a concentrate cube or added loose to a loose-mixed concentrate).

B. Free access magnesium supplements.

C. Dairy cows receiving individual magnesium supplementation.

The experiments described in Sections A and B are therefore concerned with two common farm practices for the provision of supplementary magnesium to herds of cattle outdoors, namely the addition of magnesium supplements to a concentrate food (Section A) and giving magnesium as part of a free-access supplement (Section B).

Finally, there is a general discussion and comparison of the supplementary magnesium intake data obtained in the experimental work in Sections A, B and C.

A. Group feeding beef cows from troughs.

Experiment 5.1. The availability of supplementary magnesium in a concentrate cube group-fed to beef cows outdoors.

Introduction

The present experiment describes three trials with beef cows in each of which the same four magnesium supplements (three oxides and one hydroxide) were given in a concentrate cube. The animals were group-fed in troughs outside. Part 1 used lactating beef cows given hay and concentrates outside in early spring, Part 2 used lactating beef cows at spring pasture, and Part 3 employed beef cows during the calving period (i.e. late pregnancy and early lactation) at grass in the late summer. Apparent availability was calculated for each supplement in each situation, and variations in individual intakes were noted.

Materials and Methods

Magnesium supplements

Three calcined magnesites (MgO) and one magnesium hydroxide were investigated in these trials. Random samples of the commercial granular products (1979) from Spain (A; Agma FG85) and Greece (B; Grecian Magnesite) were obtained. Particle size analyses, given in Appendix 1, show that A contains particles only up to 1·0 mm while B contains less fine particles and some coarser granules up to 2·0 mm. A random sample of the Greek commercial powdered calcined magnesite (C; Grecian Magnesite), which would normally be used for pasture-dusting, was also used. From Table 1 it can be seen that the Greek products contain less magnesium than the Spanish. Lastly for comparative purposes, a sample of commercial finely powdered English sea-water derived magnesium hydroxide (D; Steetley Minerals Ltd.) was investigated.

Table 1. Expt.5.1. Magnesium supplements.

	Supplement	gMg/kg
A	Spanish calcined magnesite (0 - 1 mm)	516.2
B	Greek calcined magnesite (0 - 2 mm)	436.6
C	Greek powdered calcined magnesite	510.3
D	Powdered magnesium hydroxide	396.0

Experimental design

Part 1. Fourteen lactating beef cows (mainly Hereford cross) outside in April-May, just over six weeks after calving, were used in the trial. They had been indoors throughout the winter, given oat straw and sugar beet pulp, and as the pasture was still sparse at turn-out, they were now fed once daily hay (1·06 g Mg/kg DM) and concentrates (1·78 g Mg/kg DM). In addition they were group fed in three 2·75 m troughs (with access from both sides) about 1 kg/head/day of a supplementary high magnesium, cubed concentrate at approximately 09.00 hours. This comprised approximately 842·5 g/kg FM ground barley, 50 g/kg calcined magnesite†, 7·5 g/kg chromic oxide and 100 g/kg soya bean meal (to facilitate the cubing process). These ingredients were well mixed, especially the calcined magnesite with the chromic oxide, prior to cubing. Representative samples were taken from each batch of cubes, dried and analysed for magnesium and chromium; the mean magnesium/chromium concentration ratio for each supplement is given in Table 2.

†i.e. a daily individual allowance of 50 g (approx. 2 ozs) calcined magnesite which is the generally recommended level of magnesium supplementation.

The trial lasted four weeks, and during each week a different magnesium supplement was used in the supplementary concentrate cubes (in the order A, C, B, D). After each seven-day feeding period, rectal grab samples of faeces were taken (during the morning) dried and ground for magnesium and chromium analyses. Blood samples were also taken at about 10.00 h for plasma magnesium and calcium analyses.

Part 2. In the following May-June the same cattle were transferred to lush spring pasture (1·6 g Mg/kg DM), together with an additional ten similar lactating cows and heifers, forming a herd of twenty-four. Supplement cubes were made up as in Part 1 and were given at 1 kg/head/day as before for one week each (in the order A, C, B, D) in five 2·75 m troughs (with access from both sides). Faeces and blood samples were taken at the end of each week at about 10.00 h.

Part 3. A separate herd of seventeen mainly Hereford-cross beef cows was used during the calving period at pasture (2·10 g Mg/kg DM) in August. They were given daily 0·75 kg/head[+] of the magnesium-rich cubes as given in Parts 1 and 2, in the order D, C, B, A, in four 2·75 m troughs (with access from both sides). Faeces and blood samples were taken at the end of each week. However individual cows were undisturbed if they had very recently calved or calving was imminent.

Results

Faecal data and availabilities (Table 2).

The apparent availability of each supplement was calculated from the appropriate regression coefficient of faecal magnesium on faecal chromium concentration, by the method described in Section II B. The amount of magnesium supplied by the barley in the supplementary cubes was

[+] i.e. About 37 g calcined magnesite/head/day or 75% of the usual recommended allowance

minimal (average 1·3 g/cow/day in Parts 1 and 2, 1·0 g in Part 3) compared with that provided in the calcined magnesite (average 25 g/cow/day in Parts 1 and 2, 19 g in Part 3), i.e. under 5% of the total, and was therefore disregarded in the availability calculations. Examples of regression graphs obtained in this experiment are given in Figures 1 and 2.

Table 2. Expt.5.1. Faecal magnesium and chromium regression data, and apparent availabilities of supplements.

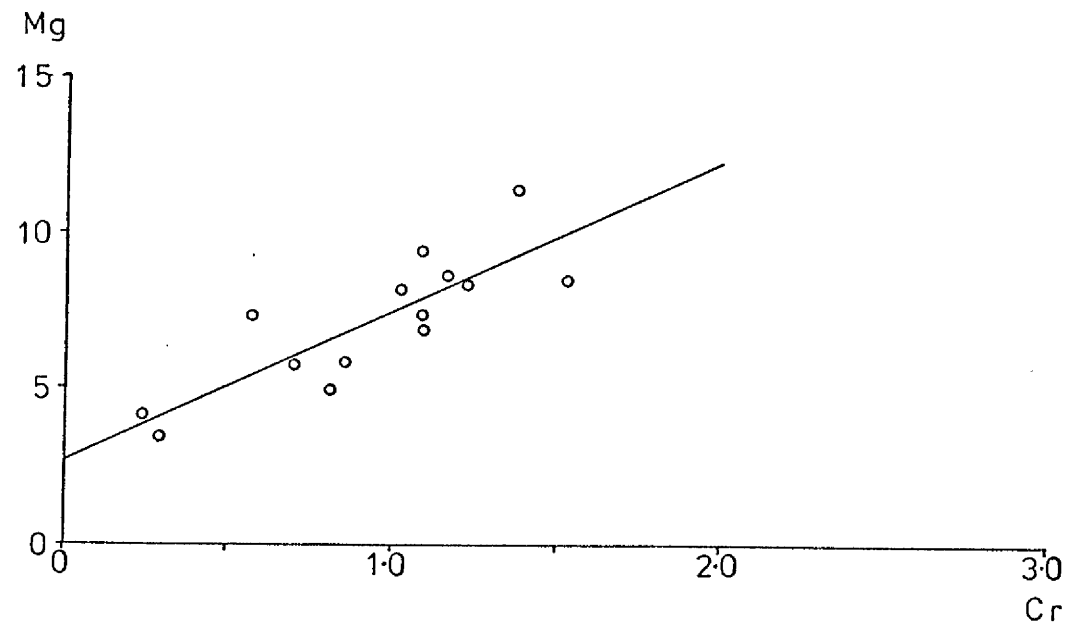
		Regression of faecal Mg on Cr				
Supplement	Supplement Mg/Cr ratio	Correlation coefficient	Regression coefficient (b)	SEb	Apparent availability %	SE avail.
PART 1						
A	6.4068	0.9571***	6.5804	0.5749***	- 2.7	8.97
B	5.2915	0.8316***	4.7020	0.9066***	11.1	17.13
C	5.4121	0.7423**	2.4271	0.6324**	55.2	11.68
D	4.5604	0.7213**	2.2985	0.6372**	49.6	13.97
					A < C**, D*; B < C*	
PART 2						
A	5.8842	0.8590***	2.8024	0.3483***	52.4	5.92
B	5.2349	0.9322***	3.7338	0.3191***	28.7	6.10
C	5.4131	0.9284***	3.0726	0.2564***	43.2	4.74
D	4.7048	0.7379***	2.2781	0.4442***	51.6	9.44
					B < A*	
PART 3						
A	4.8821	0.5250*	2.3406	1.0142*	52.0	20.77
B	5.1008	0.8845***	2.5600	0.4336***	49.0	8.50
C	6.0980	0.7329***	3.0077	0.7208***	50.7	11.82
D	4.1480	0.6048*	1.2075	0.4410*	70.9	10.63
					N.S.	

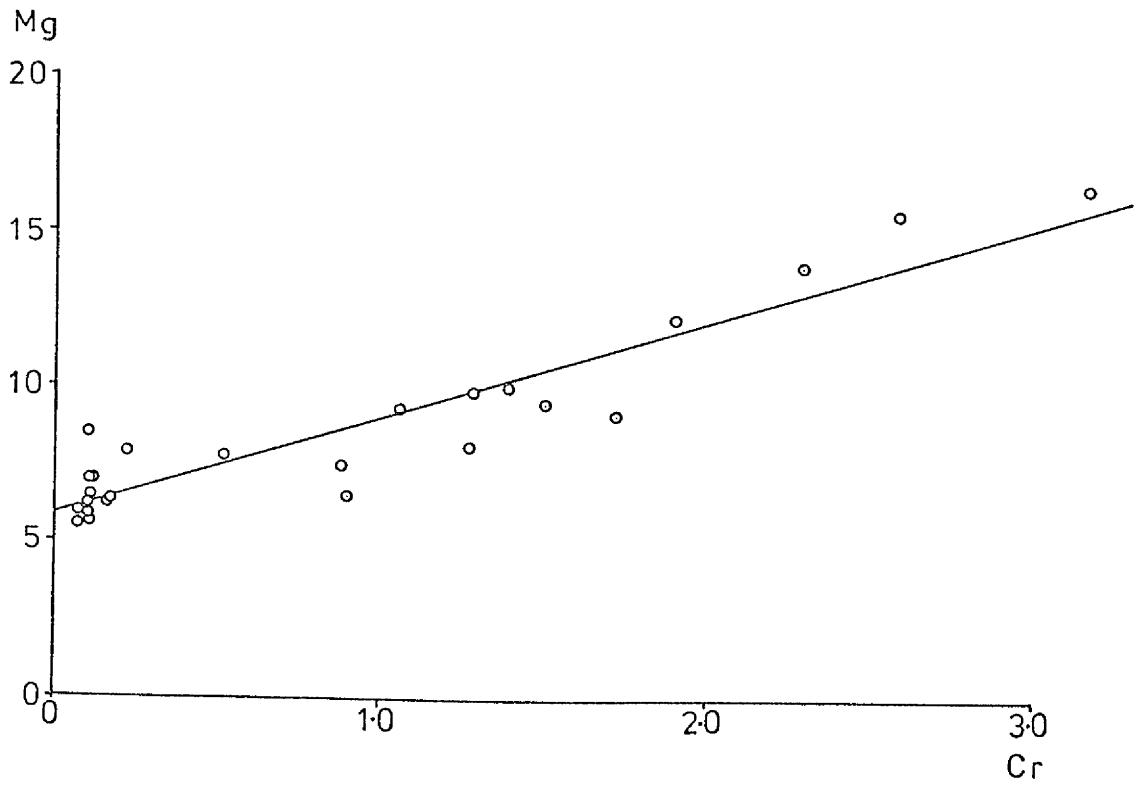
Figure 1. Expt.5.1. Regression between faecal magnesium and faecal chromium concentrations (g/kg DM) for cows given a basal diet of hay and concentrates (Part 1) supplemented with concentrate cubes containing Greek granular calcined magnesite (B).

$$y = 2.7081 + 4.7020x \; ; \quad r = 8316^{***}$$

Figure 2. Expt.5.1. Regression between faecal magnesium and faecal concentrations (g/kg DM) for cows at spring grass (Part 2) supplemented with concentrate cubes containing Greek powdered calcined magnesite (C).

$$y = 5.9276 + 3.0726x \; ; \quad r = 0.9284^{***}$$


Mg
15
10
5
0
0
1·0
2·0
3·0
Cr


Mg
20
15
10
5
0
0
1·0
2·0
3·0
Cr

Part 1. Regression and correlation coefficients were significant (A,B, $P<0.001$; C,D, $P<0.01$) and the apparent availability of A (-2·7%) was significantly less than that of D (49·6%, $P<0.05$) and C (55·2%, $P<0.01$). B was significantly ($P<0.05$) less available, at 11·2%, than C. The variation in faecal chromium contents of the cows, expressed as the coefficient of variation (CV%) was 46·2% over all periods (41·5, 51·8, 40·8 and 53·2% in periods 1, 2, 3 and 4, respectively).

Part 2. All regression and correlation coefficients were very highly significant ($P<0.001$). Availabilities were similar for A, C and D, at 52·4, 43·2 and 51·6%, respectively, but B was less available, at 28·7% ($B<A$, $P<0.05$). The CV% of faecal chromium concentration was 90·8% over all periods (91·3, 104·1, 91·3 and 75·9% for periods 1, 2, 3 and 4 respectively).

Part 3. Regression and correlation coefficients were less significant ($P<0.05$) for A and D, but highly significant ($P<0.01$) for supplements B and C. Availabilities were similar for all four supplements at 52·0, 49·0, 50·7 and 70·9% respectively for A, B, C and D, with no statistically significant differences. The CV% of faecal chromium concentration was 87·9% over all periods (88·5, 96·9, 85·7 and 80·3%, respectively, in periods 1, 2, 3 and 4).

Blood data

Mean plasma magnesium and calcium concentrations for the cows at the end of each seven-day feeding period are given, with standard deviations, in Table 3. However, it must be noted that these mean concentrations do not necessarily reflect the relative efficiencies of the magnesium supplementary treatments as individual intakes of both supplements and grass varied. They do, however, give an indication of the general magnesium status of the herd. Also, individuals with particularly high (or low) blood magnesium concentrations are usually seen to be those with particularly high (or low) faecal chromium, i.e. those

that ate markedly more (or less) than the average supplementary cube intake.

Table 3. Expt.5.1. Mean plasma magnesium and calcium concentrations (mmol/l).

Period of supplementation	1	SEM	2	SEM	3	SEM	4	SEM
PART 1								
Treatment	A		C		B		D	
Mg	0.84	0.026	0.75	0.029	0.80	0.020	0.87	0.023
Ca	2.66	0.072	2.55	0.033	2.38	0.030	2.21	0.033
PART 2								
Treatment	A		C		B		D	
Mg	0.68	0.024	0.81	0.030	0.75	0.037	0.75	0.022
Ca	2.66	0.041	2.45	0.047	2.30	0.029	2.64	0.021
PART 3								
Treatment	D		C		B		A	
Mg	0.63	0.028	0.55	0.044	0.51	0.053	0.42	0.042
Ca	2.49	0.036	2.61	0.044	2.45	0.021	2.31	0.030

Part 1. Mean plasma magnesium concentration remained within the normal range throughout, at 0.75 - 0.87 mmol/l. Calcium levels declined slightly (though remaining fully normal) with each subsequent feeding period, from 2.66 to 2.21 mmol/l.

Part 2. Mean plasma magnesium concentration was slightly sub-normal, at 0.68 mmol/l, after the first seven-day period following transfer to spring grass, but thereafter remained normal, at 0.75 - 0.81 mmol/l. Plasma calcium concentrations again remained normal, at 2.30 - 2.66 mmol/l.

Part 3. Mean plasma magnesium concentration was low throughout this part of the experiment, falling from 0.63 mmol/l in the first week to 0.42 mmol/l in the fourth. However, the mean plasma calcium remained well within the normal range, at 2.31 - 2.61 mmol/l.

Discussion

The range of individual intakes of supplementary cubes can be seen visually in the regression graphs given in Figures 1 and 2 as the scatter of individual faecal chromium concentrations. A high faecal chromium is associated with high faecal magnesium content, indicating a high intake of supplement, and a low or zero faecal chromium indicates low or zero supplement intake, and the faecal magnesium of such individuals is derived solely from the basal diet (hay and concentrates in Part 1, grass in Parts 2 and 3). This range of values aids the calculation of significant regression lines from which availabilities are calculated.

In Parts 1 and 2 of the experiment, the Greek granular calcined magnesite (C.M.) was less available than the Greek powdered product, whereas their availabilities were similar in Part 3. However, over the three parts of the experiment their mean availabilities were 29·6% and 49·7% respectively, indicating that the latter was generally more available. The powdered magnesium hydroxide was also consistently highly available, with a mean value of 57·4%. The Spanish C.M. provided an anomalous result in Part 1 (-2·7% available) especially in view of the extremely close values of 52·4 and 52·0% in Parts 2 and 3 (which were significantly ($P<0·05$) greater than the first result). The overall mean availability was 33·9%, but this is increased to 52·2% if the anomalous result is excluded. The Spanish granular C.M. was therefore overall generally similar or slightly better than the coarser Greek granular product in this experiment, indicating the importance of the source of material affecting its availability. However, it is clear

that the powdered products are consistently more available, and as the supplements were incorporated in a concentrate cube there was no problem with decreased palatability of these materials due to dustiness.

During Part 1 none of the cows became seriously hypomagnesaemic, the lowest plasma magnesium concentrations recorded being 0·64 and 0·65 mmol/l which are subnormal but not critical with respect to clinical tetany. The mean herd plasma magnesium concentration remained within the normal range as one would expect when cows are receiving adequate hay and concentrates. There was some variation in individual intakes of the supplementary cubes as indicated by the coefficient of variation (CV) of faecal chromium concentrations, which was 46·2%, even although there was adequate trough space at 0·6 m/cow (with access from both sides). If the mean faecal chromium concentration is taken to reflect a mean intake of 1 kg cubes, i.e. 50 g C.M./day, it can be seen that some individuals consumed 1·4 - 1·8 times as much, i.e. 72 - 92 g C.M., while others ate 0 - 0·1 times as much, i.e. under 5 g C.M. One individual (cow no.9) consistently consumed negligible quantities of supplementary cubes (negligible chromium in the faeces) but her plasma magnesium content remained normal, at 0·78 - 0·93 mmol/l. Also, individuals which consumed the largest quantities of supplement were not necessarily those with the highest plasma magnesium concentrations.

In Part 2 the mean plasma magnesium concentration of the herd dropped during the first week after transfer to spring pasture but thereafter rose to just within the normal range for the following three weeks. In each period there were several individuals (5 to 17) with plasma magnesium concentration below 0·74 mmol/l, of which up to 5 cows had levels below 0·60 mmol/l. The lowest recorded plasma magnesium was 0·375 mmol/l, but plasma calcium levels were normal, over 2.0 mmol/l throughout, and no cases of clinical tetany occurred. Three individuals had low plasma magnesium values throughout (0·375 - 0·70 mmol/l) and also extremely low

faecal chromium, indicating negligible supplement intake. However, four other cows (including cow no.9 as in Part 1) had low faecal chromium values but entirely normal plasma magnesium, while one or two individuals in each period had low blood magnesium but markedly high faecal chromium concentrations. In each period each individual cow maintained a similar intake of supplementary cubes, especially the noticeably "greedy" and noticeably "shy" feeders. The CV of faecal chromium was 90·8% in this Part of the experiment, i.e. about twice that in Part 1 despite a similar trough space of 0·6 m/cow. This indicates a higher variation in supplement intake when ample grazing is available compared with when concentrates are being given in the basal diet. Some individuals apparently consumed 2·3 - 3·6 times the mean supplementary ration allowance, i.e. 113 - 180 g C.M., whereas others ate under 5 g C.M./day. The greedy cows were therefore consuming excess magnesium and were in danger of scouring (Care, 1960d) while some shy feeders were indeed those that had the greatest need for supplementary magnesium to raise their blood magnesium levels.

In Part 3 this separate herd of cows during their calving period at relatively poor autumn pasture were apparently under greater magnesium stress and were generally hypomagnesaemic throughout. Mean plasma magnesium decreased from periods 1 to 4 in spite of the provision of supplementary magnesium (albeit at only 37·5 g C.M./cow/day, 75% of the standard recommended allowance), possibly due to the advancement of the calving period and consequent increase in the proportion of lactating cows (with heavier magnesium demand). Several individual plasma magnesium values below 0·40 mmol/l were recorded (but with associated normal calcium concentrations), which would be considered at risk with respect to clinical tetany, but only one case of tetany occurred, in a very recently calved cow at the end of period 4 (treated, with subcutaneous magnesium sulphate and calcium borogluconate, and cured). This cow never consumed any supplementary cubes and her plasma magnesium concentration

prior to calving was 0·45 mmol/l (with normal calcium 2·54 mmol/l), thus again giving an example of a shy feeder being one with a demonstrable need for supplementary magnesium. Indeed, four individuals consistently had very low blood magnesium (0·1 - 0·5 mmol/l) and negligible faecal chromium. However, one individual in each period had zero faecal chromium but relatively higher plasma magnesium (0·6 - 0·7 mmol/l). Visual observations at feeding time with respect to "greedy" and "shy" cows were invariably reflected in high or low faecal chromium contents. Some individuals were apparently consuming 2·1 - 3·2 times the mean intake of 0·75 kg cubes (37·5 g C.M.), i.e. 80 - 120 g C.M., whereas five or six individuals in each period consumed no cubes at all. One individual cow consistently ate the most cubes but, surprisingly, her plasma magnesium was not particularly high (0·495 - 0·71 mmol/l). The CV of faecal chromium was very similar, at 87·9%, to that obtained for the separate herd at grass in Part 2, and the trough space allowance (0·65 m/cow) was certainly similar. From this Part, however, it is clear that offering 37·5 g C.M./cow/day in trough-fed cubes was an unsatisfactory method for the prevention of hypomagnesaemia in productive cows in a risk situation, as some cows consumed an excess of magnesium supplement while others remained wholly unprotected.

Thus this experiment has given useful comparative availability data which showed differences due to source and particle size of the magnesium supplement. In addition it has demonstrated the wide range of individual intakes in a group-feeding situation (in spite of more than adequate trough space allowances), especially with animals at pasture (CV = c.90%) when they are most in need of supplementary magnesium, whereas intakes varied less (CV 46%) when hay and concentrate feeding was in practice and the need for supplementary magnesium was less.

Experiment 5.2. Availabilities of different particle sizes of calcined magnesite given with concentrates in troughs to lactating beef cows at spring grass.

Introduction

Inwintered, lactating beef cows are known to be at risk of hypomagnesaemia and hypomagnesaemic tetany immediately following transfer to spring pasture. The present experiment follows the blood magnesium and calcium levels of such a herd divided during the first week into cows that received supplementary magnesium (calcined magnesite) and unsupplemented control animals. For the subsequent three weeks all cows were offered supplementary magnesium, and availabilities of different particle size grades of calcined magnesite were determined. In addition, data on individual supplement intakes in this group-feeding situation were obtained.

Materials and Methods

Magnesium supplements

The four magnesium supplements used in this tiral were different particle size ranges of Spanish calcined magnesite, Agma FG85 (1979 product). Appropriately large and representative quantities of each fraction were provided by Chance and Hunt from the large scale sieving of several sacks of calcined magnesite obtained at random from the production plant over a period of one month. "Fine" (under 75 μm, F), "medium" (150 - 250 μm, M) and "coarse" (500 - 1,000 μm, C) fractions were used, as well as a sample of the "whole" Spanish product, W (particle size analysis given in Appendix 1) obtained from several riffled sacks. Each calcined magnesite (C.M.) was thoroughly mixed with chromic oxide (673·3 g Cr/kg), in the ratio 1·00 kg C.M. with 150 g chromic oxide, prior to administration to the animals.

Table 1. Expt.5.2. Magnesium supplements.

	Supplement	gMg/kg
F	Spanish calcined magnesite, 75 μm	506.7
M	" " " , 150 - 250 μm	524.2
C	" " " , 500 - 1,000 μm	524.1
W	" " " , whole product, 0 - 1,000 μm	524.6

Experimental Design

Twenty-three lactating beef cows, mainly Hereford cross and about 8 weeks after calving, were transferred to spring grass after being in-wintered on a diet of oat straw and sugar beet pulp. During the first eight days after turn-out (Period 1) the herd was divided into two (12 cows in group (a), 11 in (b)) for supplementary feeding at approximately 10.00 hours. Both groups were offered about 1 kg/head/day rolled "Propcorn-treated" barley in troughs and group (a) also received, added to the surface of the barley as evenly as possible, a mixed supplement of "medium" calcined magnesite (M) and chromic oxide to provide about 56 g (2 oz) calcined magnesite/head/day. After feeding and the troughs had been cleared all cows were allowed to graze together. For the following three weeks two additional similar cows joined the herd, and all 25 cows were now fed together. In addition to the barley they received a different calcined magnesite in each seven-day period, at 56 g/head/day : C, F and W in Periods 2, 3 and 4 respectively.

Blood samples were taken at about 10.00 h on the day of transfer to grass, and after three and eight days. On the eighth day, in addition, the cows were bled at 14.00 and 20.00 h, and again at 10.00 h on the ninth day. Thereafter the cows were bled at the end of each feeding period at 10.00 h, (i.e. all the blood samples were obtained 24 hours after each feed of supplement). All blood samples were analysed for plasma magnesium and calcium concentrations.

Faecal grab samples were taken on the last day of each period, dried, ground and analysed for magnesium and chromium.

Results

The magnesium content of the grass was low in the first week, at 1·6 g Mg/kg DM, but increased during the experiment to 2·4 g Mg/kg DM in period 4.

Blood data (Table 2)

The mean plasma magnesium concentration fell rapidly after transfer to spring grass and in three days that of the supplemented group had fallen from 0·78 to 0·66 mmol/l while that of the control group fell from 0·82 to 0·55 mmol/l, a significantly less reduction ($P<0.05$) for the supplemented cows. However, after eight days of grazing, mean plasma magnesium concentrations had returned to normal for both groups, 0·85 mmol/l for the supplemented group and 0·82 mmol/l for the controls, and were not significantly different. Serial bleeding on day 8 showed there to be no marked rise or fall in plasma magnesium during the 24 hours between feeds for both supplemented and unsupplemented cows. After the second week at grass, plasma magnesium was 0·93 mmol/l after all cows had been offered supplement C, but in the third and fourth periods mean plasma magnesium was 0·83 and 0·85 mmol/l, respectively (supplements F and W).

Mean plasma calcium concentration rose slightly from 2·20 - 2·25 mmol/l at the start to 2·47 - 2·52 mmol/l on day 9 and thereafter remained between 2·34 - 2·50 mmol/l, i.e. entirely normal throughout.

Faecal data and availabilities (Table 3)

Faecal magnesium and chromium regression data are given in Table 3, and regression and correlation coefficients are very highly significant in all four periods ($P<0.001$). The apparent availabilities for F, M and C are 48·4, 27·5 and 7·9%, respectively, and that for W was 22·7%

F was significantly more available than C ($P<0.001$), W ($P<0.01$) and M ($P<0.05$).

The coefficient of variation (CV) of faecal chromium concentrations was 128·8% for the twelve supplemented cows in period 1, and 101·7% for all twenty-three animals over periods 2 to 4 (121·0%, 84·4% and 108·7% in periods 2, 3 and 4, respectively).

Table 2. Exot.5.2. Mean plasma magnesium and calcium concentrations (mmol/l) throughout the experiment.

	MEDIUM C.M.				CONTROLS			
	Mg	SEM	Ca	SEM	Mg	SEM	Ca	SEM
Day 0	0.78	0.041	2.20	0.082	0.82	0.039	2.25	0.026
Day 3	0.66	0.030	2.34	0.031	0.55	0.054	2.33	0.058
Fall in Mg, day 0-3	0.12	0.039			0.27*	0.038		
Day 8: 10.00 h	0.85	0.034	2.44	0.024	0.82	0.042	2.48	0.032
14.00 h	0.89	0.034	2.40	0.020	0.81	0.039	2.46	0.038
20.00 h	0.88	0.031	2.43	0.016	0.80	0.034	2.48	0.034
Day 9: 10.00 h	0.88	0.020	2.47	0.018	0.81	0.035	2.52	0.043
	COARSE C.M.							
After 1 week	0.93	0.033	2.34	0.016				
	FINE C.M.							
After 1 week	0.83	0.031	2.50	0.026				
	WHOLE C.M.							
After 1 week	0.85	0.031	2.47	0.032				

Table 3. Expt.5.2. Faecal magnesium and chromium regression data, and apparent availabilities.

		Regression of faecal Mg on Cr					
Supplement	Supplement Mg/Cr ratio	Correlation coefficient (r)	Regression coefficient (b)	SE_b	Apparent availability (%)	SE avail.	Significance
M	5.1906	0.9061***	3.7637	0.3835***	27.5	7.39	
C	5.1901	0.9273***	4.7823	0.4153***	7.9	8.00	
F	5.0172	0.9282***	2.5888	0.2264***	48.4	4.51	F>M*; W**; C***
W	5.1946	0.9159***	4.0153	0.3839***	22.7	7.39	

Discussion

The barley and the added mixed calcined magnesite (C.M.)/chromic oxide supplements were generally well eaten throughout. The barley contained 1·3 g Mg/kg D.M., providing an average of only about 1·1 g Mg/cow/day which was considered negligible in comparison with the 28 g Mg offered/cow/day from the C.M. and was therefore ignored in the availability calculations. However the supplement availabilities quoted are technically the composite availabilities of the magnesium in the supplement plus some from the barley. The availability values obtained confirm previous findings that "fine" (0 - 75 μm), "medium" (150 - 250 μm) and "coarse" (500 - 1,000 μm) C.M.'s are in decreasing order of merit, at 48·4, 27·5 and 7·9% respectively, with the "whole" product (0 - 1,000 μm) having an availability of 22·7%. The fine, medium and coarse ranges comprise, respectively, about 10, 23 and 21% of the whole product, with the remaining material being 18% 75 - 150 μm (between "fine" and "medium") and 28% 250 - 500 μm (between "medium" and "coarse"). Thus an overall availability for the whole product of 22·7%, indicating a fairly high proportion of medium to coarse particles, could be expected. However, it is interesting that the fine particle size range is significantly more available ($P<0·01$) that the whole commercial product itself.

The plasma magnesium concentrations of all the cows dropped rapidly at transfer to spring grass. This is characteristically a period of major risk of hypomagnesaemic tetany but no clinical cases occurred despite extremely low plasma magnesium values. In the unsupplemented group, nine of the eleven cows had plasma magnesium values less than 0·74 mmol/l after 3 days, and four had levels below 0·40 mmol/l which were considered at risk, especially as one cow (plasma magnesium 0·26 mmol/l) also had low plasma calcium at 1·77 mmol/l. In the supplemented group, however, only seven of the twelve cows had plasma magnesium contents below 0·74 mmol/l and none were below 0·40 mmol/l. However, after a further

five days, on day 8, all plasma magnesium levels increased so that only three of the unsupplemented cows had values below 0·74 mmol/l, the lowest being 0·60 mmol/l. Only one of the supplemented group had plasma magnesium under 0·74 mmol/l, at 0·48 mmol/l, and interestingly this particular cow consumed the largest quantity of supplementary magnesium (highest faecal chromium concentration). Provision of supplementary magnesium at 56 g C.M./cow/day with a carrier concentrate in troughs for cows immediately after turn-out to grass evidently did not prevent development of some degree of hypomagnesaemia in the herd during the first week. However individual intakes of supplement were extremely varied, as shown by the CV of faecal chromium of 129%. Five of the twelve cows consumed no supplementary feed at all whereas three consumed two to four times the mean intake, i.e. calculated to be about 112 - 224 g C.M./day. The former five were observed not to approach the troughs and indeed had no faecal chromium, however their plasma magnesium levels remained relatively high, all being over 0·82 mmol/l on day 8, although two had values of 0·62 and 0·69 mmol/l on day 3. Anomalously, the three greedy cows had lower plasma magnesium concentrations on day 3 (below 0·60 mmol/l) and although two had levels over 0·81 mmol/l on day 8, one individual had a level as low as 0·54 mmol/l. Thus the variations in individual supplementary magnesium intake were, perhaps surprisingly, not necessarily reflected in individual plasma magnesium concentrations, although the supplementary group as a whole suffered a lesser initial fall ($P<0·05$) in mean plasma magnesium following transfer to grass than did the controls.

The average CV of faecal chromium concentrations over periods 2, 3 and 4 (together), when all twenty-three cows were supplemented, was slightly less than in the first week, at 102%, but it varied from period to period. The CV was generally greater than in the previous trial with cows at grass (Experiment 5.1., when it was 90%) but the supplement in this present experiment (5.2.) was inevitably less evenly admixed with

the barley (because it was sprinkled on the top of the braley in the trough) and this therefore introduced further variation. The change in the CV between periods 2 - 4 may also be an indication of the relative acceptabilities of the "loose-fed" supplements. If greater intake variation is taken to reflect reduced acceptability of the supplement, the fine particle size range is apparently more acceptable than either the coarse particles or the whole granular product, which is contrary to the popular belief that powdered materials are relatively more unpalatable to livestock.

In periods 2, 3 and 4, one cow consistently ate the most supplement, 3·5 to 4·7 times the mean intake, i.e. 200 - 260 g C.M. daily, and this cow also had a consistently high plasma magnesium concentration, 0·86 - 1·06 mmol/l. However two cows continually failed to consume any appreciable amounts of supplement and their plasma magnesium concentrations fell from 0·74 to 0·60 mmol/l and from 0·72 to 0·44 mmol/l. One cow ate no supplement in periods 2 and 3 and had plasma magnesium values of 0·62 and 0·57 mmol/l (respectively), but she had a high intake in period 4 when her plasma magnesium was raised to 0·82 mmol/l. In period 2, of the four cows with plasma magnesium concentrations less than 0·74 mmol/l, only two had markedly low supplement intakes, whereas seven of the eight hypomagnesaemic cows in period 3 and all five hypomagnesaemic cows in period 4 did have markedly low intakes. Thus although 56 g C.M. was offered/cow/day on a group basis, individual consumptions could vary from 0 to 260 g C.M. therefore leaving some animals unprotected with respect to hypomagnesaemia and others in possible danger of scouring due to excess magnesium intakes. Generally cows are not very interested in trough-feeding immediately after transfer to lush spring grazing, at a time when they all require supplementary magnesium.

In contrast to the consistent findings with sheep (Experiment 3.3., Ritchie & Hemingway, 1963) there was little evidence of an immediate transient response to magnesium supplementation within the 24 hours following administration, as seen by serial blood samples taken over days 8/9. Mean plasma magnesium concentration increased only very slightly from 0·85 to 0·89 mmol/l in the 4 hours following supplementary feeding and remained at 0·88 mmol/l after 24 hours. The effect may have been masked by the extremely varied intakes of supplement, and the fact that the mean plasma magnesium value was already high. However, there was still no diurnal pattern when the shy feeders were excluded from the mean plasma magnesium concentrations. The mean plasma magnesium value remained relatively constant over the 24 hours for the control unsupplemented cows. Five weeks following the completion of this present trial, a further attempt was made to detect any possible diurnal pattern in plasma magnesium concentration following magnesium supplementation. Fifteen cows from the same herd, on the same (summer) grazing, were drenched with 56 g Spanish C.M. (1979 commercial product as used in this Experiment 5.2.) at 10.00 h and blood samples were taken at 10.00, 14.00, 20.00 and the following 10.00 hours. The initial mean plasma magnesium value ($\pm$ SEM) was low for these cows, at 0·61 $\pm$ 0·043 mmol/l. This increased slightly to 0·65 $\pm$ 0·038 mmol/l in 4 hours, 0·66 $\pm$ 0·036 mmol/l in 10 hours and was significantly greater than the 10.00 h level ($P<0.05$) by the following 10.00 h, at 0·73 $\pm$ 0·032 mmol/l. It therefore appears that for slightly hypomagnesaemic cows at grass there is a more gradual rise in plasma magnesium in response to magnesium supplementation compared with the sharp 4-hour rise observed in hypomagnesaemic ewes fed indoors (Experiment 3.3.). This may be due to the fact that the cows were grazing continually over the 24 hours whereas the ewes had two distinct feeds per day. However Ritchie and Hemingway (1963) have reported a consistent 4-hour peak in plasma magnesium concentration following

magnesium supplementation of grazing sheep. There is possibly, therefore, a species difference in response but the reasons for this are far from clear.

B. Free access magnesium supplements.

Magnesium supplements in farm practice can be offered in various ways. Whereas the last two experiments (5.1. and 5.2.) described were concerned with the common practice of adding supplementary magnesium to a concentrate food, the following experiments (6.1., 6.2., 6.3. and 6.4.) are concerned with an alternative common practice of providing magnesium as part of a free-access supplement.

(i) Magnesium supplement contained in a molassed meal

Experiment 6.1. The use of a free-access calcined magnesite/molassed sugar beet pulp mixture for cows at autumn grass

Introduction

Beef cows at pasture during the autumn are often considered at risk of hypomagnesaemia and hypomagnesaemic tetany, and they generally require supplementary magnesium. The present short experiment investigates the use of a free-access mix containing calcined magnesite in terms of the range of individual intakes and the apparent magnesium availability.

Experimental

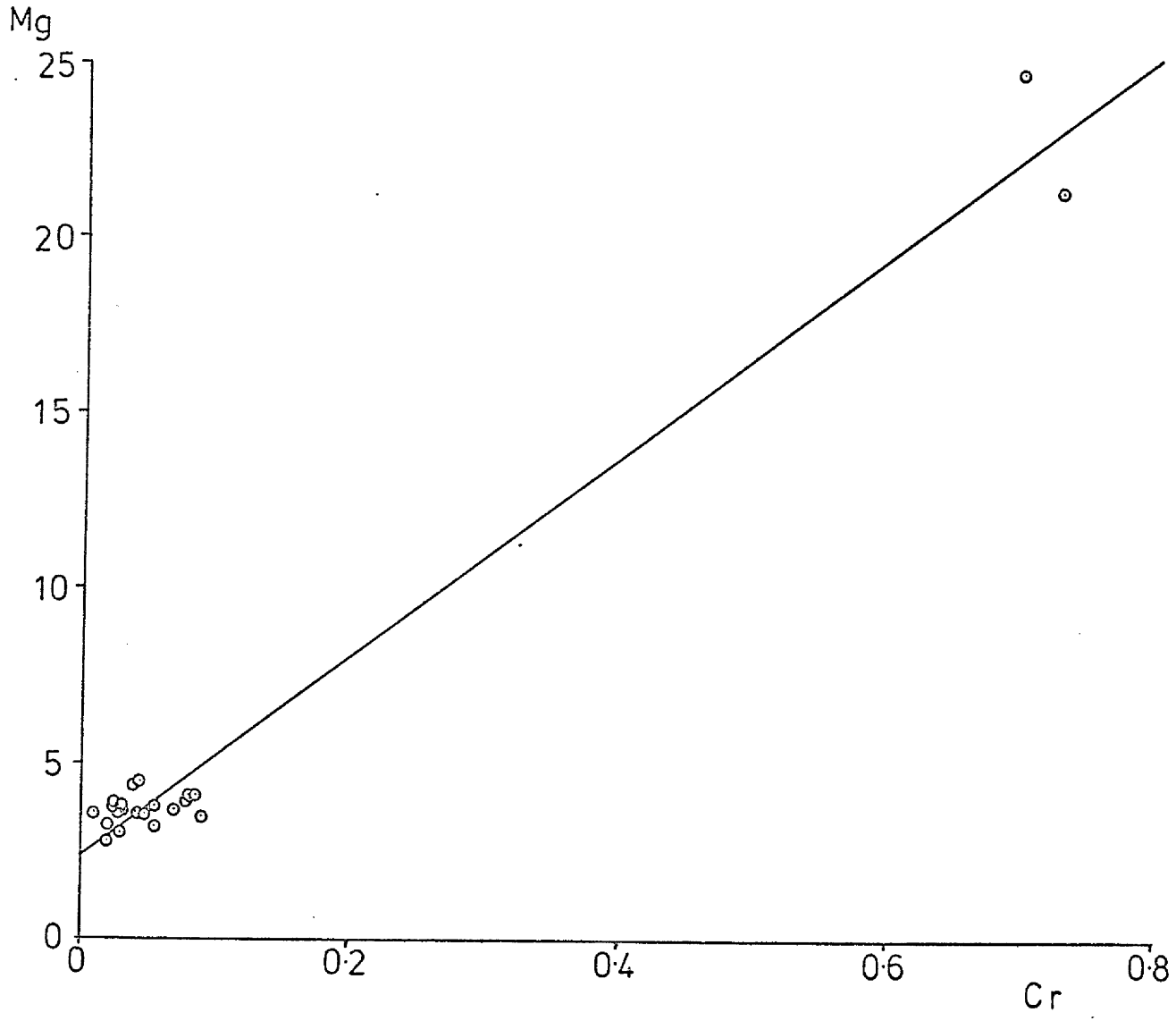
Twenty-two pregnant beef cows (mainly Hereford cross) at grass in the autumn were given free access to two tubs containing a 50 : 50 mixture of extra-molassed sugar beet pulp and Spanish (Agma FG85) calcined magnesite (trade name "Beemag," Chapman & Frearson, Ltd.). The mixture contained 304·8 g Mg/kg, and also 10·30 g Cr/kg as chromic oxide. After about 2 weeks the cows were brought in for in-wintering and grab samples of faeces were taken, dried, ground and analysed for magnesium and chromium.

Figure 1. Exot.6.1. Regression between faecal magnesium and faecal chromium concentrations (g/kg DM) for 22 cows offered a calcined magnesite/molassed sugar beet pulp mixture.

$y = 2.393 + 28.653x$; $r = 0.987^{***}$

Supplement Mg/Cr = 29.588

Availability = 3.2% (SE = 3.54)


Mg
25
20
15
10
5
0
0
0·2
0·4
0·6
0·8
Cr

Results and Discussion

The regression of faecal magnesium on faecal chromium concentration is given in Figure 1. The correlation is statistically very significant ($P < 0.001$, $r = 0.987$) but it can be seen that the slope is largely determined by the faecal concentrations of just two cows that had consumed any appreciable quantities of supplement. The calculated availability for the Spanish calcined magnesite (C.M.) was 3·2% but this was consequently largely determined by the two greedy cows and is probably not representative. However it is interesting to note that the coefficient of variation (CV) of faecal chromium concentrations was very high, 187·2%, indicative of the wide range in individual intakes of magnesium supplement in a free-access feeding situation. Although not 100% effective, Todd (1967) reports average intakes of 28 - 84 g of magnesia daily by hill cows offered ad lib a mixture in equal proportions of magnesia and molasses. Thus more cows were protected from hypomagnesaemic tetany in his trial than in the present trial where only two cows from a herd of twenty-two consumed any marked quantities of magnesia.

(ii) Feedblock trials

The following trials investigate the intake and magnesium availability of two formulations of a high enery molasses-based feedblock (Crystalyx, Pfizer Ltd.). Each feedblock weighed 16·6 kg and contained 65 g/kg oil, 130 g/kg protein (108 g/kg protein equivalent of urea), zero fibre, 350 g/kg sugar (expressed as sucrose) and 12·1 MJ ME/kg DM. They also contained additional magnesium, calcium, phosphorus and trace minerals. (i) Block M6 (old formulation) contained 52·2 g Mg/kg as the powdered grade Spanish calcined magnesite (Agma powder, which is widely used for pasture dusting), 44·9 g Ca and 5·3 g P/kg. (ii) Block M3 (new high mineral formulation) contained 107·1 g Mg, 23·1 Ca and 14·5 g P/kg.

In this case the magnesium is provided by both Spanish calcined magnesite powder (87%) and magnesium phosphate (6%) (the remaining 7% of the magnesium being in the other block constituents). In addition the blocks contained chromic oxide as an indigestible marker for availability and intake studies (M6 : 1·43 g Cr/kg; M3 : 2·54 g Cr/kg).

Experiment 6.2. The consumption of magnesium-containing feedblocks by lactating beef cows at grass in the autumn

Introduction

Three studies of feedblock intake and magnesium availability were undertaken with lactating beef cows. In previous years clinical cases of hypomagnesaemic tetany had been recorded in the three groups of cows concerned when at grass in the autumn. The present studies aimed to investigate the efficacy of the free-access feedblock method for supplying the necessary extra magnesium to grazing cows, and to determine the apparent availability of this source of supplementary magnesium in this situation.

Experimental

Three separate herds of lactating beef cows at grass in the autumn were used in these feedblock trials:-

Herd 1. 20 Autumn calving beef cows and their calves maintained on 6 ha of moderate grazing.

Herd 2. 8 Spring calving beef cows and their calves, plus 3 pregnant heifers maintained on 3 ha of moderate grazing.

Herd 3. 6 Spring calving beef cows and their calves, plus 3 pregnant heifers and 2 pregnant cows maintained on 6 ha of grazing (4 ha of aftermath grass and 2 ha of rough grazing).

Particular blocks were placed centrally in the respective fields for *ad lib* consumption, at about 1 block per 10 cows (being replaced as

necessary to maintain a continual supply) for periods of up to 3 weeks. Separate detailed studies were conducted on block consumption, involving weather recording (rainfall and temperature) and daily block weighing. Towards the end of each period when a herd was receiving a particular block, blood and faeces (rectal grab) samples were taken from the cows. The bloods were analysed for plasma magnesium and calcium concentrations, and faeces were dried and ground for subsequent magnesium and chromium analyses.

Herd 1 was offered 2 x M6 Blocks for a 3 week period, followed by 2 x M3 Blocks for 2 weeks.

Herd 2 was given one M6 Block for 3 weeks, then one M3 Block for 4 weeks (with sampling after 2 weeks). However the M3 Blocks used were later discovered to contain no chromium and thus provided only daily intake and blood data but no availability figures.

Herd 3 was given one M3 Block for 3 weeks (but faeces and blood samples were taken after one week).

Results (See Tables 1 and 2)

Herd 1

The mean (3-day rolling average) intake of the M6 Block was 327 g/head/day (ranging from 235 - 421 g) i.e. 17·1 g Mg/head/day. The coefficient of variation of faecal chromium (CV) was 53·3% and from the significant regression of faecal chromium on faecal magnesium ($P<0·001$) the apparent magnesium availability was calculated as 18·6%. The mean plasma magnesium and calcium concentrations were 0·52 and 2·33 mmol/l, respectively.

The mean (3-day rolling average) intake of the M3 Block in the next two weeks was 408 g/head/day (range 332 to 480 g), i.e. 43·6 g Mg/head/day. The CV of faecal chromium was 59·0%. There was, however, no close correlation between faecal chromium and magnesium concentrations (r =

0·19) unless three extremely anomalous results were excluded from the regression analysis (then, $r = 0.65$, $P<0.01$) and the magnesium availability was then calculated as 24·4%. Mean plasma magnesium and calcium concentrations were 0·63 and 2·22 mmol/l, respectively, the magnesium level being significantly greater ($P<0.05$) than that when the cows had access to the M6 Block.

Table 1. Expt.6.2. Mean plasma magnesium and calcium concentrations (mmol/l) (SEM), CV of faecal chromium concentrations, magnesium availability and mean daily block intakes.

Herd	1	2	3
Plasma Mg			
M6 Block	0.52 (0.022)	0.87 (0.068)	-
M3 Block	0.63 (0.033)	0.84 (0.027)	0.90 (0.022)
Significance	M3>M6*	NS	
Plasma Ca			
M6 Block	2.33 (0.025)	2.34 (0.037)	-
M3 Block	2.22 (0.057)	2.31 (0.019)	2.33 (0.019)
Significance	NS	NS	
CV% of faecal Cr			
M6 Block	53.3	54.6	-
M3 Block	59.0	-	62.8
Mg availability (%)	(See Table 2)		
M6 Block	18.6	20.5	-
M3 Block	24.4	-	-22.5
Mean Daily block intake (g/head/day)			
M6 Block	327	188	-
M3 Block	408	297	258

Table 2. Expt.6.2. Faecal magnesium and chromium regression data and apparent availabilities.

Herd	Feedblock	Feedblock Mg/Cr ratio	Regression of faecal Mg on Cr: Correlation coefficient	Regression of faecal Mg on Cr: Regression coefficient (b)	Regression of faecal Mg on Cr: SE_b	Apparent Availability (%)	$SE_{avail.}$
1	M6	36.53	0.8239***	29.7157	5.2768***	18.6	14.44
	M3	42.15	0.6495**	31.8573	9.9662**	24.4	23.64
2	M6	36.53	0.9184***	29.0586	4.1742***	20.5	11.43
3	M3	42.15	0.8698***	51.6309	9.7644***	-22.5	23.17

Herd 2

The mean (3-day rolling average) intake of the M6 Block was 188 g/head/day (range 62 - 333 g), i.e. 9·8 g Mg/head/day. The CV of the faecal chromium was 54·6%, and from the significant regression of faecal magnesium on faecal chromium ($P < 0.001$), the magnesium availability was calculated as 20·5%. Mean plasma magnesium and calcium concentrations were 0·87 and 2·34 mmol/l.

The mean (3-day rolling average) intake of the M3 Block was 297 g/head/day (range 182 - 418 g), i.e. 31·8 g Mg/head/day. Unfortunately there was negligible chromium detectable in the M3 blocks used here and so CV and availability data were not obtainable. Mean plasma magnesium and calcium concentrations were 0·84 and 2·31 mmol/l, respectively.

Herd 3

The mean (3-day rolling average) intake of the M3 Block was 258 g/head/day (range 240 to 276 g), i.e. 27·6 g Mg/head/day. The CV of faecal chromium was 62·8%, and the magnesium availability was calculated, as -22·5%, from the significant ($P < 0.001$) regression of faecal magnesium on faecal chromium concentrations. The mean plasma magnesium and calcium concentrations were 0·90 and 2·33 mmol/l.

Discussion

Weather conditions were generally very wet throughout the trials. Some rain fell on virtually every day, and strong winds often accompanied heavy rainfall, although there were few very cold days. For all three groups of cows block intake tended to increase on days with heavy rainfall, but this was thought to reflect both an increased individual consumption of block and the loss of some block material due to weathering. As the amount of grass declined during the trials, there was a progressive increase in daily block consumption (irrespective of the type of block on offer) in all three groups.

The lowest plasma magnesium concentrations were seen in Group 1 when the herd had access to the M6 block, the mean concentration being only 0·52 mmol/l. None of the cows had plasma magnesium values greater than 0·74 mmol/l at this time (which would be considered normal) and two had values under 0·41 mmol/l, which would be considered at risk with respect to hypomagnesaemic tetany. However no clinical cases occurred, and plasma calcium concentrations (perhaps importantly) remained normal. Thus these cattle were generally hypomagnesaemic, with some risk of clinical tetany, but they did not consume sufficient supplementary magnesium from the ad lib feedblock to raise their plasma magnesium levels to normal. The mean daily intake of 327 g/cow over the period might seem adequate but this supplied a mean of 17·1 g Mg at 18·6% availability, i.e. only 3·2 g Mg absorbed from the supplement, which was evidently insufficient. The same cows (Herd 1) had a significantly higher mean plasma magnesium concentration (0·63 mmol/l; $P<0·05$) when they had access to the higher mineral M3 block. Five cows now had values over 0·74 mmol/l and only one had a plasma magnesium value less than 0·41 mmol/l. This latter cow had a plasma magnesium concentration of 0·31 mmol/l and calcium only 1·3 mmol/l, therefore being at great risk of tetany, and she had eaten no block at all (zero faecal chromium). In addition to the higher magnesium content of the M3 block, the average intake of 408 g/cow/day was also higher than that of the M6 block, and the herd was generally better protected from hypomagnesaemic tetany.

The cows in Herds 2 and 3 had consistently higher mean plasma magnesium concentrations (0·84 - 0·90 mmol/l) than for Herd 1. Herd 2 showed no difference in plasma magnesium between the two types of block on offer. The lowest value was 0·35 mmol/l for one cow when the herd had access to the M6 block, but this particular cow consumed very little. The same cow had the lowest value when the M3 block was subsequently on offer but it was now 0·725 mmol/l, and although faecal chromium data were

not available, it was evident from her faecal magnesium concentration that she had consumed a certain amount of this block. Apart from this cow, all other individuals in Herds 2 and 3 were at less risk of hypomagnesaemic tetany than was Herd 1, and, interestingly, their mean daily block intakes were also less. In Herd 2, as in Herd 1, intake of the M3 block was higher than that of the M6 block by about 100 g/cow/day, but it was possible that this was due to the decreased grazing later in the season rather than the relative acceptabilities of the two types of block.

The CVs of faecal chromium, indicative of the range of individual block intakes, were very similar for both Herds 1 and 2 receiving the M6 block, at about 54%, and for both Herds 1 and 3 receiving the M3 block, at about 60%. However all CVs are low, indicating relatively uniform individual intakes in the herd. This is surprising for a free-access feed situation, especially compared with the CV 187% reported in Experiment 6.1. (free-access), and the intake variation was even less than that in group (trough) feeding situations with animals at grass (90 - 100%, Experiments 5.1., 5.2.). Thus the palatable nature of these feedblocks appear to encourage all animals to consume a fairly uniform quantity which is desirable if all are to receive supplementary magnesium. The availabilities calculated for the fine Spanish C.M. powder in the M6 blocks agreed well between Herds 1 and 2, at 18·6 and 20·5%, respectively, but these values are low compared with previous findings for powdered Spanish C.M. (e.g. Experiment 5.2.). The availabilities of the magnesium in the M3 blocks did not agree well, being 24·4% (SEM = 23·64) for Herd 1 and -22·5% (SEM = 23·17) for Herd 3 and their standard errors were very large. It is possible that there was wide variation in the chromium content of these M3 blocks and that it was not evenly dispersed (especially as the blocks used for Herd 2 were discovered to contain no chromium). Thus the CV and availability data for the M3 block in these particular

experiments must be treated with caution. In a previous experiment (3.4., section 3), the availability for the magnesium in the M3 block was found to be quite good at 29·8% (SEM 6·41), and from the significant blood response by Herd 1 this block is presumably a fairly good source of magnesium.

Experiment 6.3. The consumption of magnesium-rich feedblocks by out-wintered cattle receiving silage

Introduction

A further study of the intake and magnesium availability of the high mineral feedblock (M3) was undertaken. This time outwintered silage-fed bulling heifers were the experimental animals, which would not be considered particularly at risk of hypomagnesaemic tetany.

Experimental

Thirteen Friesian dairy heifers plus 5 Hereford x Friesian beef heifers, together with 1 Hereford bull were maintained on an area (1·7 ha), with essentially no grazing, where they were outwintered on silage *ad lib*. Over a period of 2 - 3 weeks they also had on offer two M3 feedblocks for *ad lib* consumption. Daily block intakes and weather records were taken. Faecal grab samples were also obtained for the determination of magnesium and chromium.

Results

The mean (3-day rolling average) intake of the M3 Block was 270 g/head/day (range 214 - 338 g), i.e. 29 g Mg/day, and the coefficient of variation (CV) of faecal chromium concentrations was 49·1%. The feedblock Mg/Cr ratio was 27·7943, the regression coefficient of faecal magnesium on faecal chromium concentrations was 15·4312 (SE 1·8650), with correlation coefficient 0·896 ($P<0·001$) and the magnesium availability

was calculated as 44·5% (SE 6·71).

Discussion

The weather was generally very wet and some periods of frost and snow cover occurred. The cattle frequently sought shelter for long periods throughout the day and the mean daily block intake of 270 g/cow was therefore perhaps surprisingly low, especially compared with the similar or even higher intakes reported for cattle at grass (Experiment 6.2.). However the CV of faecal chromium in the present trial was low, at 49·1%, indicative of fairly uniform individual intakes, and all cattle were known to eat some block (i.e. all had detectable chromium in the faeces). This may be due largely to the fact that the feedblocks were situated close to the area where the principal feed (silage) was given.

The availability of the magnesium in the M3 block was very high, at 44·5%, especially compared with previous findings (Experiments 6.2. and 3.4.). However from the availabilities obtained separately for fine Spanish calcined magnesite and magnesium phosphate in, for example, Experiment 3.3. where they were 41·4 and 47·6%, respectively, an availability of 44·5% for the feedblock magnesium might be expected.

Experiment 6.4. The consumption of magnesium-rich feedblocks by housed sheep.

Introduction

The consumption of the high mineral feedblock (M3) by housed sheep (in early pregnancy) was investigated to compare with previous data obtained for cattle outdoors. Block intakes and magnesium availabilities were determined, but such animals would not normally be considered to require supplementary magnesium.

Experimental

Two groups of 20 Polled Dorset Horn ewes were housed during the trial. They were given 1·5 kg/head/day of a basal diet composed of 3·2 parts molassed sugar beet pulp and 1 part barley husk siftings, with added urea, minerals (but no additional magnesium), trace elements and vitamins. One M3 Block containing chromic oxide for each group of 20 ewes (the recommended level of block/ewe) was on offer for about 3 weeks. Daily block intakes were recorded and faecal grab samples were taken for magnesium and chromium analyses.

Results

The mean (3-day rolling average) intake of block by all sheep was 67 g/sheep/day (range 48-90g) and the CV of faecal chromium concentrations was 54·1%. The feedblock Mg/Cr ratio was 27·7943, the regression coefficient of faecal magnesium on faecal chromium concentrations was 14·3993 (SE 1·5015), with correlation coefficient 0·855 ($P<0·001$), giving a magnesium availability of 48·2% (SE 5·40).

Discussion

The low variation in individual intakes of block agrees with previous findings for cattle and would be expected for housed sheep. The high availability for the feedblock magnesium (48·2%) agrees well with the previous trial with unproductive cattle fed on silage (44·5%). However, both values are higher than those reported for hypomagnesaemic lactating ewes given a low magnesium diet (Experiment 3.4; 29·8%) and for grazing lactating cattle (Experiment 6.2., 24·4% and -22·5%).

C. Dairy cows receiving individual magnesium supplementation

Experiment 7.1. Availabilities of different particle sizes and heat-treated calcined magnesites for dairy cows at spring grass.

Introduction

Two dairy herds at spring grass where there is a potential risk of hypomagnesaemia were used in these trials. The cows received no supplementary magnesium other than the experimental products to be investigated. Previous work with sheep indoors (e.g. Expts.1.1., 2.1., 2.2., 2.3., 3.3., 3.4.) has indicated that particle size might influence the availability of calcined magnesite and the present experiment investigated this in a practical farm situation. In addition the effects of intensive heat treatment on the availabilities of the different particle sizes were tested. Dairy herds provide a useful practical farm experimental situation as the cows are fed individually at milking time, and hence supplements can be administered individually.

Materials and Methods

Magnesium supplements (Table 1)

Fine (F, under 75 μm), medium (M, 150 - 250 μm) and coarse (C, 500 - 1,000 μm) fractions of Spanish granular calcined magnesite (Agma FG 85, 1979 product) were supplied by Chance and Hunt (as in Experiment 5.2.). Samples of each (roughly 10 kg) were subjected to intensive heat treatment in a kiln for about 2 hours at about 1,300°C †. Some "burning" of the material evidently occurred, as indicated by the decreased L.O.I. (Table 1), but it was not the characteristically dark colour of "deadburnt" calcined magnesite (as seen, for example in Experiment 3.3., when burnt medium calcined magnesite became very dark in colour and indeed had an even lower L.O.I. of 0·03%). Each of the six supplements were mixed thoroughly with chromic oxide (673·3g Cr/kg), in the ratio 1·00 kg calcined magnesite to 150 g chromic oxide, and small

† At Department of Metallurgy, University of Strathclyde.

plastic scoops were made to hold about 32 g and 64 g of each mixed supplement, i.e. to give about 28 g or 56 g calcined magnesite.

Table 1. Expt.7.1. Magnesium supplements

	Supplement	gMg/kg	† L.O.I.(%)
F	Spanish calcined magnesite, 75 μm	506.7	1.96
M	" " " , 150-250 μm	524.2	1.54
C	" " " , 500-1,000 μm	524.1	2.36
Burnt F	F subjected to 1300°C	531.2	0.57
Burnt M	M " " "	534.7	0.48
Burnt C	C " " "	534.6	0.76

†L.O.I. determinations by Chance & Hunt.

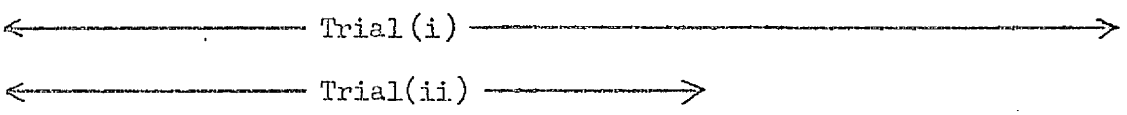
Experimental design

Trial (i). Forty cows in a Friesian dairy herd at spring grass were divided into four similar groups of ten according to their stage of lactation, and hence their level of feeding of concentrates. One group ((a)) acted as a control, with no supplementary magnesium, and over four one-week periods the three other groups ((b), (c) and (d)) were given two levels of supplements F, M and C, and one level of "burnt" F, M and C, as shown in Table 2. Each mixed calcined magnesite/chromic oxide supplement was added, by an appropriate plastic scoop, to the surface of the concentrates given individually at the afternoon milking (about 16.00 h) each day for seven days. After each seven-day period all the cows were bled at about 16.00 h, and rectal grab samples of faeces were taken. The blood samples were analysed for plasma magnesium and calcium concentrations and faeces were dried, ground and analysed for magnesium and chromium.

Trial (ii). At the same time as trial (i), a second shorter experiment of similar design was carried out using 44 dairy cows in a Friesian herd at spring grass on a separate farm (Dykescroft, Moscow, Ayrshire). They were arranged into four groups of eleven, and the two one-week experimental periods mirrored periods 1 and 2 of trial (i) (Table 2). In this trial the supplements were added to the concentrates at the morning milking. Sampling of blood and faeces were carried out at about 16.00 h at the end of each week.

Table 2. Expt.7.1. Magnesium supplements fed to the groups of cows during the four experimental periods.

		Quantity of calcined magnesite fed g/cow/day			
Groups of Cows	Magnesium Supplement	Periods 1	2	3	4
(a)	0	0	0	0	0
(b)	F	28	56	28	28 "burnt"
(c)	M	28	56	28	28 "burnt"
(d)	C	28	56	28	28 "burnt"



Results

Blood data

Trial (i). (Table 3) Mean plasma magnesium concentrations remained high and well within the normal range for all groups, including the control animals, throughout all periods (ranging 0·82 - 0·99 mmol/l). Differences between treatments were statistically significant only in period 2 when group (c) (supplement M) was greater, at 0·96 mmol/l, than (a) (0), at 0·88, ($P<0·01$) and (d) (C), at 0·84, ($P<0·05$). Group (b) also had a

higher mean plasma magnesium concentration at 0·92 mmol/l, but the differences were non-significant. Mean plasma calcium concentrations also remained high and normal throughout, 2·28 - 2·44 mmol/l. Group differences were apparent at the start, when (b) was greater than (c) ($P<0·05$; 2·41 and 2·34 mmol/l, respectively), and again in period 3, when (b) was greater, at 2·44 mmol/l, than (c) (2·34 mmol/l) and (d) (2·32 mmol/l) ($P<0·05$).

Trial (ii). (Table 4) Mean plasma magnesium concentrations were higher for the supplemented groups than for the controls in both periods. However, the differences were only significant in period 2 when (b), (c) and (d) had concentrations of 0·99, 1·03 and 1·01 mmol/l, respectively, which were greater than that of (a), at 0·85 mmol/l ($P<0·01$). Mean plasma calcium concentrations remained high, 2·35 - 2·45 mmol/l, and non-significantly different between groups throughout.

Faeces data and Magnesium Availabilities

In both trials the regression of faecal magnesium on faecal chromium concentrations for each supplementary treatment was very highly significant ($P<0·001$) and availabilities were calculated.

Trial (i). (Table 5) The availabilities of F, M and C were, respectively, 47·8, 35·1 and 0% in period 1 (F and M being more available than C, $P<0·05$); 46·1, 16·3 and 4·4% in period 2 (F being more available than M and C, $P<0·01$); and 34·0, 37·8 and 7·6% in period 3 (F and M being more available than C, $P<0·05$). The "burnt" supplements F, M and C used in period 4 had availabilities of 43·9, -5·6 and 6·4%, respectively (F being more available than M, $P<0·05$). The coefficients of variation (CV) of faecal chromium contents for all supplemented cows (i.e. groups (b), (c) and (d)) in periods 1, 2, 3 and 4 were 34·3, 37·3, 33·8 and 43·5%.

Trial (ii). (Table 6) The availabilities of F, M and C were -14·2, -5·3 and 3·3%, respectively, in period 1, with no significant differences. In period 2, F was significantly ($P<0.05$) less available, at -47·9%, than M (0·1%) and C (10·4%). The CV's of faecal chromium concentration were 32·8% in period 1 and 29·3% in period 2.

Table 3. Expt.7.1. (i) Mean plasma magnesium and calcium concentrations (mmol/l) (and SEM) of the groups of cows throughout the trial.

Period / Group	Start	1	2	3	4
Plasma magnesium concentration					
(a) O	0.90(0.027)	0.99(0.023)	0.82(0.043)	0.88(0.019)	0.92(0.029)
(b) F	0.91(0.022)	0.97(0.022)	0.92(0.022)	0.89(0.025)	0.92(0.029)
(c) M	0.92(0.016)	0.97(0.019)	0.96(0.018)	0.92(0.030)	0.96(0.024)
(d) C	0.89(0.036)	0.93(0.037)	0.84(0.042)	0.86(0.027)	0.93(0.034)
Significance	NS	NS	(c)>(a)**; (d)*	NS	NS
Plasma calcium concentration					
(a) O	2.36(0.029)	2.36(0.024)	2.43(0.051)	2.35(0.041)	2.32(0.036)
(b) F	2.41(0.016)	2.40(0.021)	2.43(0.034)	2.44(0.030)	2.36(0.037)
(c) M	2.34(0.024)	2.38(0.027)	2.44(0.038)	2.34(0.035)	2.32(0.047)
(d) C	2.34(0.031)	2.34(0.027)	2.35(0.065)	2.32(0.034)	2.28(0.046)
Significance	(b) (c)*	NS	NS	(b)>(c), (d)*	NS

Table 4. Expt.7.1.(ii) Mean plasma magnesium and calcium concentrations (mmol/l) (+ SEM) of the groups of cows throughout the trial.

Period	1	2
Group		
Plasma magnesium concentration		
(a) O	0.89 (0.050)	0.85 (0.030)
(b) F	0.95 (0.042)	0.99 (0.031)
(c) M	0.99 (0.040)	1.03 (0.044)
(d) C	1.02 (0.037)	1.01 (0.032)
Significance	NS	(a)<(b),(c),(d)**
Plasma calcium concentration		
(a) O	2.39 (0.037)	2.41 (0.030)
(b) F	2.35 (0.027)	2.45 (0.019)
(c) M	2.39 (0.033)	2.44 (0.022)
(d) C	2.42 (0.031)	2.43 (0.034)
Significance	NS	NS

Table 5. Expt.7.1.(i) Faecal magnesium and chromium regression data and apparent availabilities of supplements.

Period		Mixed supplement Mg/Cr ratio	Regression of faecal Mg or Cr			Apparent availability (%)	SE of availability	Significance (availabilities)
			Correlation coefficient(r)	Regression coefficient(b)	SE_b			
1 (28g)	F	5.0172	0.7337***	2.6183	0.6261***	47.8	12.48	
	M	5.1906	0.8268***	3.3666	0.5725***	35.1	11.03	
	C	5.1901	0.8941***	5.1914	0.6502***	0.0	12.53	M,F>C*
2 (56g)	F	5.0172	0.8579***	2.7027	0.4047***	46.1	8.07	
	M	5.1906	0.9707***	4.3461	0.2776***	16.3	5.35	
	C	5.1901	0.9381***	4.9627	0.4581***	4.4	8.83	F>C,M**
3 (28g)	F	5.0172	0.9071***	3.3100	0.3966***	34.0	7.90	
	M	5.1906	0.8413***	3.2264	0.5352***	37.8	10.31	
	C	5.1901	0.9022***	4.7935	0.5195***	7.6	10.01	M,F>C*
4 (28g)	Burnt F	5.2603	0.7230***	2.9526	0.7284***	43.9	13.85	
	Burnt M	5.2942	0.8957***	5.5899	0.6939***	-5.6	13.11	F>M*
	Burnt C	5.2937	0.8630***	4.9558	0.7286***	6.4	13.76	

Table 6. Expt.7.1.(ii). Faecal magnesium and chromium regression data and apparent availabilities of supplements

Period		Mixed supplement Mg/Cr ratio	Regression of faecal Mg on Cr			Apparent Availability(%)	SE of avail.	Significance
			Correlation coefficient(r)	Regression coefficient(b)	SE_b			
1 (28g)	F	5.0172	0.9026***	5.7291	0.6111***	- 14.2	12.18	NS
	M	5.1906	0.8810***	5.4665	0.6565***	- 5.3	12.65	
	C	5.1901	0.9002***	5.0189	0.5438***	3.3	10.48	
2 (56g)	F	5.0172	0.9136***	9.6223	0.9823***	- 47.9	19.58	F<M,C*
	M	5.1906	0.9591***	5.1865	0.3513***	0.1	6.77	
	C	5.1901	0.9049***	4.6522	0.5020***	10.4	9.67	

Discussion

On both farms the grass magnesium content was fairly low, at 1·7 g Mg/kg DM, but in spite of this potentially "risk" situation, there was no case of hypomagnesaemic tetany throughout the trials. Indeed there was no serious development of hypomagnesaemia in either herd, with individual plasma magnesium concentrations remaining above 0·74 mmol/l for all cows in both periods of trial (ii) and for all cows in periods 1, 3 and 4 of trial (i). In period 2 of trial (i) four control cows had levels 0·68 - 0·70 mmol/l (i.e. not seriously low) and one cow in group (d) (receiving coarse C.M.) had a plasma magnesium concentration of 0·61 mmol/l. The mean plasma magnesium content was lowest for the control group during this period, perhaps reflecting a change in the pasture, and it is interesting that one cow developed such a low plasma magnesium in spite of receiving 56 g/day of the coarse-grade C.M.

In trial (i), the mean plasma magnesium concentrations for all groups, including the non-supplemented controls, were similar when individuals were given 28 g C.M./day in periods 1 and 3. This was surprising considering the relatively high availability figures calculated for the fine and medium C.M.'s in these periods (34 - 48% and 35 - 38%, respectively) although this perhaps indicated successful renal clearance of excess magnesium in the blood. The coarse C.M. was poorly available in these two periods (0 - 8%) and this was reflected in a mean plasma magnesium concentration no different from that of the control group. In period 2, when supplementation was higher at 56 g/cow/day, differences in mean plasma magnesium concentrations were more apparent (though this was largely due to a lower value for the control group). Fine and medium C.M.'s produced higher plasma magnesium while the coarse C.M. (with a poor availability of 4%) failed to raise it above the unsupplemented level. Fine C.M. in period 2 had an accordingly high availability (46%), but medium C.M. was rather poorly available (16%) which was not reflected in

the high mean plasma magnesium value recorded. In period 4, as in periods 1 and 3, when 28 g/cow/day of the "burnt" supplements were given, no differences in blood magnesium concentration between the groups were detectable. In trial (ii) all supplements raised mean plasma magnesium values significantly above the control level when given at 56 g/cow/day but not when given at the lower rate of 28 g/cow/day. However the poor availabilities obtained would suggest that the supplements could provide no appreciable magnesium for absorption at all.

Trial (i) demonstrates the fundamental problem of the unrepeatability of precise availability figures. Periods 1 and 3 were identical, carried out one week apart, and the availabilities calculated for fine, medium and coarse C.M. were 47·8, 35·1 and 0%, respectively, in period 1, and 34·0, 37·8 and 7·6% in period 2. These differences between periods were not statistically significant and the values agree well within the limits of the variation involved in any animal experiment, but it is clear that a particular figure quoted singularly might be misleading, e.g. one could quote fine C.M. as being 48% or 34% available. In addition, trial (ii) mirrored periods 1 and 2 of trial (i) using different animals but the same class of stock, and the availability data obtained here were markedly different.

In trial (i) there was a clear trend of increasing availability with decreasing particle size. Availabilities were similar whether the supplement was given at 28 g or 56 g/day and the mean availabilities over periods 1 - 3 for fine, medium and coarse C.M. ($\pm$ SEM) were, respectively, 44·6 ($\pm$ 4·86), 22·4 ($\pm$ 4·26) and 4·3 ($\pm$ 5·50)%. Thus, overall, fine C.M. was more available than both medium and coarse ($P<0.001$), and medium C.M. was better than coarse ($P<0.05$). The "burnt" fine and coarse materials retained similar availabilities, at 43·9 and 6·4%, respectively, but the medium "burnt" C.M. became less available ($P<0.05$) at -5·6%. The C.M.'s were evidently not "deadburnt" (and rendered unreactive) but some burning

had occurred as indicated by the decreased L.O.I. (Table 1). It is perhaps significant that the medium "burnt" C.M. had the lowest L.O.I. and, correspondingly, its availability was decreased. It is possible that the C.M.'s were not evenly "burnt" in the kiln as large quantities were treated in a single batch, and the finished products were not evenly coloured. More intense heat treatment would probably have rendered all three C.M.'s less available.

The results obtained in trial (ii) are somewhat anomalous. Availabilities approximated to zero in all cases, except for fine C.M. given at 56 g/day, when a highly negative availability (-47·9%) was obtained. Zero availabilities are hard to believe in view of the mean plasma magnesium response to supplementation, and a high negative availability would imply that the animal was becoming rapidly depleted of its body magnesium. One explanation for these findings might be that the cows, unknowingly, had access to some other source of extra magnesium (possibly an old free-access supplement in the field). Conducting experiments in a farm situation, although extremely relevant, necessarily means that the experimental conditions cannot be under rigid control (as, for example, in indoor feeding trials), and this might well introduce some inaccuracies, particularly as in the case of trial (ii) the farm is not managed by nor under the control of the research institution.

The CV's of faecal chromium were similar in both trials at 31·0 and 37·2%, respectively, overall in trials (i) and (ii). All cows were given approximately equal quantities of chromic oxide daily and so this slight variation probably reflects different individual intakes of grass (and hence different quantities of faecal dry matter).

Thus these trials have given some availability data for cows at spring grass, but the different availabilities were not necessarily reflected in differential plasma magnesium responses. For Spanish C.M. in one trial ((i)) the fine particles are considerably better than

medium, and both are better than coarse granules.

Experiment 7.2. Availabilities of different commercial calcined magnesites for dairy cows at autumn grass

Introduction

In this short trial the same Friesian dairy herd as in Experiment 7.1. trial (i) was used, this time at autumn grass. One fatal case of hypomagnesaemic tetany occurred during the previous week, indicating that the herd was in a risk situation and required magnesium supplementation. The supplements investigated were the three most important calcined magnesites used by the animal feed trade in the U.K., administered at the recommended rate, with a view to calculating availabilities and observing any blood magnesium response.

Materials and Methods

Magnesium supplements

The three major commercial animal feed grade granular calcined magnesites (C.M.'s) in the U.K. were investigated in this trial (see Section I).

A, Spanish C.M. (Agma FG85, 1979 product) from a mixed batch of several riffled sacks produced over one month, containing 524·6 g Mg/kg.

B, Greek C.M. (Grecian Magnesite, 1979), a random sample, containing 465·1 g Mg/kg.

C, Chinese C.M. (0·3 - 2·0 mm grade, 1979), a random sample, containing 517·9 g Mg/kg.

Approximate particle size distributions of these products are given in Appendix 1.

Each C.M. was mixed thoroughly with chromic oxide, in the ratio 1 kg C.M. to 0·15 kg chromic oxide, and plastic scoops were made to hold

about 64 g of each mix, i.e. about 56 g C.M.

Experimental design

40 cows in a Friesian dairy herd at autumn grass were divided into four similar groups of ten according to their levels of concentrate feeding (and therefore to the stage of lactation). One group acted as controls receiving no supplementary feeding, and the remaining three groups were given supplements A, B and C respectively. The supplements were added, at 56 g C.M./cow/day, to the concentrates given individually at the afternoon milking (about 16.00 h) every day for seven days. On the last day of this period at about 16.00 h all cows were blood sampled and faeces grab samples were taken. The blood samples were analysed for plasma magnesium and calcium concentrations, and faeces for magnesium and chromium in the dry matter.

Results

Mean plasma magnesium concentrations were slightly higher for the groups receiving A, B and C (0·75, 0·79 and 0·79 mmol/l, respectively) than for that receiving no supplement (0·62 mmol/l) but the differences were not quite statistically significant (Table 1). Mean plasma calcium concentrations were similar between all groups and were between 2·29 - 2·34 mmol/l.

Table 1. Expt.7.2. Mean plasma magnesium and calcium concentrations (mmol/l) (and SEM's) of each group of cows.

	Mg	Ca
Supplement		
0	0.62(0.070)	2.34(0.027)
A	0.75(0.048)	2.31(0.032)
B	0.79(0.053)	2.29(0.023)
C	0.79(0.045)	2.32(0.031)
Significance	NS	NS

The regression of faecal magnesium on faecal chromium concentrations was significant ($P < 0.001$) for each supplement (Table 2) and the calculated availabilities for A, B and C were -0.3, 6.3 and 24.4%, respectively, with no significant differences.

The coefficient of variation (CV) of faecal chromium was 24.2%.

Table 2. Exot 7.2. Faecal magnesium and chromium regression data and apparent availabilities of supplements.

	Mixed supplement Mg/Cr ratio	Regression of faecal Mg on Cr: Correlation coefficient (r)	Regression coefficient (b)	SE_b	Apparent availability (%)	$SE_{avail.}$
A	5.4028	0.8033***	5.4165	0.9466***	-0.3	17.52
B	4.6056	0.8430***	4.3153	0.6489***	6.3	14.09
C	5.1282	0.7778***	3.8765	0.7383***	24.4	14.40

Discussion

For the first two days some cows were reluctant to eat all of their allocated supplement but thereafter troughs were generally well cleared. The control group demonstrated that the herd was tending to be hypomagnesaemic at the autumn grass with no supplementary magnesium. Their mean plasma magnesium was 0.62 mmol/lm with six of these ten control cows having values below 0.74 mmol/l, and two with values 0.24 and 0.31 mmol/l. However there were no cases of hypomagnesaemic tetany during the one-week trial, and it may be relevant that individual plasma calcium levels remained fully normal throughout. Six of the ten cows receiving Spanish C.M. also had plasma magnesium concentrations below 0.74, the lowest being 0.525 mmol/l, although the mean for this group was slightly greater than that for the controls. In the groups receiving Greek and Chinese C.M. there were, respectively, 2 and 3 cows with plasma magnesium values

under 0·74 mmol/l, and their mean levels were again higher than that of the control group. The relatively low mean plasma magnesium concentrations were determined from blood samples taken 24 hours after administration of supplements. However, unlike the case with grazing sheep (Ritchie & Hemingway, 1963), there was no significant rise and fall in plasma magnesium concentrations within the 24 hours detected for grazing cattle (Experiment 5.2.) and it is therefore unlikely that the present experiment (7.2.) "missed" higher mean plasma magnesium values during the day. Indeed the low mean plasma magnesium levels may be a reflection of the extremely low availability values obtained in this trial (0 - 24%, with large standard errors (of $\pm$ 14 to 17·5%)). It can only be concluded that in this particular trial all three commercial C.M.'s given at the recommended daily allowance were poorly absorbed and served little protection against hypomagnesaemia.

A discussion of supplementary magnesium intake data

The coefficients of variation (CV) of faecal chromium concentrations observed in these experiments in Section IV provide a useful indication of the range of intakes of supplementary magnesium by individual animals when presented in different ways.

The following table summarises the CV data obtained:-

	Basal diet	Supplement†	Expt.	(mean) CV(%)	Approximate Average CV(%)
INDIVIDUAL ADMINISTRATION (dairy cows)	Grass	mix	7.1(i)	37.5	30
			(ii)	31.0	
		mix	7.2	24.2	
		cubes	-	32.0	
TROUGH (GROUP) FEEDING	Hay + concs.	cubes	5.1.1	46.2	45
	Grass	cubes	5.1.2	90.8	90 - 100
			5.1.3	87.9	
		mix	5.2.	128.8	
				101.7	
FREE ACCESS FEEDING	Grass	molassed meal	6.1.	187.2	190
	Grass	Feedblocks	6.2.	(M6)53.3	50 - 60
				54.6	
				(M3)59.0	
				62.8	
	Silage		6.3.	49.1	
	Concs.		6.4.	54.1	

† Mg supplied in loose Mg/Cr mix, in barley cube, a molassed meal, or feedblocks.

In order to "calibrate" the CV data obtained in Experiment 5.1., in which barley cubes containing magnesium and chromium were trough-fed to beef cows, 1·0 kg quantities of such cubes (containing Spanish C.M.) were given daily to twelve dairy cows in a Friesian herd at grass. Unfortunately availability data were not obtained as it was later discovered that the cows had access to another self-feed magnesium source

in their fields. However, single faeces grab samples were taken after the cows had received the cubes for seven days and the CV of faecal chromium was calculated as 32·0% (which agreed well with other CV's for individual supplementation, 24 - 37%). The CV when the same cubes were trough-fed to beef cows that were given hay and concentrates was only slightly higher at 46%. However when adequate grazing was available the CV doubled to 90%. When the magnesium supplement was added as a loose mix to a trough-fed carrier concentrate, the CV was slightly higher still, at about 100%. When the supplement was given in this way, a high CV of 129% was recorded during the first week after turn-out to spring grass. Thus although relatively uniform individual intakes were achieved by trough-feeding a group of cows when they were already in receipt of trough-fed concentrates as part of their basal diet and were perhaps generally hungry with no alternative grazing available, intakes became extremely varied when the same cows had access to grazing. This was especially marked during the first week after transfer to lush spring pasture when animals are possibly at the greatest risk of hypomagnesaemic tetany.

The lowest overall average CV was obtained, as expected, when supplements were individually administered. At 30% this was fairly high and was indicative of both the variation in grass intake by the cows and sampling error. The highest CV (of 190%) was obtained when beef cows had free-access to the sugar beet pulp/calcined magnesite mix, with only two out of twenty-two cows consuming any appreciable quantities. However the range of individual intakes of the free access feedblocks was considerably less, with CV 50 - 60% in a variety of situations, indicating that these feedblocks were generally more palatable to the animals. This may be due to the higher proportion of calcined magnesite (about 50%), present as the granular material, in the sugar beet pulp/C.M. mix compared with the molasses-based feedblocks which contained about 10 to 20% C.M., present as powdered material. The variation in individual intakes of feedblock

was, surprisingly, considerably less than that of trough-fed supplementary concentrates, with animals at grass. Kendall (1977), however, consistently found that the mean CV for individual feedblock intake was significantly greater than when an equivalent amount of dry matter was given as concentrates in troughs, e.g. 82% v. 55% for cattle at grass. The blocks used by Kendall were, however, rather different (notably harder and generally less palatable) than those used in the present studies. Kendall reports slightly lower CV's for trough-fed animals than found here, with the CV being markedly lower when the animals were housed rather than at grass. The CV is known to increase with increased competition at the troughs, i.e. with decreased concentrate ratio or reduced individual trough space allowance. However, in the present experiments trough space allowances were adequate and in Experiment 5.1. the CV remained the same when 1 kg or 0·75 kg supplementary concentrate/cow/day was given. Trough-feeding is generally assumed to give a fairly uniform individual dry matter intake, however, some individuals are very slow to compete at troughs and therefore refuse the supplementary magnesium. This is obviously undesirable but shy feeders can be soon recognised and alternative measures can be taken to prevent hypomagnesaemic tetany. The intakes of palatable feedblocks, as in the present trials, are surprisingly uniform, especially in view of Kendall's findings with other types of feedblock, and their use as carriers of supplementary magnesium for the prevention of hypomagnesaemic tetany might appear to be satisfactory. However, it is also clear that there are generally one or two individuals that eat very small quantities of block, thus remaining virtually "unprotected", but these animals remain unnoticed in the self-feed situation and alternative measures are not administered. Thus it can only be concluded that these feedblocks are not totally reliable prophylactic measures against hypomagnesaemic tetany.

The ideal method to ensure that all individuals in a herd receive

supplementary magnesium is obviously individual administration. This can be recommended and put into practice for dairy cows but is impractical for beef herds at grass. In this situation the particularly palatable free-access feedblocks used in these trials gave the most uniform intakes, with trough-feeding giving a wider range of consumption. In both cases, however, there was the occasional "shy feeder", which in practice would require separate supplementation or treatment. In addition, cows immediately following abrupt transfer to spring pasture, i.e. when at greatest risk of hypomagnesaemic tetany, appear reluctant to consume supplementary feed in any form. However, it is generally considered "safer" to transfer animals gradually, over a period of days, to lush spring grass.

SECTION V

SOLUBILITY TESTS FOR CALCINED MAGNESITES IN VIVO AND IN VITRO

A. The Nylon Bag Technique

As early as 1938, Quin, Van Der Wath & Myburgh discussed the feasibility of using cylindrical bags made of very fine natural silk for measuring the digestion of feeds in the rumen of cannulated sheep. Since then many workers have developed this idea for various purposes, employing what are variously called artificial fibre, dacron, terylene, polyester or nylon bags. The technique measures the disappearance of feed constituents from a permeable bag containing the test material after suspension in the rumen for varying periods. A major advantage is that it gives a rapid *in vivo* method to measure the proportion of feed constituents which are susceptible to fermentation in the rumen, and estimates of feed digestibility, protein degradation and carbohydrate breakdown can be made.

The rumen is now widely believed to be a major site for magnesium absorption in the ruminant digestive tract (see Section I) and it follows that the amount absorbed will be directly affected by the amount of magnesium dissolved in the rumen liquor. It therefore seemed appropriate to extend the nylon bag technique to compare the rumen solubilities of different inorganic dietary magnesium supplements and, possibly, to relate these to their apparent availabilities. There is, however, no published data on the dissolution of calcined magnesite in the rumen.

Various workers have discussed the variables that can affect the results for organic feeds in nylon bags (e.g. Mehrez & Ørskov, 1977; Neathery, 1969; Van Keuren & Heinemann, 1962), some of which might also apply to inorganic materials. The time during which the bag remains in the rumen is important and will influence the digestibility value observed, but this may not be an accurate reflection of the length of time the test material would remain in the rumen had it been ingested. The dietary regimen of the fistulated animal can have significant

effects on the results, and the position of the bag within the rumen is important. Balch and Johnson (1950) showed that dry matter loss from bags was almost twice as much in the ventral sac compared with the dorsal sac of the rumen, and many investigators have found it necessary to attach weights in order to anchor the bags in the ventral sac where digestion is more rapid. Inorganic magnesium supplements have a higher density than the organic feed samples and this problem was thought not to apply in the present studies.

The method of processing the bag and contents after removal from the rumen can have a considerable effect on the reliability of the results. McManus, Manta and McFarlane (1972) reported that dry matter disappearance increased as the time of rinsing the bag in water increased. This is possibly partly because some organic constituents may be water soluble and further dissolution of the test material might occur, as well as being due to the increased removal of adhering rumen contents. A standard "rinsing technique" is apparently critical. However, in the present case, the calcined magnesites are water-insoluble and it was considered reasonable to continue washing until all traces of unwanted rumen material had been removed, without danger of losing further calcined magnesite from the bag.

The sample size, especially in relation to the size of the bag, is important (Mehrez & Ørskov, 1977; Van Keuren & Heinemann, 1962), with dry matter disappearance decreasing with larger samples. This is probably related to a decreased natural flow of digesta and agitation of the test material when it occupies a large volume of the bag, and the problem should not arise with small weights of calcined magnesites which are relatively dense. The "natural" conditions inside the bag will also be influenced by the pore size of the nylon, which can therefore affect digestibility results. Solid particles of rumen contents can pass in and out of the bags, depending on pore size, and the extent of this movement

will affect estimates of the loss of test material. The disappearance of calcined magnesite from nylon bags would represent both dissolved material and solid particles from which material has been dissolved thus rendering them sufficiently small to pass through the pores. A minimum pore size which does not interfere with the flow of rumen fluid in and out of the bags (a surface tension effect would develop with excessively small pore sizes) and yet requires the calcined magnesite to be largely dissolved when it leaves the bag, is therefore desirable. After consultation with Mrs. P.J. Strachan (Unilever Research, Colworth House, Bedford), and a consideration of available nylon materials, mesh sizes of 24 μm and 43 μm were considered appropriate in the present studies.

Mehrez and Ørskov (1977) reported variation in test material disappearance from bags between animals and between days of incubation. From this and the above discussion it is clear that the standardisation of the procedure is important, and useful comparative data is obtainable, however, the repeatability of precise results is less certain.

Experiment 8. A series of trials to investigate the solubility of different magnesium supplements in the bovine rumen.

Introduction

A number of trials were conducted to determine the relative solubilities of different dietary magnesium supplements in the bovine rumen *in vivo*, using the nylon bag technique. It was thought possible that differences in *in vivo* rumen solubility might be reflected in similar differences in dietary availability (as determined in Sections II, III and IV). Indeed it has recently been reported (Jesse *et al.*, 1981) that differences in the dietary availability of ground versus unground magnesium

oxides were reflected in differences in the solubility in vitro rumen fluid.

Materials and Methods

The nylon bags were constructed from precision-made nylon cloth (Henry Simon Ltd., Special Products Division, Stockport, Cheshire) with pore size 24 μm or 43 μm. Each bag was about 14 x 12 cm and care was taken in sewing the bags to ensure a smooth interior with no pockets or crevices, and no exposed edges of fabric to fray. A nylon cord c. 80 cm long was sewn to each bag. After introduction of 5·000 g of the test material, the open end was folded over twice and stapled closed. The free end of the cord was tied to a short length of rubber tubing to which a number of bags could be attached. The bags were thus suspended in the rumen to a depth of about 40 - 50 cm, held by the rubber tubing which remained outside the fistula.

After removal of a bag from the rumen at a particular time (anything from 1 to 14 days) it was thoroughly washed under running water until all traces of adhering rumen contents were removed and the washing water was no longer discoloured. The bags were allowed to drip and then dried for 48 hours in a small glass-drying oven at about 80°C. The partly-digested, dried sample was carefully removed from the bag, weighed and analysed for magnesium.

The experimental animals were four non-productive Ayrshire cows which had had rumen fistulae in position for a number of years. They were used either at pasture or in a byre where they received straw and concentrates.

Each cow had up to 8 bags in its rumen at any one time, and samples of rumen liquor were taken periodically for pH, sodium and potassium analyses.

Details of the supplements used in each of the twelve trials A to M, duration in the rumen, bag mesh size and basal diet of the cows are given in the following table:-

TRIAL	Basal diet	Bag mesh size (µm)	Magnesium supplements (particle sizes in µm)	No. of days in rumen
A	Grass (Summer)	24	1 Spanish C.M. <75	1,2,3,7,14
			2 75-150	
			3 150-250	
			4 250-500	
			5 500-1,000	
B	"	43	1)	2
			2)	
			3) As above	
			4)	
			5)	
C	"	43	3 Spanish C.M. 150-250	1,3,7,14
			6 English MgO 150-250	
D	"	24	6 English MgO 150-200	2
			7 200-500	
			8 500-1,000	
			9 1,000-2,000	
E	"	24	Spanish C.M.(75-500) calcined at	
			10 Raw	2,4,7,14
			11 500°C	
			12 650°C	
			13 800°C	
			14 900°C	
			15 1,100°C (as in Expt.1.2.)	

TRIAL	Basal diet	Bag mesh size (μm)	Magnesium supplements (particle sizes in μm)	No. of days in rumen
F	Grass (Autumn)	24	1 Spanish C.M. <75	2,3,7,14
			3 150-250	
			5 500-1,000	
			Spanish C.M. "burnt" at at 1,300°C (for Expt.7.1.)	
			16 <75	
			17 150-250	
			18 500-1,000	
G	"	24	19 Spanish C.M. "1979 product"	2,7,9
			20 Greek granular C.M. "1979"	
			21 Chinese granular C.M."1979"	
			22 Swedish granules	
			23 Irish granules (as in Expt.2.2.)	
H	"	24	5 Spanish C.M. 500-1,000	2,6,10,14
			24 Greek (1979) 500-1,000	
			25 Chinese (1979) 500-1,000	
			26 Greek 250-500	
			27 Chinese 1,000-2,000	
			28 Magnesium phosphate	
J	Bruised barley Barley straw	24	English MgO (<150) calcined at	2
			29 600°C	
			30 900°C	
			31 1,100°C	
			32 1,400°C	
			33 English "deadburnt" MgO 500-1,000 (as in Expt.1.3.)	

TRIAL	Basal diet	Bag mesh size (μm)	Magnesium supplements (particle sizes in μm)	No. of days in rumen
K	Bruised barley	43	3 Spanish C.M. 150-250	2
	Sugar beet pulp		34 " " " "burnt"	
	Oat straw		at 1300°C (for Expts.3.3.& 3.4.)	
L	"	24	35 Swedish C.M. > 2,000	2
			36 " 1,000-2,000	
			37 Irish C.M. > 1,000	
			38 " 250-1,000	
M	Sugar beet pulp	24	"1980 products" 500-2,000:	2,6,14
	Oat straw		39 Spanish	
			40 Greek	
			41 Chinese	
			75-500	
			42 Spanish	
			43 Greek	
			44 Chinese	
			(as in Expt.2.3.)	

Results and Discussion

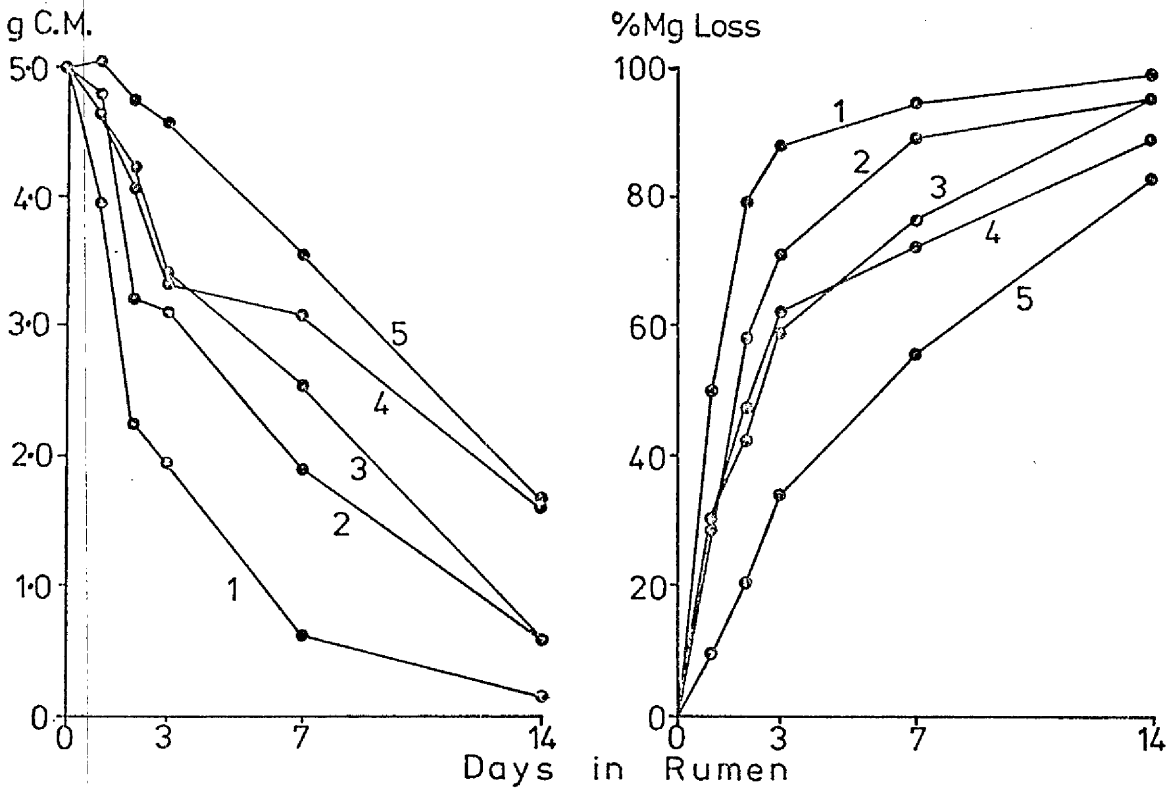
The results are presented for each trial in terms of (i) the weight of magnesium supplement (C.M.) remaining in the bag, and (ii) the percentage loss of magnesium from the original samples. The changes over a period of days are shown graphically where appropriate, and where samples remained in the rumen for a single time period the results are given in comparative histograms.

Table 1 gives the mean pH and sodium and potassium concentrations of the rumen liquor during the different trials.

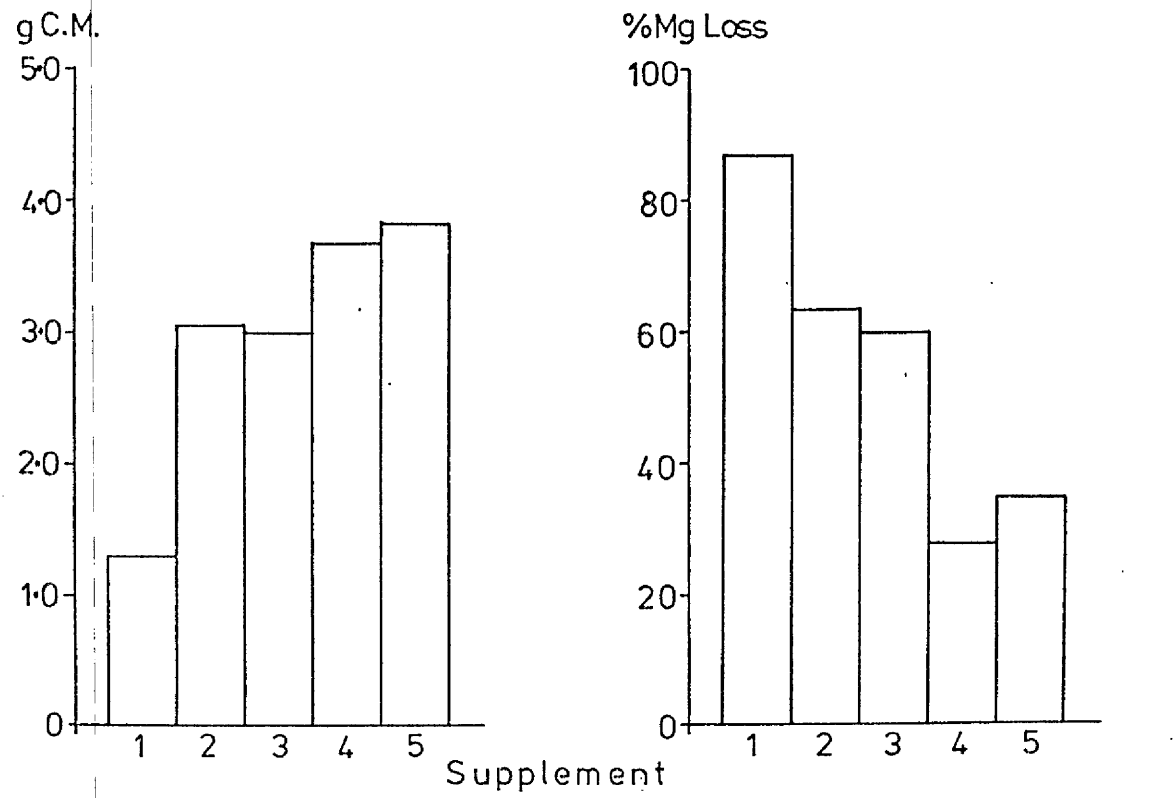
Table 1. Expt.8. Mean rumen conditions during the nylon bag trials: pH, sodium and potassium concentrations of rumen liquor.

		Concentrations (g/l) of		
TRIALS	pH	Na	K	Na : K ratio
At Grass				
A,B,C,D	6.7	2.80	1.04	2.7
E,H	6.0	2.82	0.53	5.3
F,G	5.7	2.38	0.58	4.1
Indoor diets				
J	6.8	2.72	0.50	5.5
K,L	7.0	3.15	0.39	8.1
M	6.8	3.26	0.37	8.8

TRIAL A



TRIAL B



Trials conducted with the cows at grass

TRIAL A : Different particle sizes of Spanish C.M. (Up to 14 days, 24 μm bags)

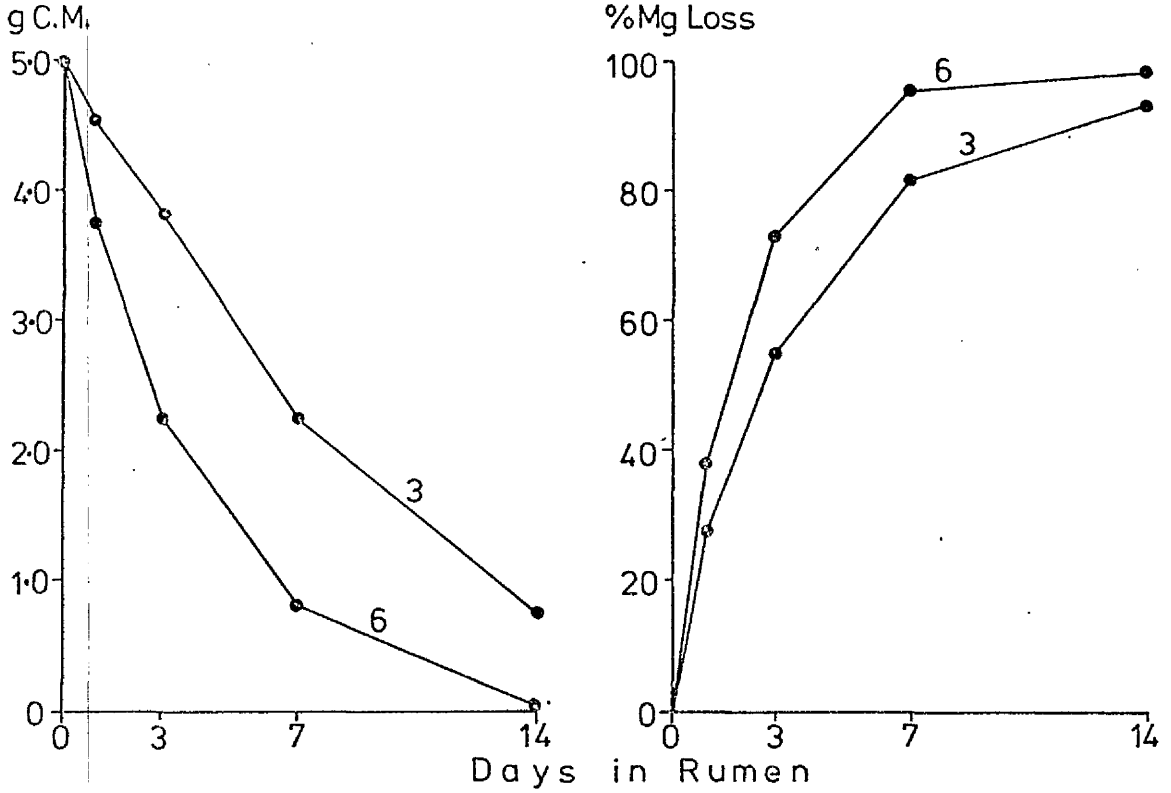
There was a progressive decrease in weight of each C.M. and a corresponding increase in the loss (%) of magnesium. The rate of loss over the 14 days decreased with increasing particle size, with the coarse 500-1,000 μm grade (5) being markedly the slowest to dissolve. This trend reflects that observed throughout this thesis that dietary C.M. availability decreases with increasing particle size.

Some disappearance of material from the bags may have been due to some particle escape through the nylon mesh without dissolution. An attempt to "calibrate" this effect was carried out, concurrently with Trial A, using acid-washed sand which is inert in the rumen. 5·000 g samples of different particle size fractions in 24 μm bags were left 3 days in the rumen, and the percentage weight loss for each fraction was only: <75 μm, 10.9%
75-150 μm, 2.2%
150-250 μm, 0.7%
250-500 μm, 0.9%
500-1000μm, 0.8%
The losses were therefore considered negligible, except for the <75 μm grade but the particle sizes within this fraction may differ between C.M. and sand. In any case, the validity of the method for powdered materials, under 75 μm, was considered doubtful.

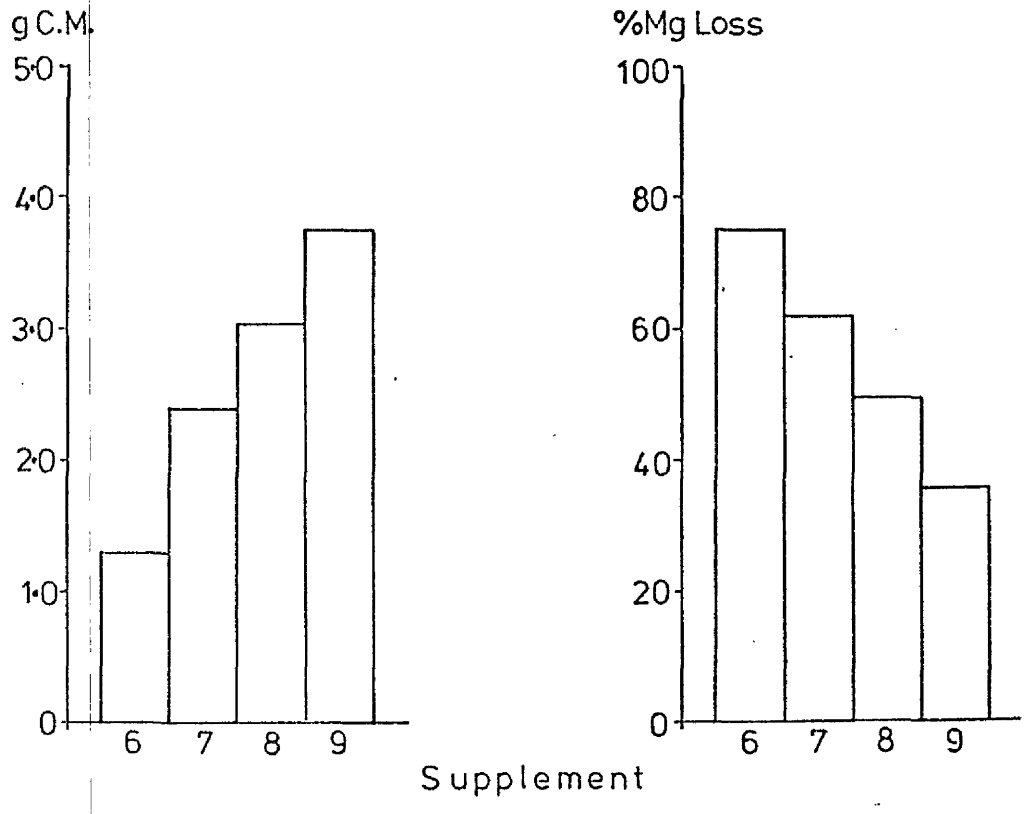
TRIAL B : Different particle sizes of Spanish C.M. (After 2 days, 43 μm bags)

After 2 days in the larger mesh size nylon bags (43 μm), the losses in weight and of magnesium showed the same trend of decreased rumen solubility with increased particle size of C.M. as seen in Trial A. The actual losses were, as expected, greater from the 43 μm mesh bags than from the 24 μm bags. Samples of sand (150 - 250 μm) in 43 μm bags after 3 and 7 days in the rumen lost 0.8 and 1.5% weight, respectively.

TRIAL C



TRIAL D



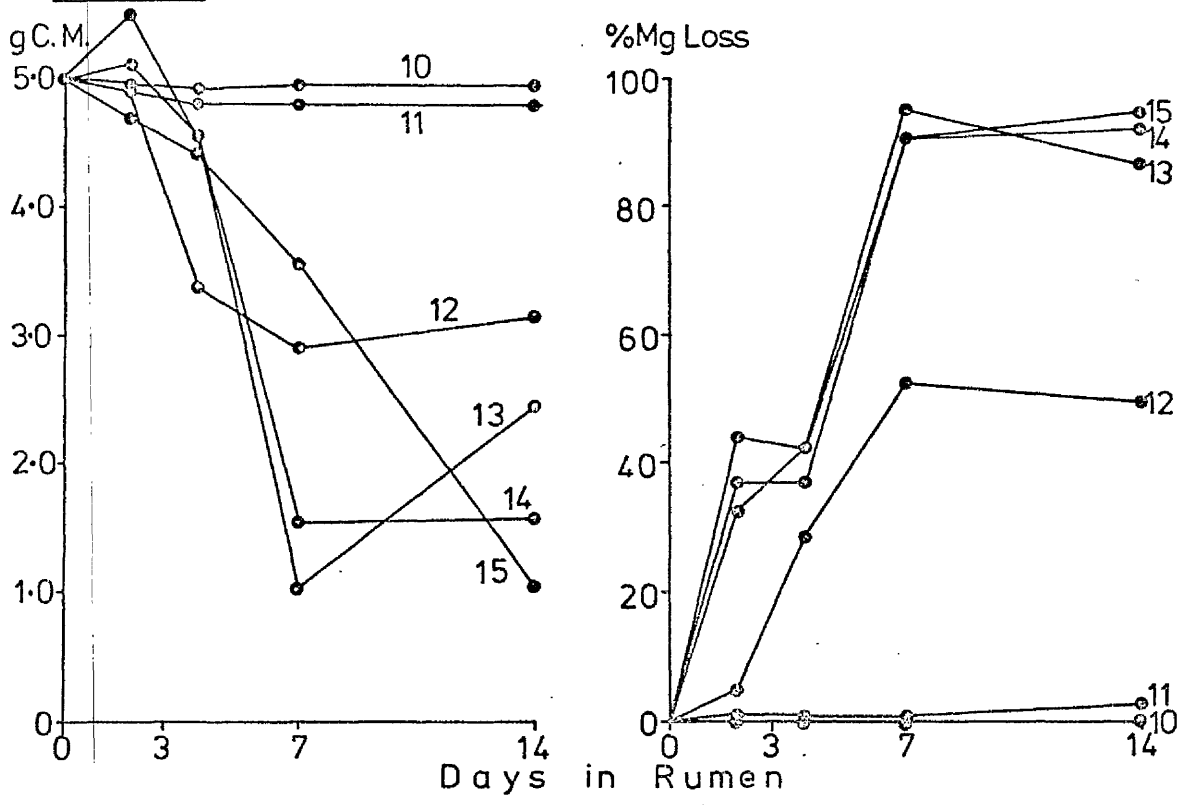
TRIAL C : Comparison of Spanish (3) and English (6) 150 - 250 μm fractions. (Up to 14 days, 43 μm bags).

The pattern of loss of magnesium from Spanish 150 - 250 μm C.M. repeated that observed previously, but the English material appeared to dissolve more rapidly. This was surprising considering the relatively poor availabilities usually observed for English material (e.g. in Expt.1.3.)

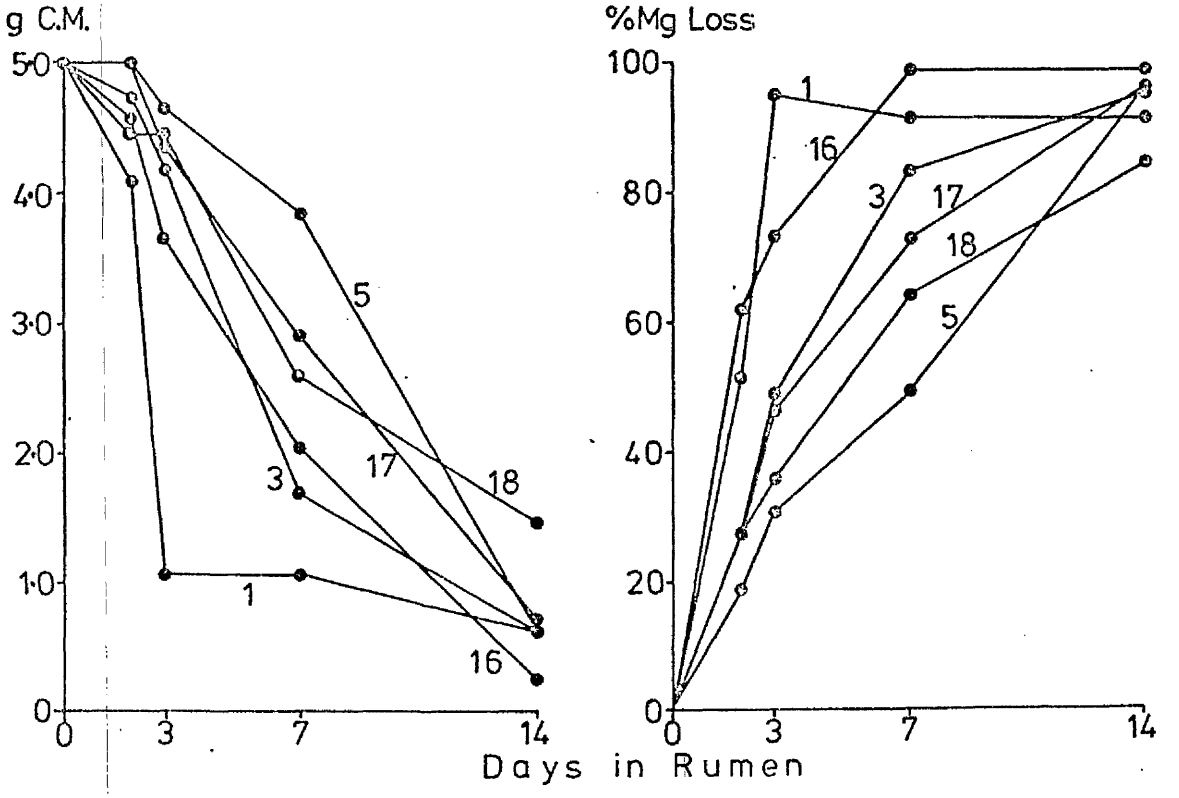
TRIAL D : Different particle sizes of English MgO. (After 2 days, 24μm bags)

As reported for Spanish C.M., solubility of English MgO tended to decrease with increasing particle size. The actual losses were again surprisingly high and it was thought likely that the English particles, being more friable than the Spanish particles, may have broken down and "escaped" from the bag without necessarily being dissolved.

TRIAL E



TRIAL F



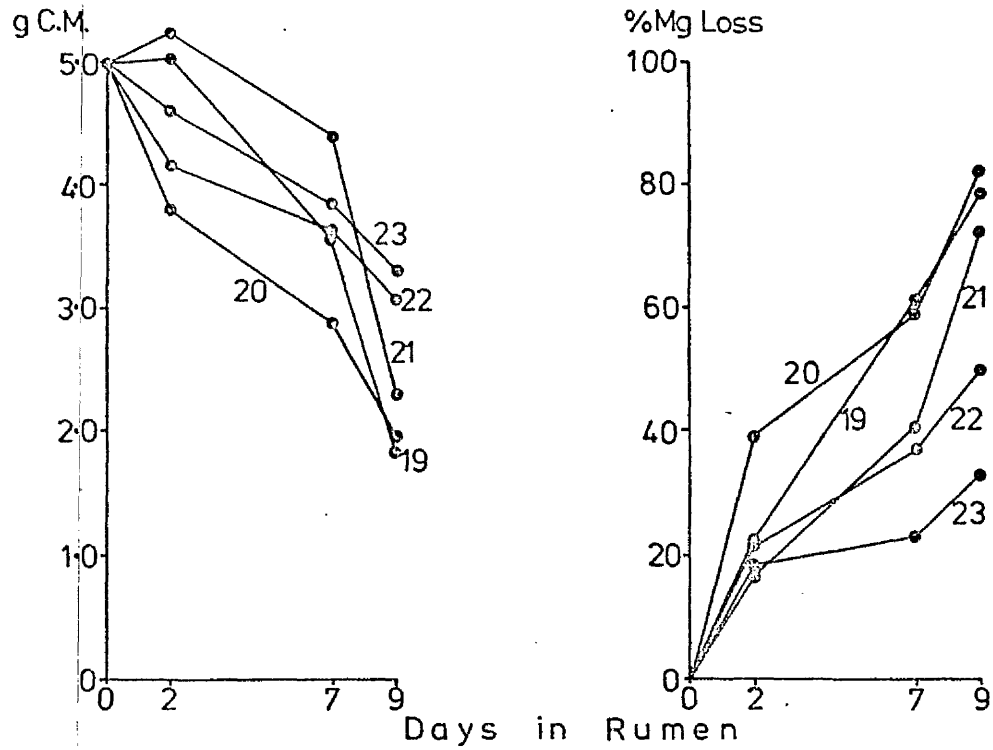
TRIAL E : Spanish magnesite calcined at different temperatures. (Up to 14 days, 24 µm bags).

Raw magnesite (10) and that calcined at 500°C (11) appeared to be insoluble in the rumen, whereas 800°C (13), 900°C (14) and 1,100°C (15) C.M.'s showed similar patterns of relatively high solubility, with 90% Mg dissolved in 1 week. 650°C (12) C.M. was of intermediate solubility with about 50% Mg loss in 1 week. This pattern satisfactorily reflects the pattern of availabilities reported in Expt.1.2. in which 800°, 900° and 1,100°C C.M.'s all had similar high availability (c. 40%) whereas raw, 500° and 650° C.M.'s were very poorly available. Subsequently Expt.3.4. has reported a better availability (25.0%) for 650° C.M., which tends to agree with its intermediate rumen solubility.

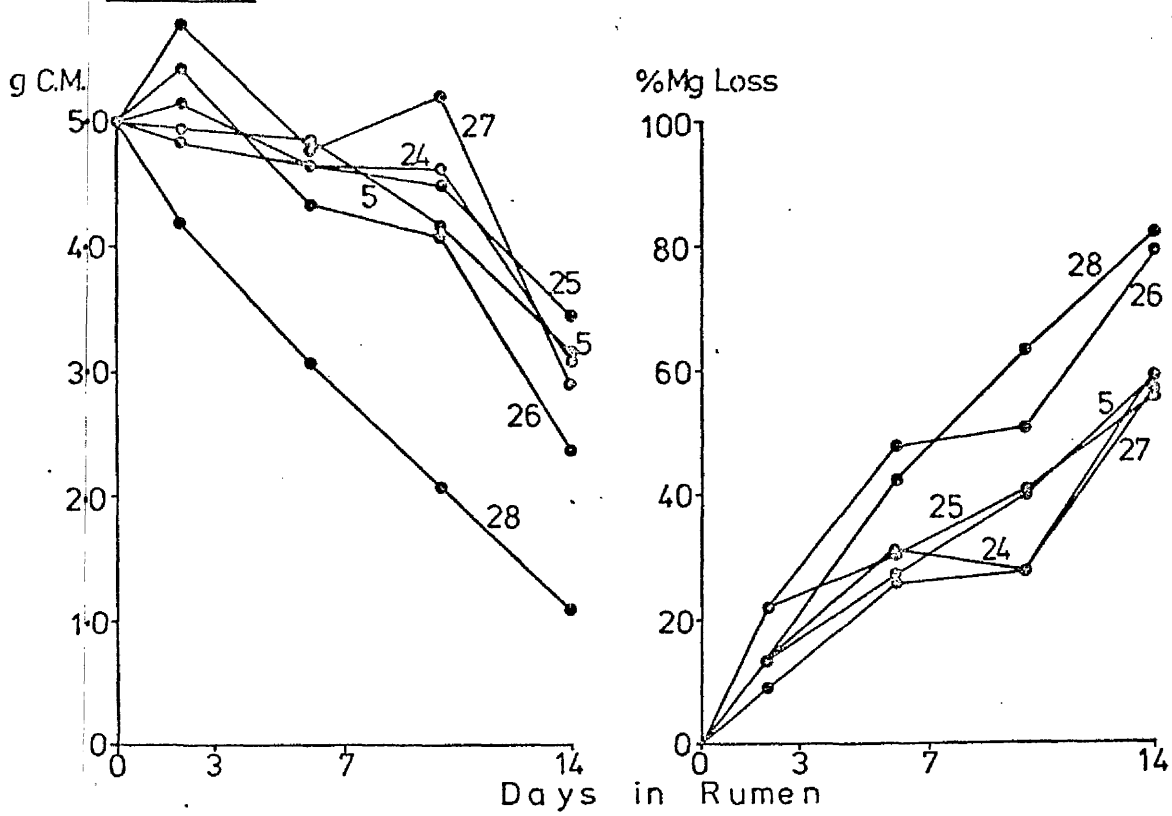
TRIAL F : Particle size grades of Spanish C.M. untreated and "burnt" at 1,300°C. (Up to 14 days, 24 µm bags).

The rates of disappearance of Mg from fine (1), medium (3) and coarse (5) grades of Spanish C.M. in the rumen agree well with Trial A. Subjecting these fractions of "burning" at 1300°C (supplements 16, 17 and 18, respectively) did not affect their rates of dissolution over the 14 days. This agreed with the findings in Expt.7.1. when "burning" did not affect the apparent dietary availabilities of fine and coarse grades. However, in Expt.7.1. the availability of medium C.M. was apparently depressed by burning, but in the present trial its rumen solubility was unaffected.

TRIAL G



TRIAL H



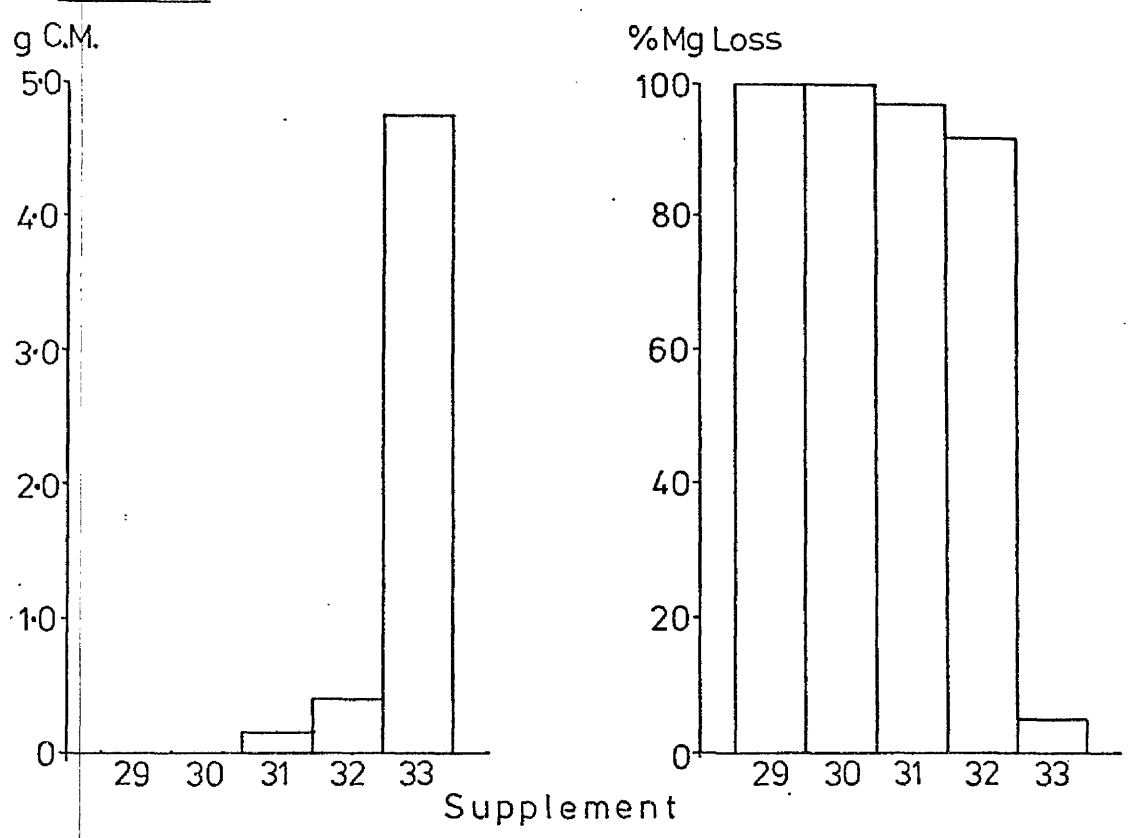
TRIAL G : Granular C.M. products from different countries. (Up to 9 days, 24 µm bags).

The Swedish (22) and Irish (23) "manufactured" granules (as used in Expt.2.2.) appeared less soluble in the rumen than the three commercial (1979) granular animal feed C.M.'s from Spain (19), Greece (20) and China (21), and of these three the Spanish and Greek C.M.'s were more soluble than Chinese. This pattern did not reflect closely the differences in availability observed in Expt.2.2., as the Irish C.M. was the most available, followed by Chinese, and the Greek C.M was the least available. It is possible that the nylon bag technique is not suitable for such "whole" products which span a wide range of particle sizes, and closer control of the particle range may give more accurate results.

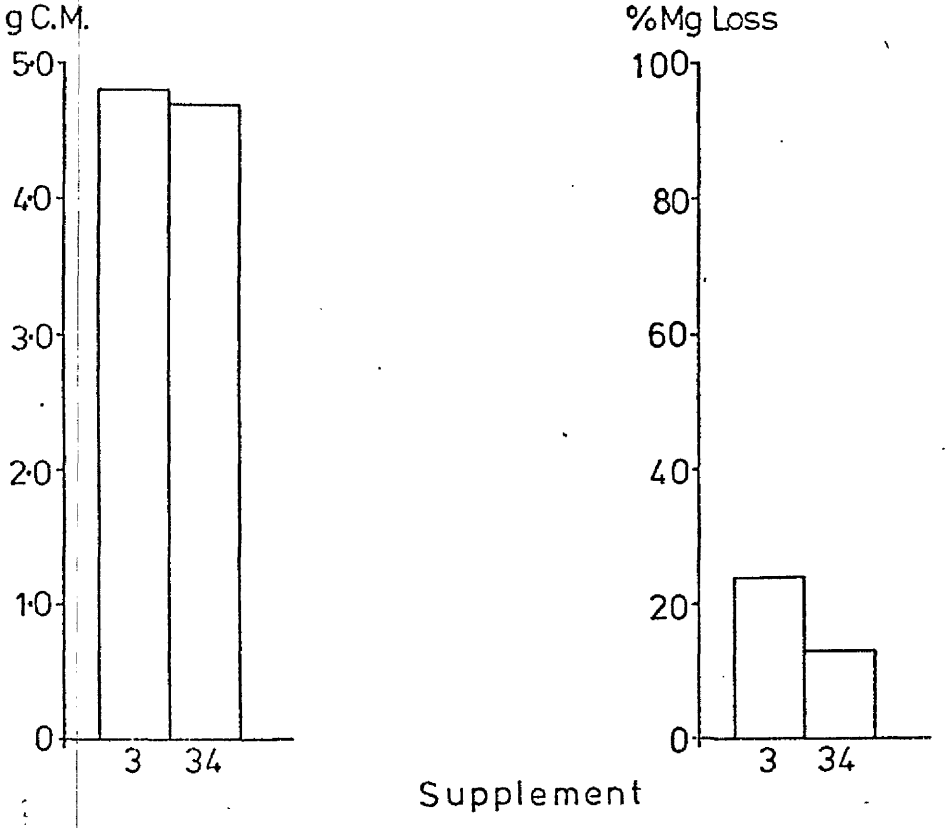
TRIAL H : Magnesium phosphate and different particle size grades of granular C.M.'s. (Up to 14 days, 24 µm bags).

Magnesium phosphate (28) was the most soluble supplement over the 14 days. Greek 250 - 500 µm (26) was more soluble than Greek 500 - 1,00 µm (24), and Chinese 500 - 1,000 µm (25) was more soluble than Chinese 1,000 - 2,000 µm (27). These respective fractions constituted the major part of the Greek and Chinese C.M. products as used in Trial G and the solubility patterns therefore agree quite well between the trials, with Greek C.M. being overall slightly more soluble than the Chinese. Of the 500 - 1,000 µm grades, the Spanish (5) and Chinese were slightly better than the Greek in this trial.

TRIAL J



TRIAL K



Trials conducted with the cows on indoor diets

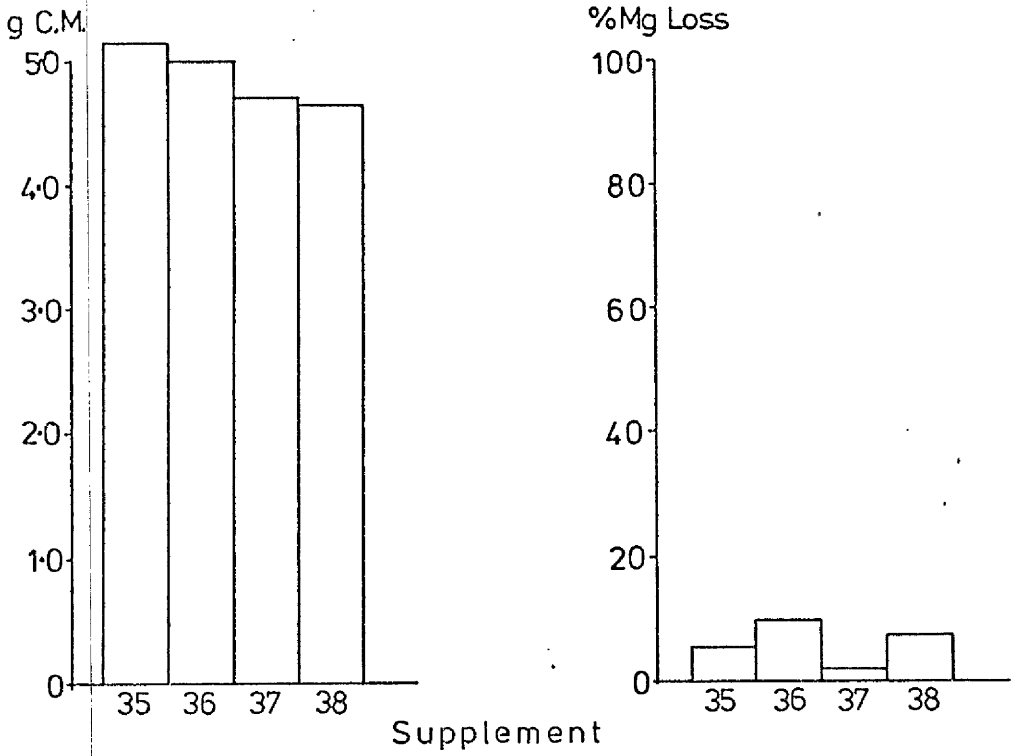
TRIAL J : English MgO calcined at different temperatures. (After 2 days, 24 μm bags).

The four English MgOs calcined at different temperatures were completely dissolved after just 2 days in the rumen. This contrasts markedly with the very poor availabilities reported for these materials in Expt.1.3. However it is possible that although the particle size was up to 150 μm there was considerable particle breakdown and dust present which could be lost from the bags. The coarse deadburnt English MgO (33) was virtually insoluble as expected.

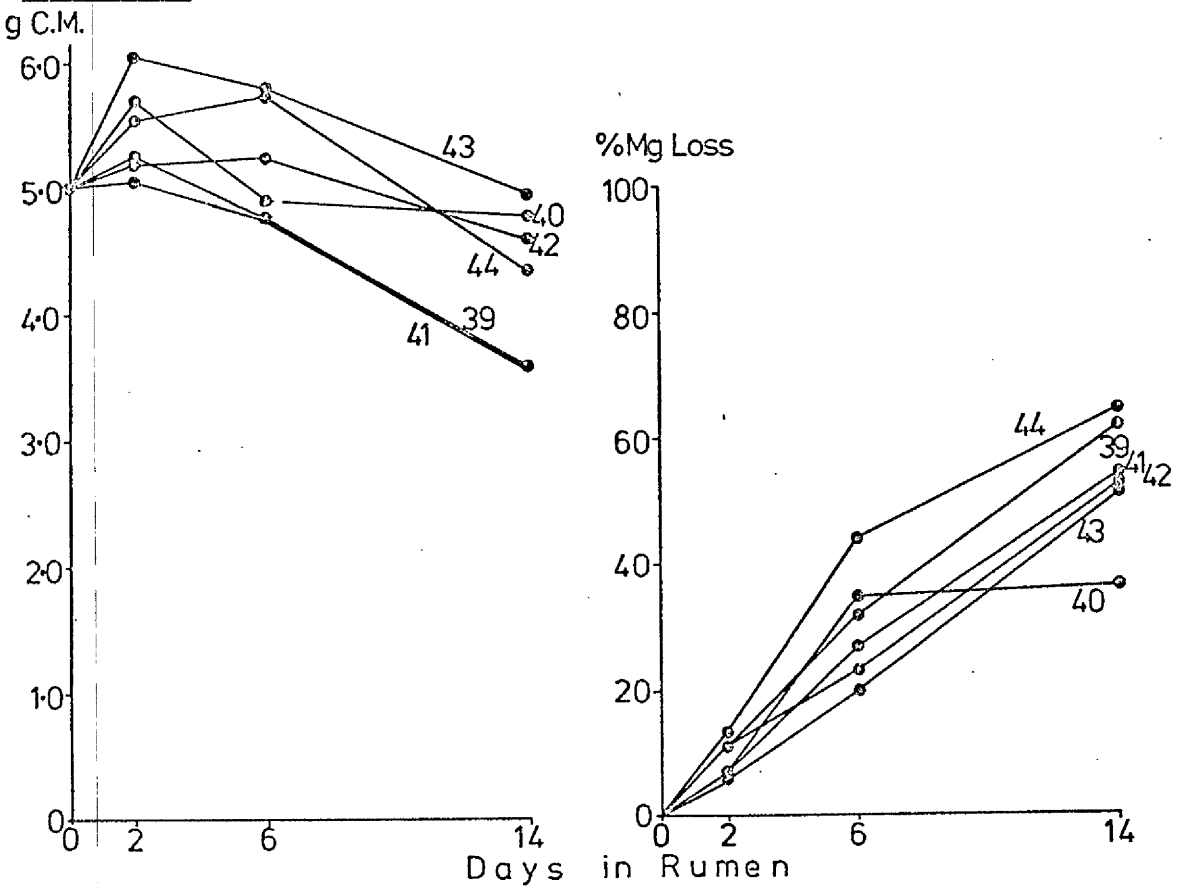
TRIAL K : Spanish C.M. 150 - 250 μm untreated and "deadburnt" (as in Expts.3.3. and 3.4.). (After 2 days, 43 μm bags).

The deadburnt material (34) was apparently only half as soluble as when untreated (3), with 13% and 24%, respectively, of the Mg disappeared in 2 days. This agrees with the difference in availability results reported in Expt.3.3. (27% and 50%, respectively) but the actual Mg loss in 2 days from Spanish 150 - 250 μm in this trial (24%) was considerably lower than that observed in Trials B and C (40 - 60%) (also using 43 μm bags) when the cows were at grass.

TRIAL L



TRIAL M



TRIAL L : Swedish and Irish C.M.'s (After 2 days, 24 μm bags).

All four C.M.'s were little dissolved in the 2 days. The Swedish C.M. over 2,000 μm (35) was less soluble than 1,000 - 2,000 μm (36), at 6% and 10%, respectively, and the Irish C.M. over 1,000 μm (37) was less soluble than 250 - 1,000 μm (38), at 2% and 7.5%, respectively. As in Trial G, the Swedish material was slightly more soluble than the Irish. Solubilities were generally lower in the present trial with the cows indoors than in Trial G when the cows were at grass.

TRIAL M : Spanish, Greek and Chinese "1980" products (as in Expt.2.3.) (Up to 14 days, 24 μm bags).

The Greek fractions appeared the least soluble, with the smaller particles 75 - 500 μm (43) being more soluble (over 14 days) than the corresponding coarse grade 500 - 2,000 μm (40). This agrees with their relative and poor availabilities reported in Expt.2.3. (2.8 and 0.4%, respectively). Spanish 75 - 500 μm (42) and Chinese 500 - 2,000 μm (41) had similar higher solubilities, again agreeing with their similar and higher availabilities in Expt.2.3. (18.2 and 20.8%, respectively). Spanish 500 - 2,000 μm (39) was more soluble, and the most soluble was Chinese 75 - 500 μm (44). These also had higher availabilities in Expt.2.3. (27.4 and 26.5%, respectively). Thus for Greek and Chinese C.M.'s, solubility in the rumen and availability decreased with increased particle size, with Chinese being better than Greek. For Spanish C.M., solubility, and indeed availability in Expt.2.3, reflected the opposite trend with the coarser fraction being better, but overall it was of intermediate merit to the Chinese and Greek.

General Discussion

For the series of magnesium supplements investigated within each trial, there were some clear differences in their relative rates of apparent dissolution in the rumen, and these generally reflected relative differences previously reported in their apparent dietary availability. Changes in the weight of the C.M.'s in the bags were relatively meaningless *per se* and changes in the amount of magnesium present, expressed as a percentage of that present in the original sample, were considered to be more accurate for comparative purposes. Indeed some C.M.'s *increased* in weight after 2 days in the rumen (despite thorough removal of adhering rumen contents during the washing procedure), possibly due to hydration of some of the magnesium oxide to hydroxide, which might be necessary prior to dissolution.

In order to demonstrate the relationship between availability and rumen solubility, the dietary availability for 27 of the C.M.'s investigated here has been correlated with the percentage loss of magnesium from each C.M. after 2 days in 24 μm mesh size nylon bags in the rumen (see Table 2 and Figure 1). The availability figure used for each C.M. is the average of all apparent availability results obtained for it earlier in this work (and the particular experiments involved are given in Table 2). This correlation excludes the English MgOs used in Trial J (due to their anomalously high apparent solubilities here, and their anomalously low availabilities reported in Expt.1.3.). It also excludes those C.M.'s for which there is no availability data, or which were investigated in the 43 μm mesh size bags. Magnesium phosphate is not included as the correlation was restricted to magnesium oxides, and thus the rumen solubility results used are from Trials A, E, F, G and M.

It can be seen that there is a highly significant positive correlation, $r = 0.572$ ($P < 0.005$), between dietary availability and rumen solubility in 2 days. This correlation is extremely close, especially in view of the

lack of standardisation of rumen conditions for the solubility tests. Rates of disappearance of C.M.'s were generally lower when the cows were given indoor rations, in Trials K, L and M (excluding the anomalous Trial J), than when they were at grass (summer or autumn). Indoors, the rumen liquor tended to have higher pH, and a higher Na : K ratio (Table 1). However this contrasts with the belief that a low Na : K ratio, as one finds in young "tetany-prone" grass, is detrimental to magnesium absorption (see Section I.3., e.g. Martens & Rayssiguier, 1979). It is possible that a lower rumen pH favours C.M. dissolution, but in addition the less solid consistency of the rumen contents when the cows were at grass may have allowed a greater flow of rumen liquor through the bags, and less impeded movement of the bags themselves within the rumen.

It should perhaps be stressed that the suspension of calcined magnesites inside nylon bags in the rumen is an unrealistic situation and actual rates of apparent dissolution observed may not necessarily apply to orally administered supplements. For example, the precise position within the rumen may be critical, natural flow of rumen liquor through the nylon bag may be slightly impeded, and the time periods for which the bags remained in the rumen were largely unrealistic. Many factors may affect the rate of flow of digesta along the ruminant digestive tract, and hence the efficiency of utilisation of the feed (e.g. Balch & Campling, 1965), but an average retention time for digesta in the rumen itself is about 2 days. More critical, perhaps, with regard to C.M. availability is the time for which the liquid phase of the rumen contents, supposedly containing some dissolved C.M., remains in the rumen. In addition, the specific gravity of C.M. particles may be important in affecting their retention times (e.g. Campling & Freer, 1962); for example, fine particles may be washed through the rumen fairly rapidly in the liquid phase in practice. However, these nylon bag trials have demonstrated that C.M.'s are at least partly

soluble in the bovine rumen, but they also show that the animal cannot make full use of all of the magnesium provided, as many C.M.'s were not fully dissolved even after 14 days.

Thus differences in the rumen solubility of C.M.'s are reflected in differences in their dietary availability. The nylon bag technique therefore provides a quick in vivo test to compare the relative merits of different magnesium oxides. It is possible that a more precise test for availability predictions could be developed if rumen conditions are more closely controlled and monitored, but, as carried out in Experiment 8, the test can provide valuable comparative data. The main disadvantage of this technique as an availability assay is that it is unsuitable for testing powdered materials.

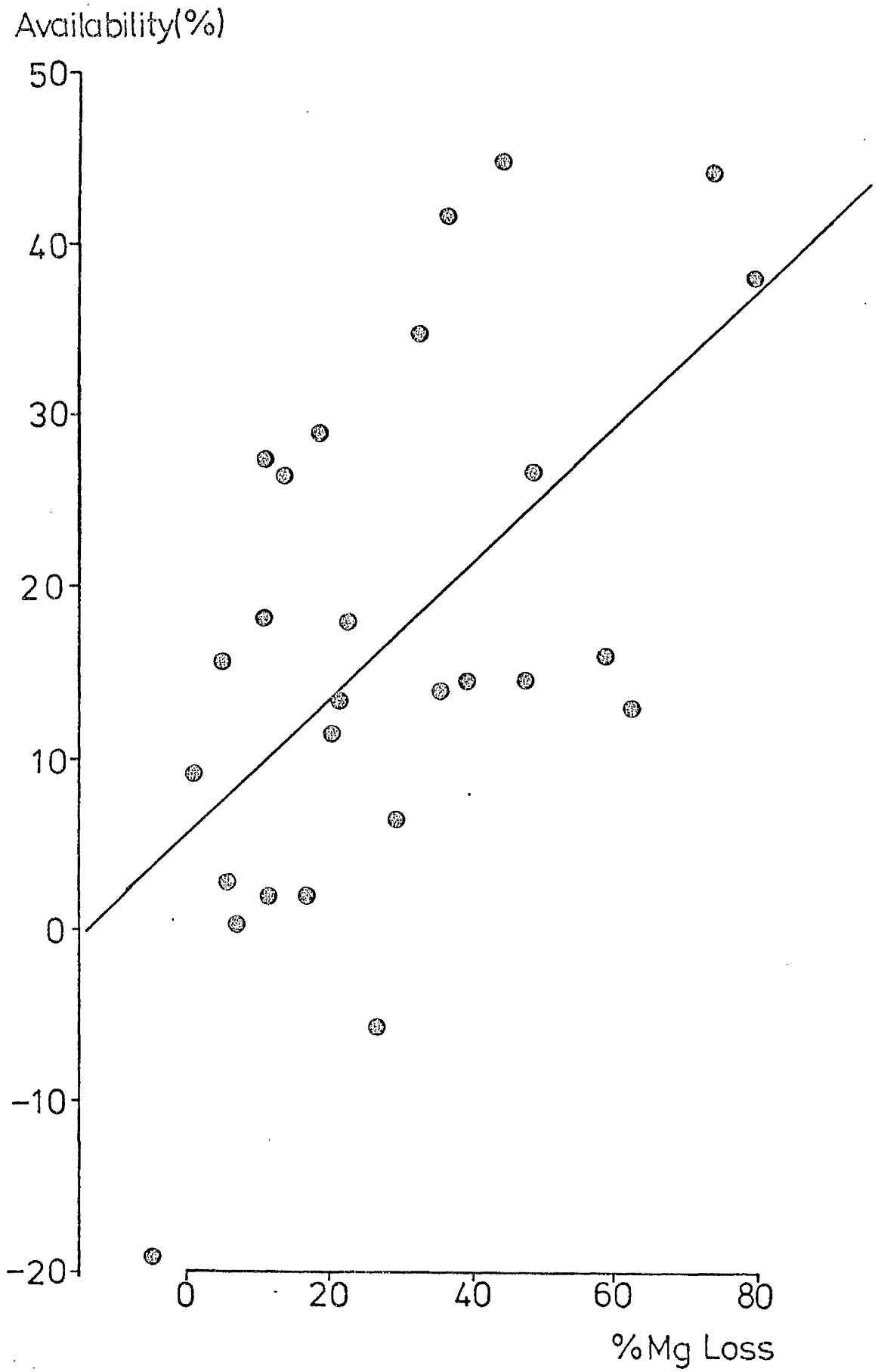
Table 2. Expt.8. Mean dietary availability (%) of calcined magnesites and the loss (%) of magnesium after 2 days in 24 μm mesh nylon bags in the rumen.

Supplement	Mean Availability (%)	Expt. Nos. used for mean availability	% Mg Loss
1 Spanish <75	38.0	1.1,2.2,2.3, 3.3,5.2,7.1.	78.9+
2 75-150	16.5	1.1	58.2+
3 150-250	26.6	1.1,1.4,3.3, 5.2,7.1.	48.6+
4 250-500	14.6	1.1,2.3.	47.4
5 500-1,000	11.5	1.1,2.2,3.3, 5.2,7.1.	20.1
7 English 200-500	12.9	1.3,2.3.	62.0
9 1,000-2,000	13.9	2.3	35.7
10 Raw Spanish magnesite	-19.3	1.2,2.1	-4.9
11 Spanish 500°C	9.2	1.2	0.9
12 650°C	15.7	1.2,3.4	5.2
13 800°C	34.7	1.2,3.4	32.5
14 900°C	44.6	1.2	43.9
15 1,100°C	41.5	1.2,3.4	36.7
16 Spanish <75 "burnt"	43.9	7.1	73.5
17 150-250 "burnt"	-5.6	7.1	26.7
18 500-1,000 "burnt"	6.4	7.1	29.2
19 Spanish C.M. "1979"	18.0	2.1,5.1,5.2, 7.2	22.7
20 Greek C.M. "1979"	14.6	2.1,2.2,5.1, 7.2	39.2
21 Chinese C.M. "1979"	2.0	2.1,2.2,7.2	16.6
22 Swedish granules	13.5	2.2	21.4
23 Irish granules	29.1	2.2	18.7
39 500-2,000 Spanish	27.4	2.3	11.0
40 " Greek	0.4	2.3	6.8
41 " Chinese	2.0	2.3	11.5
42 75-500 Spanish	18.2	2.3	10.8
43 " Greek	2.8	2.3	6.2
44 " Chinese	26.5	2.3	13.6

+ Results from Trial A.

Figure 1. Expt.8. Correlation between mean apparent availability (%) and the % Mg loss from different calcined magnesites after 2 days in 24 µm mesh nylon bags in the rumen.

$$y = 5.5 + 0.40x \; ; \quad r = 0.572^{**}$$


Availability(%)
50
40
30
20
10
0
-10
-20
0
20
40
60
80
%Mg Loss

B. Laboratory tests

There are various laboratory tests employed to characterise different magnesium oxides; for example, total magnesium content, weight loss on ignition (L.O.I.), specific gravity, surface area, Iodine number, water-soluble magnesium content (i.e. pH or electrical conductivity of water extract), and rate of dissolution in an organic acid such as citric or acetic (Britton et al., 1952; Durrant & Draycott, 1976; Harper, 1967; and Steetley Minerals Ltd., Magnesia Division). Some workers have attempted to relate a particular in vitro "chemical reactivity" test to a particular biological use of magnesium. For example, NH_4^+-exchangeable magnesium in soil (determined by extraction with ammonium nitrate) is shown to be reflected in crop magnesium uptake (Bolton & Penny, 1968; Draycott & Durrant, 1970), and NH_4^+-exchangeable magnesium in magnesium fertilisers (oxide and sulphate) gives a good indication of their plant-available magnesium content (Draycott & Durrant, 1972; Durrant & Draycott, 1976). Some Danish workers (Jensen & Jensen, 1980) advocate a dissolution test carried out at body temperature and constant pH 6·5 (pH-static titration) for in vitro estimation of the availability of dietary magnesium oxides to ruminant animals. The titration curve, called the "reactivity profile," gives the rate at which a particular magnesium oxide is dissolved and theoretically available for absorption by the animal. However, these workers did not include experimentally-determined in vivo availability data with which to compare their results, and merely assumed that their in vitro test predicts availability.

The method for determining total magnesium in feedingstuffs (including magnesium oxide) involves prolonged boiling in strong acid which is meaningless with respect to availability to the animal. It would therefore be useful to find a quick in vitro laboratory test which could determine "animal-available" magnesium. The following two experiments (9.1. and 9.2.) describe two dissolution tests for magnesium oxides

carried out with a view to comparing the results with the extensive *in vivo* availability data reported in this thesis.

Experiment 9.1. The determination of NH_4^+-exchangeable magnesium in various magnesium supplements.

Introduction

The determination of NH_4^+-exchangeable magnesium using Molar ammonium nitrate is a recognised method for determination of extractable magnesium in soils (M.A.F.F., 1973), and Durrant and Draycott (1976) have used the method to determine plant-available magnesium in magnesium oxide fertilisers. It was therefore decided to investigate this method as a possible assay for animal-available magnesium, using virtually all the magnesium supplements previously investigated *in vivo* in this thesis.

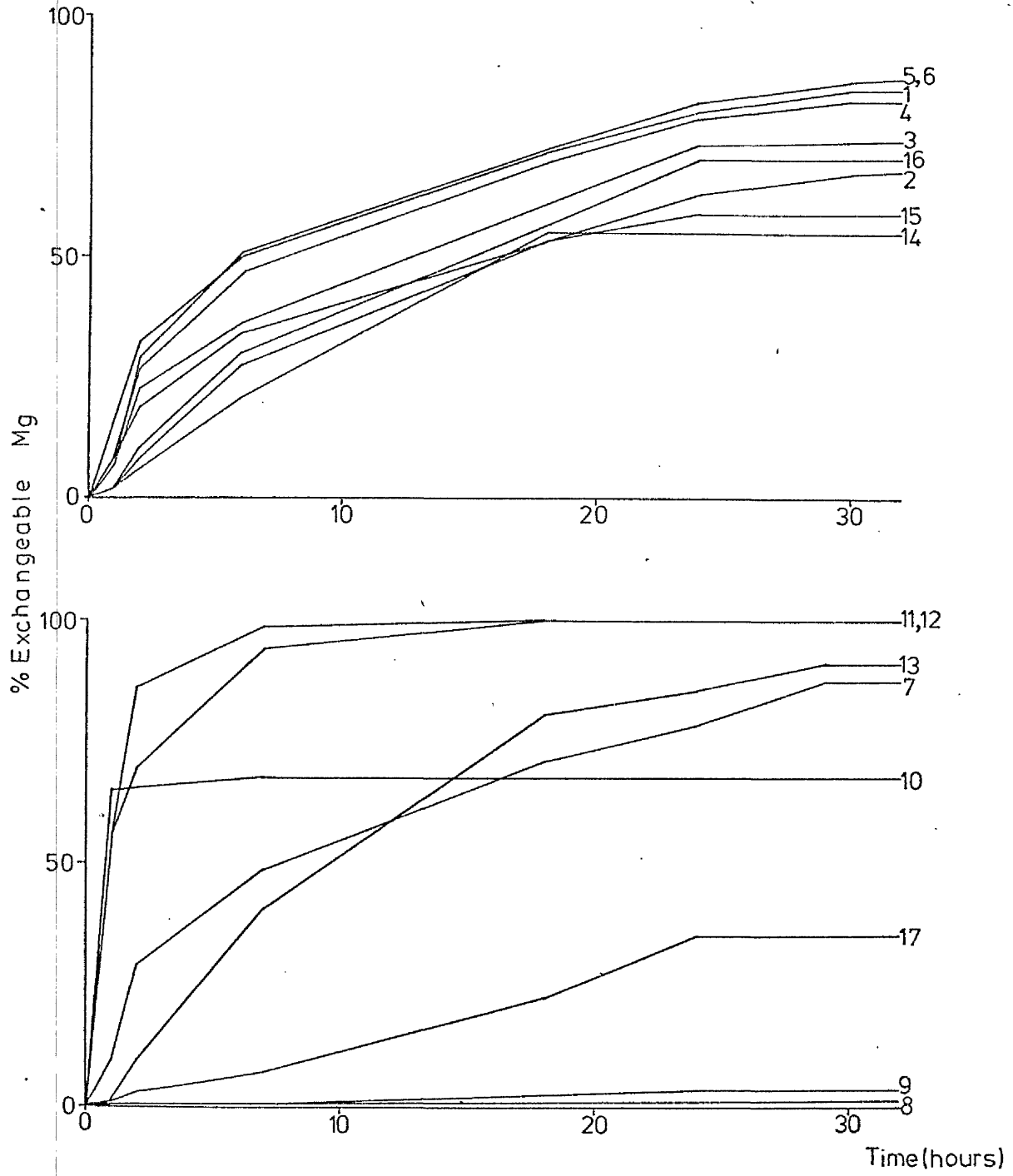
Materials and Methods

0·500 g samples of each magnesium supplement were placed in 250 ml screw-topped glass bottles, 100 ml 1 Molar ammonium nitrate solution was added to each bottle and the lids were secured (proportions after Durrant & Draycott, 1976). The bottles, fifteen at a time, were arranged on their sides on a laboratory shaker machine. They were then shaken at medium speed for a specified time period of 1, 2, 6, 18, 24, 30 or (some products only) 44 hours. Following this samples of all extracts were filtered off and analysed for magnesium (diluted with strontium chloride solution prior to atomic absorption spectroscopy, as for blood plasma magnesium analysis).

Results

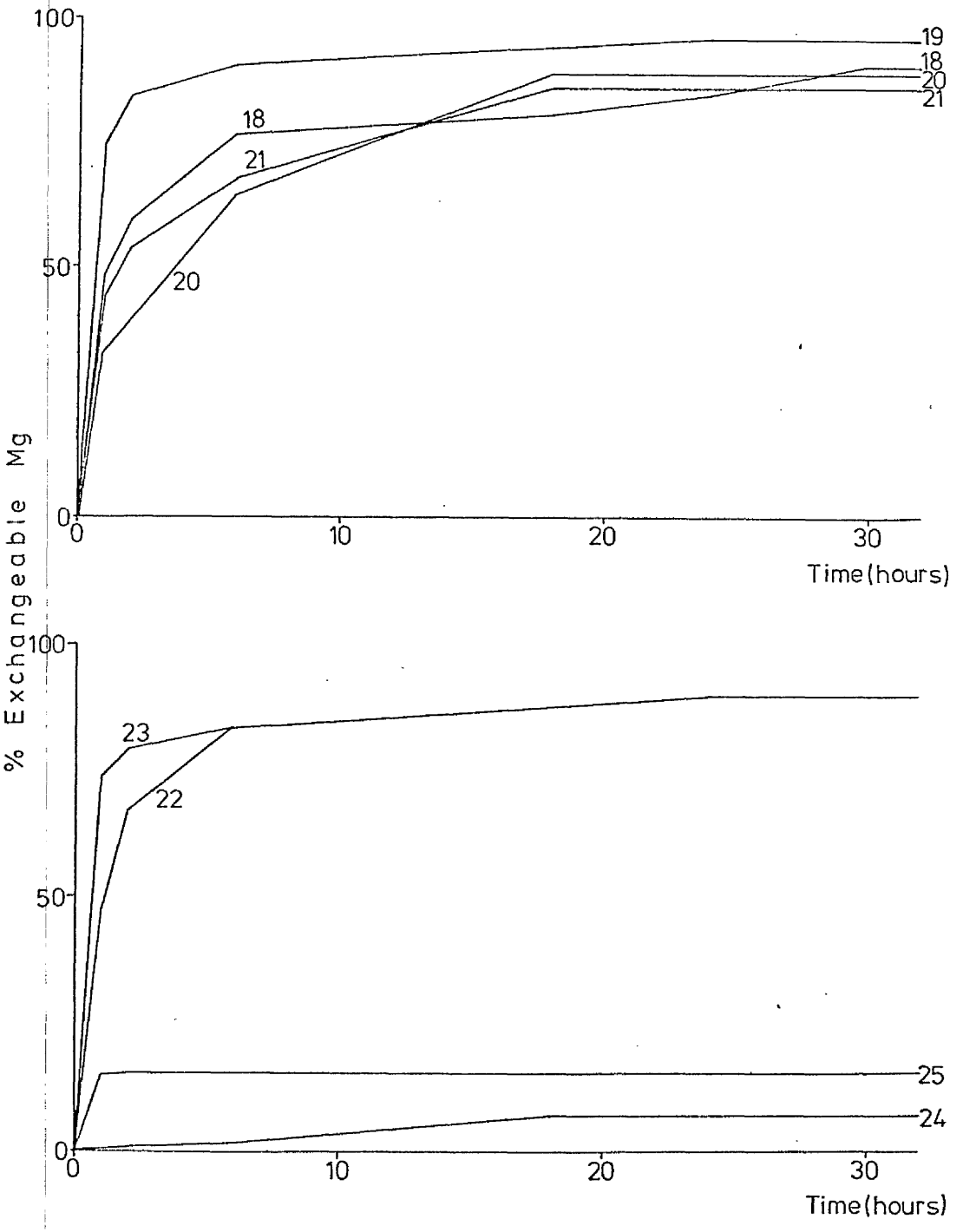
The following Figures 1-7 show the NH_4^+-exchangeable magnesium (as a percentage of total magnesium) as a function of extraction time for the

different magnesium supplements. In addition, for comparative purposes, the mean availability of each supplement is given (from a number of previous experiments whose numbers are also given).

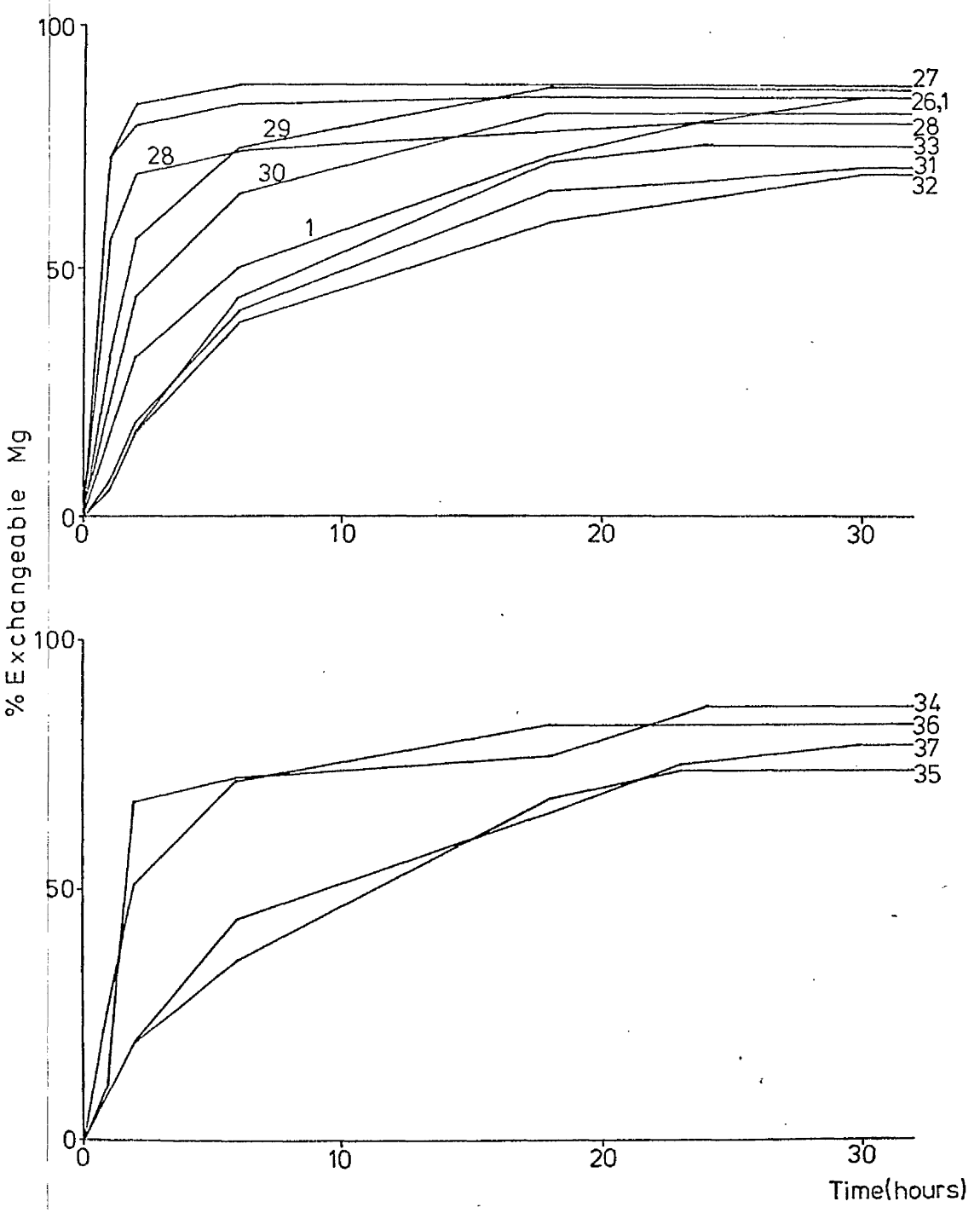

100
50
0
% Exchangeable Mg
0
10
20
30
5,6
1
4
3
16
2
15
14
11,12
13
7
10
17
9
8
Time (hours)

Figures 1 - 7. The release of NH_4^+-exchangeable Mg (as a % of total Mg) from different magnesium supplements as a function of time.

		Mean availability (%)	Availability calculated using Expt. Nos.
	Figure 1		
Spanish C.M. (μm)			
1	<75	38.0	1.1,2.2,2.3,3.3,5.2,7.1
2	75 - 150	16.5	1.1
3	150 - 250	26.6	1.1,1.4,3.3,5.2,7.1
4	250 - 500	14.6	1.1,2.3
5	500 - 1,000	11.5	1.1,2.2,3.3,5.2,7.1
6	1,000 - 2,000	-2.8	1.1
Spanish C.M. "burnt" at c. 1300°C (Expt.7.1.)			
14	<75	43.9	7.1
15	150 - 250	-5.6	7.1
16	500 - 1,000	6.4	7.1
	Figure 2		
7	Spanish C.M. "1979 product"	18.0	2.1,5.1,5.2,7.2
Spanish C.M. (75-500 μm) calcined for 0.75 h at:-			
8	Raw	-19.3	1.2,2.1
9	500°C	9.2	1.2
10	650°C	15.7	1.2,3.4
11	800°C	34.7	1.2,3.4
12	900°C	44.6	1.2
13	1,100°C	41.5	1.2,3.4
17	Spanish C.M. 150-250 μm deadburnt at 1300°C (Expt.3.3.)	29.7	3.3,3.4

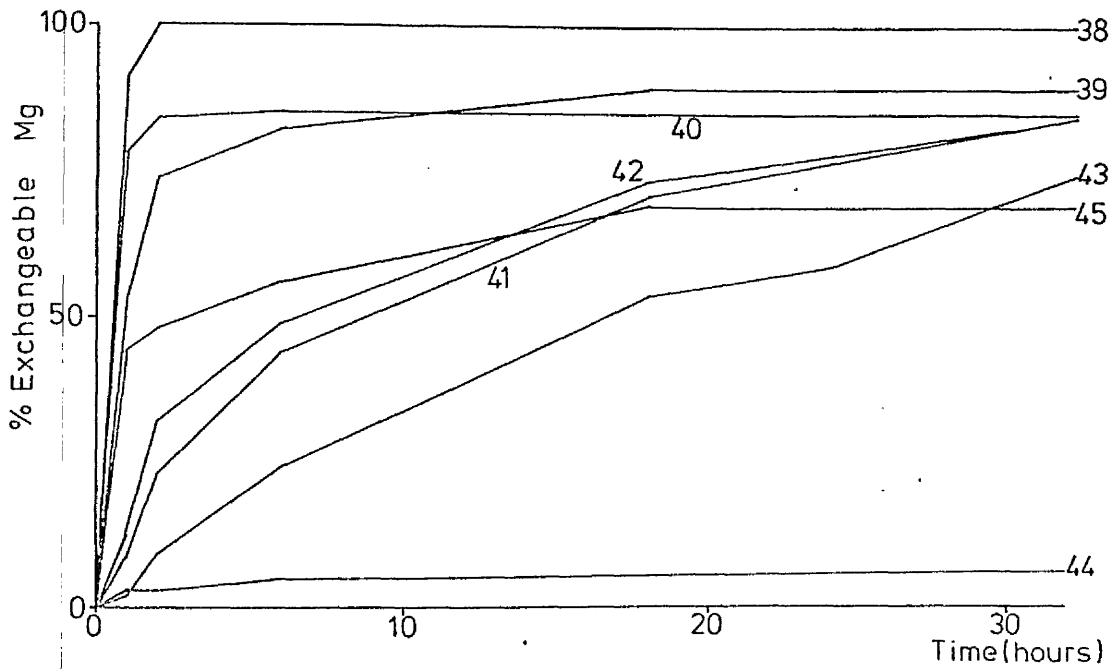

100
50
0
% Exchangeable Mg
19
18
20
21
18
21
20
0
10
20
30
Time (hours)
100
50
0
23
22
25
24
0
10
20
30
Time (hours)

			Mean availability (%)	From Expt. Nos.
	Figure 3			
18	Greek C.M. "1979"	granular	14.6	2.1,2.2,5.1,7.2
19	" "	powder	39.9	2.1,2.2,5.1
20	Chinese C.M. "1979"	granular	2.0	2.1,2.2,7.2
21	" "	powder	37.3	2.1,2.2
	Figure 4			
22	Swedish C.M.	granules	13.5	2.2
23	" "	powder	61.0	2.2
24	Irish C.M.	granules	29.1	2.2
25	Magnesium phosphate		47.6	3.3


100
50
0
0
10
20
30
% Exchangeable Mg
27
26,1
28
33
31
32
29
28
30
1
100
50
0
0
10
20
30
Time(hours)
34
36
37
35

		Mean availability (%)	From Expt. Nos.
Figure 5			
"1980" C.M. Products (μm particle size)			
26 Greek	<75	22.2	2.3,3.4
27	75-500	2.8	2.3
28	500-2,000	9.7	2.3,3.4
29 Chinese	<75	47.6	2.3,3.4
30	75-500	26.5	2.3
31	500-2,000	24.3	2.3,3.4
1 Spanish	<75	38.0	1.1,2.2,2.3,3.3,5.2,7.1
32	75-500	18.2	2.3
33	500-2,000	32.7	2.3,3.4

Figure 6			
"1980" whole C.M. Products			
34 Greek (granular)		24.9	2.3
35 Chinese granular (0·3 - 2·0mm)		29.5	2.3
36 "	gritty (30 mesh)	32.0	2.3
37 Spanish (granular)		37.3	2.3


100
50
0
% Exchangeable Mg
0
10
20
30
Time(hours)
38
39
40
42
43
45
41
44

	Mean availability (%)	From Expt. Nos.
Figure 7		
38 Analar MgO	36.9	2.1,3.4
39 English MgO powder	25.0	2.3,3.4
40 " $Mg(OH)_2$ powder	40.5	2.1,2.3,3.3,5.1
41 English MgO 1,000-2,000 µm	13.9	2.3
42 " 200-500 µm	12.9	1.3,2.3
43 American magnesia	20.5	2.3,3.4
44 English dolomite powder	14.2	2.3,3.4
45 Spanish Cyclone dust	34.4	2.1,3.3

Discussion

There are some clear differences in the patterns of NH_4^+-exchange for the different magnesium supplements in ammonium nitrate solution, in terms of both the rate of exchange and the proportion of magnesium exchangeable. However, these differences are evidently not necessarily reflected in the pattern of *in vivo* availability results.

Of the different particle size fractions of Spanish C.M. (Figure 1) the under 75 µm range and the 250-500, 500-1,000 and 1,000-2,000 µm ranges show very similar rates and extents of magnesium release, with the intermediate particle sizes showing a slightly lower pattern. This, therefore, does not reflect the trend of decreased availability with increased particle size. The "burning" of fine, medium and coarse grades (for Experiment 7.1.) had a slight detrimental effect on the NH_4^+-exchange for all three supplements, although for the fine and coarse fractions this is not associated with a decrease in apparent availability.

Raw Spanish magnesite and that "calcined" at 500°C were virtually totally unexchangeable (Figure 2), indicating that the magnesium in magnesium carbonate is not NH_4^+-exchangeable. 65% of the C.M. calcined at 650°C was exchangeable, and this occurred extremely rapidly, within 1 hour. Possibly 65% of the magnesium present is as magnesium oxide whereas the remaining 35% is as carbonate in this under-calcined material. The magnesites calcined at 800°C and 900°C are rapidly and highly exchangeable (100%), with that calcined at 1,100°C being slower and less exchangeable (90%). The Spanish "1979 commercial product" (Agma FG85) shows a similar pattern to the 1,100°C C.M., indicating that the commercial material is possibly subjected to this slightly higher temperature in the kiln (although kiln temperatures are quoted as 800-900°C). The order of increasing initial rate of Mg release is from 650°, 800°, 900° then 1,000°C C.M.'s, indicating that perhaps the most rapidly exchangeable Mg is in <u>just</u> calcined magnesium oxide, and prolonged exposure to temperature decreases its rate of exchange. Indeed the "deadburnt" C.M. from Experiment 3.3. (subjected to 1300°C) is very poorly NH_4^+-exchangeable. This detailed pattern of NH_4^+-exchange is not reflected in all availabilities, as the 800°, 900° and 1,100°C C.M.'s are of similar availability. However, raw magnesite and 500°C C.M. are indeed very poorly available, and 650°C C.M. is intermediate.

Greek granular C.M., "1979 product", is less exchangeable than the powdered Greek C.M. (Figure 3), but Chinese granular "1979" C.M. releases Mg only slightly more slowly than Chinese powdered C.M. This generally agrees with the lower availability of granular compared with powdered products. However, although the two powders have similar availability (Greek 39.9% and Chinese 37.3%), the Chinese material is less NH_4^+-exchangeable. Swedish C.M. granules are only initially marginally slower to exchange than the corresponding powder (Figure 4), in spite of the vast difference in their availabilities (13.5% and 61.0%,

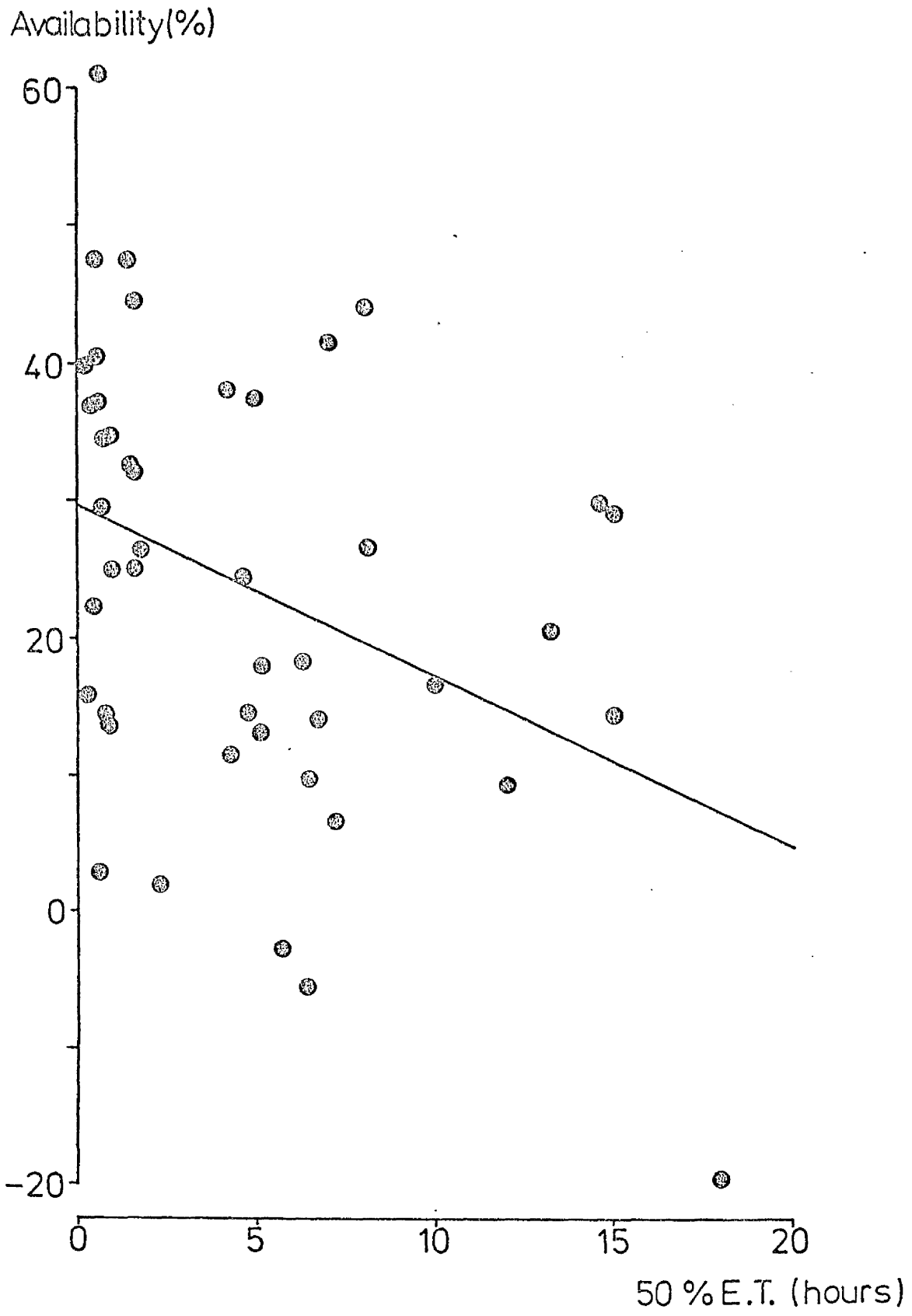
respectively). Irish granules, which have an intermediate availability of 29.1%, are hardly exchangeable at all (7%). Magnesium phosphate, although highly available (47.6%) is also poorly exchangeable (15%).

The samples of the Chinese and Spanish "1980 granular products" show virtually the same NH_4^+-exchange pattern, with Greek "1980" granular C.M. and Chinese "gritty" C.M. being more exchangeable (Figure 6). This is also the general pattern shown when the different particle size fractions of these particular products are compared (Figure 5). For Chinese C.M., in terms of NH_4^+-exchange, fine particles (under 75 μm) are better than medium (75-500 μm) and then coarse (500-2,000 μm), and this is reflected in availability differences (47.6, 26.5 and 24.3%, respectively). However, Greek fine and medium grades show similar exchange patterns although the fine fraction is considerably more available (22.2%) than medium (2.8%), but the coarse fraction is slower to exchange as well as being poorly available (9.7%). Spanish fine and coarse grades are similarly exchangeable with the medium fraction slightly poorer, and this agrees generally with availability data (38.0; 32.7 and 18.2%, respectively). However these NH_4^+-exchange results would suggest that the "whole 1980 products" in order of decreasing availability are Greek, Chinese and Spanish, but the reverse order has been observed in in vivo studies.

Analar magnesium oxide, as expected, was very rapidly and totally NH_4^+-exchangeable (100% in 2 hours, 91% in 1 hour) and it has a fairly high availability of 36.9% (Figure 7). However, many other supplements, notably powdered C.M.'s such as Swedish, Chinese under 75 μm or Spanish under 75 μm, have higher availabilities but a considerably poorer rate and extent of NH_4^+-exchange. The English MgO powder is rapidly exchangeable (but only 85% of the total Mg), with the $Mg(OH)_2$ powder showing a similar pattern despite its higher availability ($Mg(OH)_2$ 40.5%, MgO 25.0%). The granular particle fractions of English MgO are poorly

Figure 8. Expt.9.1. Correlation between mean apparent availability (%) of supplements and their 50% extraction times (E.T.) (hours) in M ammonium nitrate solution.

$$y = 29.8 - 1.25x \; ; \quad r = 0.370^{*}$$


Availability(%)
60
40
20
0
-20
0
5
10
15
20
50 % E.T. (hours)

available (1,000-2,000 μm 13.9%, 200-500 μm 12.9%) and also fairly slow to release Mg. The extremely pure but dense granules (150-250 μm) of American magnesia are very slow to exchange and also have a fairly low availability of 20.5%. Powdered dolomite is poorly exchangeable, as expected as the magnesium is present as the carbonate, and has poor availability (14.2%). The allegedly "under-calcined" Spanish cyclone dust is initially fairly rapdily exchanged, but only 70% of the Mg is exchangeable in spite of its relatively good availability of 34.4%.

The different magnesium supplements varied in both rate and extent of magnesium release, the general pattern being a rapid initial rate which decreased with time. The major differences were apparent during the first two hours of NH_4^+-exchange and in the proportion of the total Mg that was exchangeable. Poor correlation coefficients (non-significant) resulted from attempts to correlate availability with the percentage release of Mg in 1 hour ($r = 0.26$) or 2 hours ($r = 0.29$). The best correlation was that between availability and the time taken to extract 50% of the exchangeable Mg, and this is shown in Figure 8. The correlation coefficient was significant ($P<0.05$) at $r = 0.370$. This low level of significance indicates that the relationship is of doubtful value as the basis for availability predictions although the NH_4^+-exchange test is not totally without meaning. The correlation would perhaps improve with more extensive, repeated availability results.

Experiment 9.2. Determination of Citric Acid Reactivity of various magnesium supplements.

Introduction

Dissolution in citric acid is a well-known method for the determination of extractable potassium and phosphates in fertilisers, and Ammerman et al. (1972) used citric acid as one of a range of solvents

to determine relative solubilities of different inorganic magnesium salts. It was decided, in the present experiment, to determine the solubilities in citric acid of the range of magnesium supplements (mostly oxides) investigated in this thesis, and possibly to relate these to *in vivo* availability results.

Materials and Methods

The citric acid reactivity tests were carried out by Steetley Minerals Ltd., Magnesia Division, using the following method.

100 ml 0.4 N citric acid solution were measured into a 250 ml tall-form beaker containing a magnetised follower. The citric acid solution was made by dissolving 28 g Analar citric acid, 0.25 g sodium benzoate and 2 ml 1% phenol-phthalein (in ethanol) in distilled water and diluting to 1 litre. The beaker and its contents were heated to $30^{\circ}C \pm 0.2^{\circ}C$. 2.00 g of the magnesium supplement sample were added to the beaker and a stopwatch started simultaneously. The beaker was stoppered immediately and after 5 seconds shaken by hand. After 10 seconds the beaker was placed on a magnetic stirrer. The watch was stopped when the solution changed colour from colourless to pink and the activity was expressed as the time for the solution to change colour. The test was stopped after 30 minutes, and results are quoted as >30 minutes where the solution had not yet changed colour.

The supplements tested are listed in the table of results (Table 1).

Results

The citric acid reactivity (C.A.R.) results are given in Table 1.

Table 1. Expt.9.2. Citric Acid Reactivity (C.A.R.) for different magnesium supplements (supplement numbers as in Expt.9.1. for easy comparisons).

	Supplement	C.A.R. (minutes)
1	Spanish C.M. < 75	11 min 10 sec
2	75-150	> 30
3	150-250	> 30
4	250-500	30
5	500-1,000	> 30
6	1,000-2,000	30
7	Spanish "1979 product"	> 30
8	Raw Spanish magnesite	> 30
9	500°C Spanish C.M.	> 30
10	650°C	> 30
11	800°C	7 min 25 sec
12	900°C	11 min 10 sec
13	1,100°C	> 30
14	Spanish "burnt" (Expt.7.1.) < 75	> 30
15	150-250	> 30
16	500-1,000	> 30
17	Spanish "deadburnt" (Expt.3.3.) 150-250	> 30
18	Greek C.M. "1979" granular	29
19	" powdered	3 min 30 sec
20	Chinese C.M. "1979" granular	15 min 35 sec
21	" powdered	6 min 25 sec
22	Swedish C.M. granules	> 30
23	" powdered	2 min 45 sec
24	Irish C.M. granules	> 30
25	Magnesium phosphate	> 30

Supplement	C.A.R. (minutes)
26 "1980 C.M." Greek < 75	1 min 27 sec
27 " 75-500	12 min 34 sec
28 " 500-2,000	30
29 "1980" Chinese < 75	11 min 45 sec
30 " " 75-500	20 min 10 sec
31 " " 500-2,000	> 30
32 "1980" Spanish 75-500	> 30
33 " 500-2,000	> 30
34 Greek (granular)"1980 product"	21 min 42 sec
35 Chinese "granular" (0·3 - 2·0 mm) 1980	> 30
36 " "gritty" (30 mesh) 1980	18 min 35 sec
37 Spanish (granular) 1980	> 30
38 Analar MgO	1 min 36 sec
39 English MgO powder	2 min 3 sec
40 " $Mg(OH)_2$ powder	1 min 18 sec
41 English MgO 1,000-2,000	> 30
42 " 200-500	> 30
43 American magnesia	58 sec
44 English dolomite powder	> 30
45 Spanish Cyclone dust	> 30

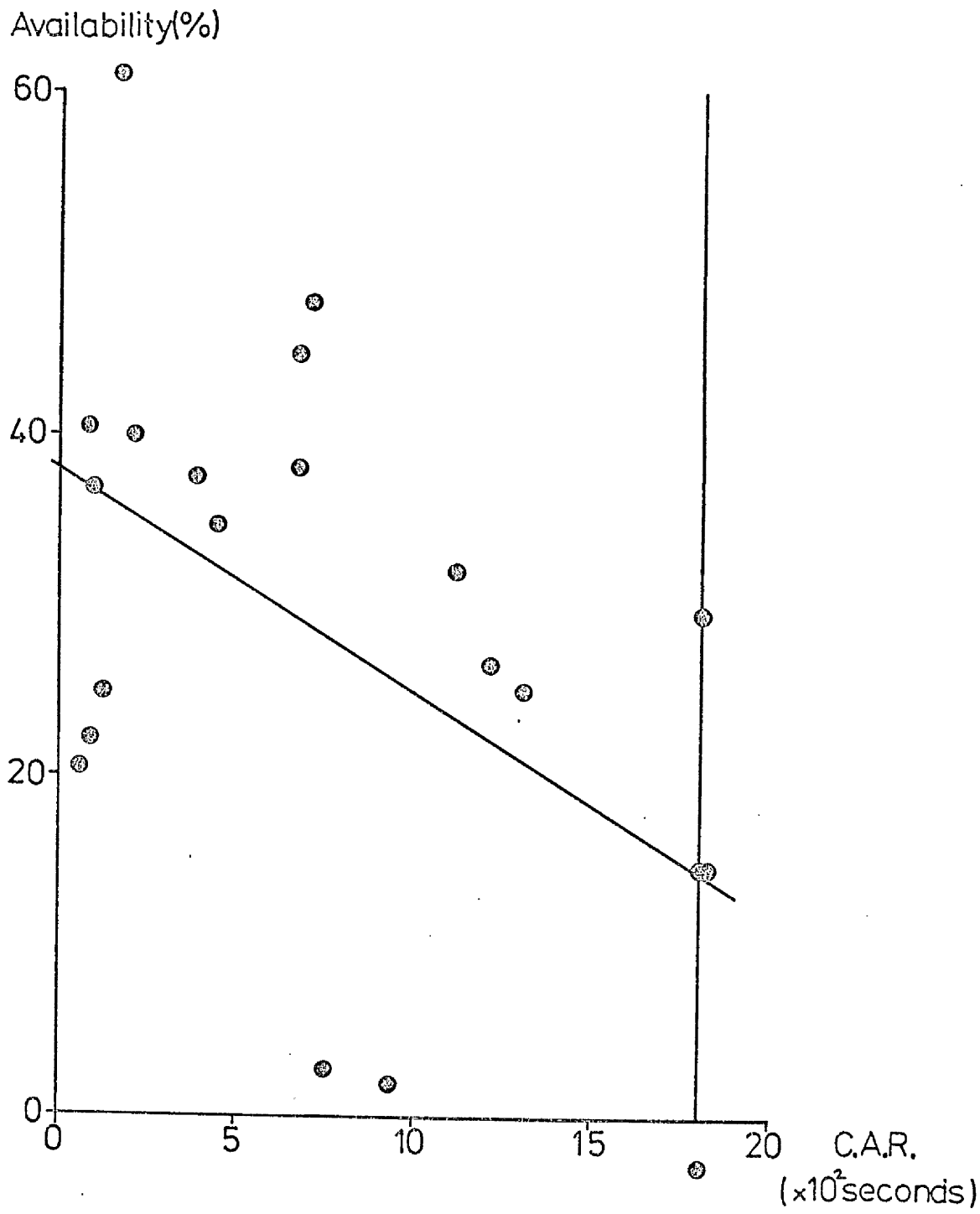
Discussion

For the 21 magnesium supplements with citric acid reactivities (C.A.R.) of up to 30 minutes, mean dietary availability is correlated with C.A.R. in Figure 1. The availability figures used are given in Experiment 9.1 (the mean of all relevant results reported earlier in this thesis). The correlation coefficient, $r = -0.540$ ($P < 0.01$) demonstrates a significant negative relationship between availability and C.A.R., however, the value

of this test for availability predictions is doubtful when the availabilities of the 24 supplements with C.A.R. over 30 minutes are considered. These range from -19.3 to +47.6%, with mean availability 21.2% (SD 15.81%). This is not appreciably lower than the mean availability of 28.2% (SD 15.98%) for the 21 supplements with C.A.R. under 30 minutes (range -2.8 to +61%). Particular supplements with poor C.A.R. (over 30 minutes) and yet relatively high availability are magnesium phosphate (47.6% available), "burnt" Spanish C.M. under 75 μm (43.9%), 1,100°C Spanish C.M. (41.5%), Spanish C.M. "1980 product" (37.3%) and Spanish cyclone dust (34.4%). Further anomalies are those supplements with poor availability and yet relatively fast C.A.R. (under 30 minutes). These include American magnesia (20.5% available), English magnesium oxide powder (25.0%), Greek C.M. under 75 μm (22.2%), Greek 75-500 μm (2.8%), Chinese "1979" granular C.M. (2.0%) and Spanish C.M. 1000-2,000μm (-2.8%). Interestingly, the American magnesia had the best C.A.R. (58 seconds) and yet appeared relatively poor in the NH_4^+-exchange test (Experiment 9.1.). Thus, although there is a slight trend to decreased C.A.R. (i.e. longer) with decreased dietary availability, it is by no means a close relationship which could allow availability predictions from C.A.R. A so-called "chemically reactive" magnesium oxide, as determined by this test, is not necessarily "active" in terms of absorption in the ruminant digestive tract.

Figure 1. Exot.9.2. Correlation between mean apparent availability (%) and C.A.R. (seconds) for supplements with C.A.R. of 30 minutes or less.

$$y = 37.9 - 0.013x ; \quad r = 0.540^{***}$$


Availability(%)
60
40
20
0
0
5
10
15
20
C.A.R.
(×10² seconds)

GENERAL CONCLUSIONS

The experimental work in this thesis has demonstrated that the apparent dietary availability of different calcined magnesites can vary considerably. Some wide differences were detected in the availabilities of material from different sources, i.e. different countries of origin, although it was recognised that the samples tested may not have been representative of the particular countries. The temperature of calcination of the product appeared to be important in so far as complete conversion of carbonate to oxide was desirable, and severe over-burning was undesirable. Under-calcined magnesite (produced at temperatures up to 650°C for 0.75 h) was poorly available, whereas magnesites calcined over the range 800-1,100°C (for 0.75 h) were equally highly available (Expt.1.2.). Thus the kiln temperatures employed commercially for calcination of magnesite (c. 800-900°C) would appear to be optimal. However, more severe heat treatment of calcined magnesite, at 1,300°C for 3 hours, appeared to be detrimental to its availability (Expt.3.3.).

The most marked differences in availability were observed between calcined magnesites of different particle sizes. Fine particle grades were generally more available than coarse granules, and this was a consistent finding in different experimental situations, i.e. with sheep indoors, with hypomagnesaemic ewes, and with grazing cattle outdoors. In order to quantify this conclusion, a consideration was made of the overall average availability results for different calcined magnesites (as given in Expt.9.1., Section V). Excluding the laboratory-calcined products, magnesium phosphate, magnesium hydroxide and dolomite, the mean availability for all eleven powdered magnesium oxides (i.e. any magnesium oxide below 75 μm particle size) was 38.8% (SEM = 3.15), and that for the twenty-six granular materials (i.e. any magnesium oxide above 75 μm particle size, including the commercial granular products which also contained some fine particles) was 17.7% (SEM = 2.23). These

availabilities were significantly different ($P<0.001$). Interestingly the mean availability of 17.7% for granular calcined magnesites agreed closely with the value of 17.0% recommended by the A.R.C.(1980) for use in the calculation of dietary magnesium allowances (i.e. the lower decile value of a range of magnesium availabilities in different feeds). However this A.R.C. (1980) "lower decile" figure and indeed their quoted mean magnesium availability, of 29.4%, severely underestimate the efficacy of powdered calcined magnesites (apparently 38.8% available) and the published recommended dietary allowances (A.R.C., 1980) may be considered over-generous where such magnesium supplements are used. Powdered materials are reputed to be "unpalatable" or "unacceptable" to animals, but there was no evidence in the experiments conducted for this thesis to support this. They tend to create a dust problem when handled and are consequently disliked in feedstuffs manufacturing mills. Calcined magnesite powders are currently used in the animal feed trade almost solely for pasture dusting. Indeed this might therefore seem to be the best method to prevent hypomagnesaemic tetany in practice, both because it ensures that all individuals in the herd or flock receive supplementation and because powders have a high availability.

There was no substantial evidence from the experimental work that the dietary availability of magnesium supplements differed when they were given with different basal indoor diets or to animals at grass. The following table lists the average availabilities of eight supplements which were used in experiments both inside and with animals outside at pasture:

	Average availabilities (%)			
	Indoor diets	(Expt. Nos.)	At Pasture	(Expt. Nos.)
Spanish granular C.M.(1979)	15.2	2.1	18.8	5.1,5.2,7.2
Greek " " "	11.2	2.1,2.2	18.0	5.1,7.2
Chinese " " "	-9.2	2.1,2.2	24.4	7.2
Greek powdered C.M.	35.0	2.1,2.2	49.7	5.1
Spanish C.M. <75 µm	33.8	1.1,2.2,2.3,3.3	46.5	5.2,7.1
" 150-250 µm	27.8	1.1,1.4,3.3	25.0	5.2,7.1
" 500-1,000 µm	15.0	1.1,2.2,3.3	6.1	5.2,7.1
Magnesium hydroxide	34.9	2.1,2.3,3.3	57.4	5.1

The mean availability ($\pm$ SEM) for the eight supplements given with indoor diets was 20.5 ($\pm$ 5.47)%. For the same eight given to grazing animals it was 30.7 ($\pm$ 6.41)%, and these figures did not differ significantly. Thus supplementary magnesium availability appeared unaffected by the basal diet, and this parallels the conclusions of the A.R.C. (1980) that the availability of magnesium in grasses and forages was on average no different from that in mixed concentrate diets.

The chromium marker technique was found to be a reliable and convenient method for availability determinations, with results comparing well with those obtained by traditional balance trial. It allowed the relatively rapid "screening" of large numbers of magnesium supplements with sheep indoors, and it was the only suitable method for use with livestock at grass. The linear regressions between faecal concentrations of magnesium and chromium (which were obtained by this technique) were invariably highly significant, and thus also provided evidence that supplementary magnesium availability remains constant over a range of supplementary intakes.

A low magnesium diet was developed which induced hypomagnesaemia in lactating ewes and also maintained realistic levels of production in these animals. When different dietary magnesium supplements were given, the different responses in plasma magnesium concentration were reflected in differences in apparent availabilities as assessed by the chromium technique. This therefore served further to validate the chromium technique, as well as providing a useful experimental design for comparing the relative efficacies of different dietary magnesium supplements. In these experiments with lactating hypomagnesaemic ewes indoors, it was observed repeatedly that a dietary magnesium supplement given once daily had only a transient effect on plasma magnesium concentrations. In all cases a peak concentration was observed at four hours after administration of the supplement, and thereafter plasma magnesium decreased towards the original level. This would suggest that in practice administration of magnesium supplements more than once daily may offer animals greater protection against hypomagnesaemic tetany. However, a similar diurnal trend in plasma magnesium values following one daily supplementation was not observed in grazing cattle (although such a trend <u>was</u> observed in grazing sheep by Ritchie & Hemingway, 1963). Nevertheless it is clear that <u>regular</u> provision of supplementary magnesium to animals at risk of hypomagnesaemic tetany is critical.

In farm practice when supplementary magnesium is on offer to grazing livestock, it is desirable to ensure that all individual animals consume a similar (and appropriate) quantity. Data were obtained in this thesis on the variation in individual magnesium intake when supplements were offered in various forms currently used. Obviously the ideal method of supplementation is to administer the magnesium individually. This is possible with dairy cows (when supplements can be added to the concentrate feed given at milking time) but is impractical for non-lactating herds at pasture. Group-feeding such herds with a magnesium supplement with a

concentrate carrier in troughs resulted in a wide range of individual intakes, especially when adequate grazing was available, and a free-access magnesium-containing molassine meal was very poorly acceptable. A very palatable free-access feedblock carrier resulted in surprisingly uniform individual magnesium intakes, whether animals were given forage/concentrate diets or had access to adequate pasture. However in both trough-feeding and free-access situations individual animals were observed to be "shy" or, alternatively, "greedy" feeders. The shy animals should ideally be detected and offered some alternative protection against hypomagnesaemic tetany, and in the farm environment detection would be more likely during trough-feeding.

In the laboratory tests, solubilities of magnesium supplements in ammonium nitrate solution and in citric acid both reflected dietary availability differences up to a point, but neither was well enough correlated to provide a predictive test for availability. The so-called "chemically reactive" magnesium oxides were evidently not necessarily the most available to the animals. However, the *in vivo* rumen solubility tests, using the nylon bag technique, gave a very close correlation with apparent dietary availability, and therefore provides a useful assay. The major disadvantage is that the nylon bags are unsuitable for powdered products, but the wealth of experimental evidence from the animal work suggests strongly that powdered calcined magnesites are generally highly available. However in order to include powdered materials, the test could perhaps be adapted so that, for example, solubilities in *in vitro* rumen fluid could be determined (as by Jesse *et al.*, 1981). Alternatively, a simulated rumen liquor or a simple mixture of the volatile fatty acids (acetate, butyrate and propionate) might be used.

Bibliography

A.D.A.S. (1976) Advisory Paper No.11. 2nd Edition.

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

AGRICULTURAL RESEARCH COUNCIL (A.R.C.) (1980) The Nutrient Requirements of Ruminant Livestock. Commonwealth Agricultural Bureaux, Slough SL2 3BN.

ALLCROFT, R. (1953) Proc. XVth Int. Vet. Congress, Stockholm. 1 : 573.

ALLCROFT, R. (1954) Vet. Rec. 66 : 517.

ALLCROFT, R. (1960) B.V.A. Conference on Hypomagnesaemia, London. p.102.

ALLCROFT, R. and BURNS, K.N. (1968) New Zealand Vet. J. 16 : 109.

ALLCROFT, W.M. (1947a) Vet. J. 103 : 2.

ALLCROFT, W.M. (1947b) Vet. J. 103 : 30.

ALLCROFT, W.M. (1947c) Vet. Rec. 103 : 73.

ALLCROFT, W.M. and GODDEN, W. (1934) Biochem. J. 28 : 1004.

ALLCROFT, W.M. and GREEN, H.H. (1934) Biochem. J. 28 : 2220.

ALLCROFT, W.M. and GREEN, H.H. (1938) J. Comp. Path. 51 : 176.

ALLEN, T. (1980) Laboratory Equipment Digest 18, No.2 : 81.

ALLSOP, T.F. and PAULI, J.V. (1975) Proc. N.Z. Soc. Anim. Prod. 35 : 170.

ALLSOP, T.F. and ROCK, J.A.F. (1979) J. agric. Sci., Camb. 92 : 403.

ALTEN, F., ROSENBERGER, G. and WELTE, E. (1958) Zentr. Veterinärmed. 5 : 201.

AMMERMAN, C.B., CHICCO, C.F., LOGGINS, P.E. and ARRINGTON, L.R. (1972) J. Anim. Sci. 34 : 122.

AXFORD, R.F.E., TAS, M.V., EVANS, R.A. and OFTER, N.W. (1975) Res. Vet. Sci. 19 : 333.

BAILEY, C.B. (1961) Br. J. Nutr. 15 : 443.

BALCH, C.C. and CAMPLING, R.C. (1965) In "Physiology of Digestion in the Ruminant," Edit. Dougherty, R.W. et al. p.108.

BALCH, C.C. and JOHNSON, V.W. (1950) Br. J. Nutr. 4 : 389.

BARLET, J.P., RAYSSIGUIER, Y. and LARVOR, P. (1974) C.r. hebd. Séanc. Acad. Sci. Paris. Serie D 278 : 2661.

BARRENTINE, B.F. and MORRISON, E.G. (1953) Proc. Southern Agr. Workers. 50 : 64 (Abstr.).

BARTLETT, S., BROWN, B.B., FOOT, A.S., ROWLAND, S.J., ALLCROFT, R. and PARR, W.H. (1954) Br. Vet. J. 110 : 3.

BARTLETT, S., BROWN, B.B., FOOT, A.S., HEAD, M.J., LINE, C., ROOK, J.A.F., ROWLAND, S.J. and ZUNDEL, G. (1957) J. agric. Sci. 49 : 291.

BELL, M.C., OLUO KUN, J.A., RAMSEY, N. and BECKMANN, G.E. (1978) Feedstuffs, U.S.A. 50(6) : 24.

BEN-GHEDALIA, D., TAGARI, H., ZAMWEL, S. and BONDI, A. (1975) Br. J. Nutr. 33 : 87.

BENZIE, D., BOYNE, A.W., DALGARNO, A.C., DUCKWORTH, J. and HILL, R. (1959) J. agric. Sci., Camb. 52 : 1.

BERTONI, G., WATSON, M.J., SAVAGE, G.P. and ARMSTRONG, D.G. (1976) Zoot. Nutr. Anim. 2 : 107.

BIRCH, J.A. and WOLTON, K.M. (1961) Vet. Rec. 73 : 1169.

BLACK'S VETERINARY DICTIONARY (1979) 13th Edition. Edit. G.P. West.

BLAKEMORE, F. and STEWART, J. (1934-35) Univ. Camb. Inst. Animal Pathol. 4th Rep. of Director, p.103.

BLAXTER, K.L. (1956) Ciba Symposium on Bone Structure and Metabolism. J. & A. Churchill Ltd., London, p.117.

BLAXTER, K.L. and McGILL, R.F. (1956) Vet. Rev. Annot. 2 : 35

BLAXTER, K.L. and ROOK, J.A.F. (1954) J. Comp. Path. 64 : 176.

BLAXTER, K.L. and SHARMAN, G.A.M. (1955) Vet. Rec. 67 : 108.

BLAXTER, K.L., COWLISHAW, B. and ROOK, J.A.F. (1960) Anim. Prod. 2 : 1.

BLAXTER, K.L., ROOK, J.A.F. and MacDONALD, A.M. (1954) J. Comp. Path. 64 : 157.

BLOOM, S., JACOBSON, N.L., ALLEN, R.S., McGILLIARD, L.P. and HOMEYER, P.G. (1959) J. Dairy Sci. 40 : 240.

BOHMAN, V.R., LESPERANCE, A.L., HARDING, G.D. and GRUNES, D.L. (1969) J. Anim. Sci. 29 : 99.

BOLTON, J. and PENNY, A. (1968) J. agric. Sci., Camb. 70 : 303.

BOULD, C. (1964) Vet. Rec. 76 : 1377.

BRANDT, J.L., GLASER, W. and JONES, A. (1958) Metabolism. 7 : 335.

BRITTON, H.T.S., GREGG, S.J. and WINSOR, G.W. (1952) J. Appl. Chem. 2 : 693.

BRODAUF, H., KOPP, C., MUELLER, W. and SCHMIDT, B. (1970) Berl. Munch. Tierarztl. Wschr. 83 : 101.

BROWN, R.C., CARE, A.D. and PRCKARD, D.W. (1978) J. Physiol. 276 : 62P.

BRYANT, G.W., LEYMAN, G. and KNOEFEL, P.K. (1939) J. Pharmacol. and exper. Ther. 65 : 318.

BURAU, R. and STOUT, P.R. (1965) Science. 150 : 766.

BURNS, K.N. and ALLCROFT, R. (1967) Br. Vet. J. 123 : 340.

BUTLER, E.J. (1963) J. agric. Sci., Camb. 60 : 329.

CAMPBELL, R.W. (1972) Aust. Vet. J. 48 : 440.

CAMPLING, R.C. and FREER, M. (1962) Br. J. Nutr. 16 : 507.

CARE, A.D. (1960a) B.V.A. Conference on Hypomagnesaemia, London. p.8.

CARE, A.D. (1960b) B.V.A. Conference on Hypomagnesaemia, London. p.34.

CARE, A.D. (1960c) Res. Vet. Sci. 1 : 338.

CARE, A.D. (1960d) Vet. Rec. 72 : 517.

CARE, A.D. and ROSS, D.B. (1963) Res. Vet. Sci. 4 : 24.

CARE, A.D. and VAN'T KLOOSTER, A.Th. (1965) J. Physiol., Lond. 177 :174.

CARE, A.D., ROSS, D.B. and WILSON, A.A. (1965) J. Physiol. 176 : 284.

CARR, C.W. (1955) Proc. Soc. Exp. Biol. Med. 89 : 546.

CARR, C.W. and WOODS, K.R. (1955) Arch. Biochem. Biophys. 55 : 1.

CAVELL, A.J. (1955) J. Sci. Fd. Agric. 6 : 479.

CHARTON, A., FAYE, P., HERVY, A. and LEFRANÇOIS, C. (1959) C.R. Acad. Sci. 248 : 2407.

CHICCO, C.F., AMMERMAN, C.B. and LOGGINS, P.E. (1973) J. Dairy Sci. 56 : 822.

CHICCO, C.F., AMMERMAN, C.B., HILLIS, W.G. and ARRINGTON, L.R. (1972) Amer. J. Physiol. 222 : 1469.

CHRISTIAN, K.R. and WILLIAMS, V.J. (1960) N.Z. J. Agric. Res. 3 : 389.

CHURCH, D.C. and FONTENOT, J.P. (1979) In "Digestive Physiology and Nutrition of Ruminants," (Ed. D.C. Church) Vol.2 - Nutrition, 2nd Edition. O & B Books, Inc., Oregon, U.S.A.

COLLINS, F.C. (1960) B.V.A. Conference on Hypomagnesaemia, London. p.115.

CUNNINGHAM, I.J. (1933) N.Z. J. Sci. Technol. 15 : 191.

CUNNINGHAM, I.J. (1934) N.Z. J. Sci. Technol. 15 : 414.

CUNNINGHAM, I.J. (1936a) N.Z. J. Sci. Technol. 18 : 419.

CUNNINGHAM, I.J. (1936b) N.Z. J. Sci. Technol. 18 : 424.

DANIEL, O., HATFIELD, E.E., SHREWSBERRY, W.C., GIBSON, M.E. and MacVICAR, R. (1952) J. Anim. Sci. 11 : 790.

DAVEY, L.A. (1968) Vet. Rec. 82 : 142.

de GROOT, T. and MARTIN, M.A. (1967) Tijdschr. Diergeneesk. 92 : 452.

DEL CASTILLO, J. and ENGBAEK, L. (1953) J. Physiol. 120 : 54P.

DEL CASTILLO, J. and STARK, L. (1952) J. Physiol. 116 : 507.

DEVENDRA, C. and LEWIS, D. (1974) Anim. Prod. 19 : 67.

DIJKSHOORN, W. and t'HART, M.L. (1957) Neth. J. Agric. Sci. 5 : 18.

DIRKSEN, G., KAUFMANN, W. and PFEFFER, E. (1972) Fortschr. Tierphysiol. Tierernähr. No.1.

DISHINGTON, I.W. (1965) Acta. Vet. Scand. 6 : 150.

DOUGLAS, T.A. (1960) B.V.A. Conference on Hypomagnesaemia, London. p.4.

DRAYCOTT, A.P. and DURRANT, M.J. (1970) J. agric. Sci., Camb. 75 : 137.

DRAYCOTT, A.P. and DURRANT, M.J. (1972) J. agric. Sci., Camb. 79 : 455.

DRAYCOTT, A.P. and HUTCHISON, B.J. (1979) Chance & Hunt Report 1979. Broom's Barn Experimental Station (pers. comm).

DRAYCOTT, A.P., DURRANT, M.J. and BENNET, S.N. (1975) J. agric. Sci., Camb. 84 : 475.

DRYERRE, H. (1932) Vet. Rec. 12 : 1163.

DUCKWORTH, J. and GODDEN, W. (1941) Biochem. J. 35 : 816.

DUCKWORTH, J. and GODDEN, W. (1943) Biochem. J. 37 : 595.

DUNCAN, C.W., HUFFMAN, C.F. and ROBINSON, C.S. (1935) J. Biol. Chem. 108 : 35.

DUNCAN, C.W., LIGHTFOOT, C.C. and HUFFMAN, C.F. (1938) J. Dairy Sci. 21 : 689.

DURRANT, H.J. and DRAYCOTT, A.P. (1976) J. agric. Sci., Camb. 36 : 543.

DUTHIE, I.F. (1959) Lab. Pract. 8 : 408.

EATON, H.D. and AVAMPTO, J.E. (1952) J. Anim. Sci. 11 : 761.

EDIN, H. (1918) Meddelanden från Centralanstalten för Försöksväsendet på Jordbruksområdet No.105.

EDRISE, B.M., SMITH, R.H. and BUTTLE, H.L. (1977) Proc. Nutr. Soc. 36 : 8A.

EGAN, D.A. (1969) Irish Vet. J. 23 : 8.

ENDER, F., DISHINGTON, I.W. and HELGEBOSTAD, A. (1957) Nord. Vet.-Med. 9 : 881.

ERDMAN, R.A., BOTTS, R.C., HEMKEN, R.W. and BULL, L.S. (1980) J. Dairy Sci. 63 : 923.

EVELETH, D.F. and MILLEN, T.W. (1940) Vet. Med. 35 : 30.

FELDBERG, W. (1945) J. Physiol. 103 : 367.

FELDBERG, W. and HEBB, C. (1945-46) J. Physiol. 104 : 42P.

FELDBERG, W. and HEBB, C. (1947) J. Physiol. 106 : 8.

FIELD, A.C. (1959) Nature, Lond. 183 : 983.

FIELD, A.C. (1960) Nature, 188 : 1205.

FIELD, A.C. (1961) Br. J. Nutr. 15 : 349.

FIELD, A.C. (1967) Br. J. Nutr. 21 : 631.

FIELD, A.C. and MUNRO, C.S. (1977) J. agric. Sci., Camb. 89 : 365.

FIELD, A.C., McCALLUM, J.W. and BUTLER, E.J. (1958) Br. J. Nutr. 12 : 433.

FISHER, E.W. (1960) Br. J. Nutr. 14 : 9.

FISHWICK, G. (1978) N.Z. J. Agr. Res. 21 : 571.

FISHWICK, G. and HEMINGWAY, R.G. (1973) J. agric. Sci., Camb. 81 : 44.

FISHWICK, G., FRASER, J., HEMINGWAY, R.G., PARKINS, J.J. and RITCHIE, N.S. (1977) J. agric. Sci., Camb. 88 : 143.

FISKE, C.H. and SUBBAROW, Y. (1925) J. Biol. Chem. 66 : 375.

FITT, T.J., HUTTON, K. and ARMSTRONG, D.G. (1979) Proc. Nutr. Soc. 38 : 65A.

FITT, T.J., HUTTON, K., THOMPSON, A. and ARMSTRONG, D.G. (1972) Proc. Nutr. Soc. 31 : 100A.

FONTENOT, J.P., GERKEN, H.J.Jr., KALISON, S.L., WATSON, D.F. and ENGEL, R.W. (1965) Feedstuffs, 37(12) : 66.

FONTENOT, J.P., MILLER, R.W., WHITEHAIR, C.K. and MacVICAR, R. (1960) J. Anim. Sci. 19 : 127.

FONTENOT, J.P., WISE, M.B. and WEBB Jr., K.E. (1973) Fed. Proc. 32 : 1925.

FORBES, A.J. (1972) Aust. Vet. J. 48 : 444.

FRYE, T.M., FONTENOT, J.P. and WEBB, K.E. Jr., (1977) J. Anim. Sci. 44 : 919.

GARRETT, O.F. and OVERMAN, O.R. (1940) J. Dairy Sci. 23 : 13.

GERKEN, H.J. and FONTENOT, J.P. (1967) J. Anim. Sci. 26 : 1404.

GILBERT, I.G.F. (1961) Biochim. biophys. Acta. 54 : 489.

GRACE, N.D. and BODY, D.R. (1979) N.Z. J. Agric. Res. 22 : 405.

GRACE, N.D. and MACRAE, J.C. (1972) Br. J. Butr. 27 : 51.

GREEN, H.H. (1939) Vet. Rec. 51 : 1179.

GREEN, H.H. and ALLCROFT, R. (1951) Rept. Natl. Inst. Dairying, Reading. p.35.

GROENWALD, J.W. (1935) Onderstepoort J. Vet. Res. 4 : 93.

GRUND, H. (1961) Thesis, University of Hanover.

GRUNES, D.L., STOUT, P.R. and BROWNELL, J.R. (1970) Adv. Agron. 22 : 331.

GUEGUEN, L. and SALMON-LEGAGNEUR, E. (1959) C.R. Acad. Sci., 249 : 784.

HALSE, K. (1970) Acta. Vet. Scand. 11 : 394.

HANNA, S. (1961) J. clin. Path. 14 : 410.

HANNA, S. and MacINTYRE, I. (1960) Lancet II : 348.

HARPER, F.C. (1967) J. Appl. Chem. 17 : 5.

HARRISON, F.A. (1971) Phil.Trans. R. Soc. B 262 : 301.

HARRISON, F.A., HILL, K.J. and MANGAN, J.L. (1963) Biochem. J. 89 : 99.

HEAD, M.J. and ROCK, J.A.F. (1955) Nature 176 : 262.

HEMINGWAY, R.G. (1961) J. Brit. Grassld. Soc. 16 : 106.

HEMINGWAY, R.G. and BROWN, N.A. (1967) Proc. Nutr. Soc. 26 : xxiv.

HEMINGWAY, R.G. and McLAUGHLIN, A.M. (1979) Br. Vet. J. 135 : 411.

HEMINGWAY, R.G. and RITCHIE, N.S. (1963) J. Sci. Fd. Agric. 14 : 162.

HEMINGWAY, R.G. and RITCHIE, N.S. (1965) Proc. Nutr. Soc. 24 : 54.

HEMINGWAY, R.G. and RITCHIE, N.S. (1969) Vet. Rec. 3 : 465.

HEMINGWAY, R.G., RITCHIE, N.S. and BROWN, N.A. (1965) J. agric. Sci., Camb. 64 : 109.

HEMINGWAY, R.G., RITCHIE, N.S., RUTHERFORD, A.R. and JOLLY, G.M. (1963) J. agric. Sci., Camb. 60 : 307.

HERD, R.P. (1966) Aust. Vet. J. 42 : 369.

HERD, R.P. and PEEBLES, R.M. (1962) Aust. Vet. J. 38 : 455.

HERD, R.P., SCHUSTER, N. and COLTMAN, M. (1965) Aust. Vet. J. 41 : 142.

HICKS, J.D. and PAULI, J.V. (1976) N.Z. Vet. J. 24 : 225.

HIDES, D.H. and THOMAS, T.A. (1981) Society of Chemical Industry Symposium (Agriculture Group). J. Sci. Fd. Agric. (in press).

HJERPE, C.A. (1968) Cornell. Vet. 58 : 193.

HOFF, H.E., SMITH, P.K. and WINKLER, A.W. (1940) Amer. J. Physiol. 130 : 292.

HOLMES, A.D., SPELMAN, A.F. and WETHERBEE, R.T. (1949) J. Nutr. 37 : 385.

HOPKIRK, C.S.M., MARSHALL, D. and BLAKE, T.A. (1933) Vet. Rec. 13 : 355.

HORN, J.P. and SMITH, R.H. (1978) Br. J. Nutr. 40 : 473.

HORVATH, D.J. (1964) J. Anim. Sci. 23 : 879.

HOUSE, W.A. and BIRD, R.J. (1975) J. Anim. Sci. 41 : 1134.

HOUSE, W.A. and MAYLAND, H.F. (1976a) J. Anim. Sci. 42 : 506.

HOUSE, W.A. and MAYLAND, H.F. (1976b) J. Anim. Sci. 43 : 842.

HOUSE, W.A. and VAN CAMPEN, D. (1971) J. Nutr. 101 : 1483.

HUFFMAN, C.F., CONLEY, C.L., LIGHTFOOT, C.C. and DUNCAN, C.W. (1941) J. Nutr. 22 : 609.

HVIDSTEN, H., ODELLIEN, M., BAUERUG, R. and TOLLERSRUD, S. (1959) Acta. Agr. Scand. 9 : 261.

INGLIS, J.S.S., WEIPERS, M. and MARR, A. (1954) Vet. Rec. 66 : 353.

INGLIS, J.S.S., WEIPERS, M. and PEARCE, P.J. (1959) Vet. Rec. 71 : 755.

INSKEEP, E.K. and KENNY, A.D. (1968) Endocrinology 83 : 183.

JENSEN, A.T. and JENSEN, E. (1980) Ugeskrift for Jordburg 17 : 444.

JESSE, B.W., THOMAS, J.W. and EMERY, R.S. (1979) J. Dairy Sci. 62 (suppl.1) : 177 (abstract).

JESSE, B.W., THOMAS, J.W. and EMERY, R.S. (1981) J. Dairy Sci. 64 : 197.

KANE, E.A., JACOBSEN, W.C. and MOORE, L.A. (1950) J. Nutr. 41 : 583.

KANE, E.A., JACOBSEN, W.C. and MOORE, L.A. (1952) J. Nutr. 47 : 211, 48 : 131.

KÄSEBIETER, J. (1978) Diss. Tierärztliche Hochschule Hannover.

KAYA, S. (1932) Jap. J. Gastroenterol. 4 : 128.

KELLY, P. (1979) ADAS Quarterly Rev. No.34, 151.

KEMP, A. (1958) Neth. J. Agric. Sci. 6 : 281.

KEMP, A. (1960) Neth. J. Agric. Sci. 8 : 281.

KEMP, A. and t'HART, M.L. (1957) Neth. J. Agric. Sci. 5 : 4.

KEMP, A. and TODD, J.R. (1970) Vet. Rec. 86 : 463.

KEMP, A., DEIJS, W.B., HEMKES, O.J. and VAN ES, A.J.H. (1960) B.V.A. Conference on Hypomagnesaemia, London, p.23.

KEMP, A., DEIJS, W.B., HEMKES, O.J. and VAN ES, A.J.H. (1961) Neth. J. Agric. Sci. 9 : 134.

KEMP, A., DEIJS, W.B. and KLUVERS, E. (1966) Neth. J. Agric. Sci. 14 : 290.

KEMP, A., VAN'T KLOOSTER, A. Th., ROGERS, P.A.M. and GEURINK, J.H. (1973) Neth. J. Agric. Sci. 21 : 44.

KENDALL, P.T. (1977) Ph.D. Thesis. University of Glasgow.

KENNEDY, G.S. (1968) Aust. J. Biol. Sci. 21 : 529.

KOLB, E., ELLRICH, T.H. and GRUNDEL, G. (1976) Monastschefte fur Vet. Med. 31 : 765.

KOTB, A.R. and LUCKEY, T.D. (1972) Nutr. Abs. & Rev. 42 : 813.

LANGLEY, L.L., GRIMES, O.R. and COCKRELL, D.F. (1962) Amer. J. Physiol. 202 : 707.

LARVOR, P. (1977) Cornell Vet. 66 : 413.

LARVOR, P. and GUÈGUEN, L. (1963) Ann. Zootech. 12 : 39.

LARVOR, P. and VIOLETTE, C. (1969) Ann. Rech. Vét. 1969, No.2, 27.

LEECH, F.B., DAVIS, M.E., MACRAE, W.D. and WITHERS, F.W. (1960) Disease, Wastage and Husbandry in the British Dairy Herd. Rep. of a National Survey 1957-58. Min. Agric. Fish. & Food, H.M.S.O., London.

LEHNINGER, A.L. (1950) Physiol. Rev. 30 : 393.

L'ESTRANGE, J.L. and AXFORD, R.F.E. (1964) J. agric. Sci., Camb. 62 : 353.

L'ESTRANGE, J.L. and AXFORD, R.F.E. (1966) J. agric. Sci., Camb. 68 : 173.

L'ESTRANGE, J.L., OWEN, J.B. and WILMAN, D. (1967) J. agric. Sci., Camb. 68 : 165, 173.

LINE, C., HEAD, M.J., ROOK, J.A.F., FOOT, A.S. and ROWLAND, S.J. (1958) J. agric. Sci., Camb. 51 : 353.

LOMBA, F., CHAUVAUX, G. and BIENFET, V. (1972) Zbl. Vet. Med. A. 19 : 138.

LOMBA, F., PAQUAY, R., BIENFET, V. and LOUSSE, A. (1968) J. agric. Sci., Camb. 71 : 181.

LOMBA, F., CHAUVAUX, G., FUMIÈRE, I., BIENFET, V., PAQUAY, R., BAERE, R.DE. and LOUSSE, A. (1971) Zeit. für Tierphysiol. Tierernahrung Fittermitte 28 : 189.

M.A.F.F. (1973) Technical Bulletin No.27 "The Analysis of Agricultural Materials" H.M.S.O., London.

McALEESE, D.M. and FORBES, R.M. (1959) Nature, Lond. 184 : 2025.

McCANCE, R.A. and WIDDOWSON, E.M. (1942-3) J. Physiol. 101 : 350.

MACRAE, J.C. and ARMSTRONG, D.G. (1969) Br. J. Nutr. 23 : 15.

McDONALD, P. and JACKSON, T.H. (1955) Proc. Brit. Soc. Animal Prod. p.109.

MACGREGOR, R.C. and ARMSTRONG, D.G. (1979) Proc. Nutr. Soc. 38 : 60A.

McINTOSH, S., CROOKS, P. and SIMPSON, K. (1973) Plant and Soil 39 : 389.

MacINTYRE, I. (1959) Proc. Roy. Soc. Med. 52 : 212.

MacINTYRE, I. and DAVIDSSON, D. (1958) Biochem. J. 70 : 456.

MacINTYRE, I., DAVIDSSON, D. and LEONG, P.C. (1958) Intern. Abstr. Biol. Sci. 10, suppl.161.

MacINTYRE, I., ROSS, S. and TROUGHTON, V.A. (1963) Nature, Lond. 198 : 1058

McCONAGHY, S., McALLISTER, J.S., TODD, J.R., RANKIN, J.E.F. and KERR, J.A.M. (1963) J. agric. Sci., Camb. 69 : 313.

McMANUS, W.R., MANTA, L., McFARLANE, J.D. and GRAY, A.C. (1972) J. agric. Sci., Camb. 79 : 27.

MADSEN, F.C., LENTZ, D.E., MILLER, J.K., LOWREY-HARNDEN, D. and HANSARD, S.L. (1976) J. Anim. Sci. 42 : 1316.

M.A.F.F. (1974) Advisory Leaflet LGD54.

MALORNY, G. and OHNESORGE, F.K. (1951) Die Naturwissenschaften 38 : 481.

MALY, F. (1935) Klin. spisy šk. Zvěrolék., Brno. 12 : 39.

MARONGIU, A. (1971) Bolletino della Societa Italiana di Biologia Sperimentale 47 : 768.

MARTENS, H. and RAYSSIGUIER, Y. (1979). In "Digestive Physiology and Metabolism in Ruminants." (Edit. Ruckebusch & Thrivend) Proceedings of the 5th International Symposium on Ruminant Physiology, Clermont-Ferrand, 1979. MTP Press Ltd.

MARTENS, H., HARMEYER, J. and BREVES, G. (1976) In "Nuclear Techniques in Animal Production and Health." International Atomic Energy Agency, Vienna. p.261.

MAYLAND, H.F. and GRUNES, D.L. (1974) J. Range. Mgmt. 27 : 198.

MAYLAND, H.F., GRUNES, D.L. and STUART, D.M. (1974) Agron. J. 66 : 441.

MEHREZ, A.Z. and ØRSKOV, E.R. (1977) J. agric. Sci., Camb. 88 : 645.

MEYER, H. and BUSSE, F.W. (1975) Rectal absorption of Magnesium in ruminants. Deutsche Tierärztliche Wochenschrift 82 : 140.

MEYER, H. and GRUND, H. (1963) Tieraerztl. Umschau. 18 : 181.

MEYER, H. and SCHOLZ, H. (1972) Deutsche Tierärztliche Wochenschrift 79 : 55.

MALLOY, L.F., METSON, A.J. and COLLIE, T.W. (1973) N.Z. J. Agr. Res. 16 : 457.

MOORE, W.F., FONTENOT, J.P. and TUCKER, R.E. (1971) J. Anim. Sci. 33 : 502.

MOORE, W.F., FONTENOT, J.P. and WEBB, K.E. (1972) J. Anim. Sci. 35 : 271, 1046.

NACHMANSOHN, D. (1940) Nature 145 : 513.

NATIONAL RESEARCH COUNCIL (N.R.C.) (1971) Nutrient Requirements of Domestic Animals No.3 Nutrient Requirements of Dairy Cattle. National Academy of Science, Washington D.C.

NEATHERY, M.W. (1969) J. Dairy Sci. 52 : 74.

NEL, J.W. (1976) S. Afr. J. Anim. Sci. 6 (11-16) : 2.

NEUMAN, W.F. and WEIKEL, J.H. (1955) Ann. N.Y. Acad. Sci. 60 : 685.

NEWTON, G.L., FONTENOT, J.P., TUCKER, R.E. and POLAN, C.E. (1972). J. Anim. Sci. 35 : 440.

NICHOLSON, J.A. and SHEARER, G.D. (1938) Vet. J. 94 : 388.

O'KELLEY, R.E. and FONTENOT, J.P. (1969) J. Anim. Sci. 29 : 959.

OYAERT, W. (1962) Berlin Münch. Tierärztl. Wochschr. 75 : 323.

PARR, W.H. and ALLCROFT, R. (1957) Vet. Rec. 69 : 1041.

PATTERSON, R. and CRICHTON, C. (1960) J. Br. Grassland Soc. 15 : 100.

PAULI, J.V. and ALLSOP, T.F. (1974) N.Z. Vet. J. 22 : 227.

PAYNE, J.M. (1977) B.S.A.P. Occasional Publication No.1. p.3.

PEELER, H.T. (1972) J. Anim. Sci. 35 : 695.

PERKIN-ELMER (1976) Analytical methods for atomic absorption spectrophotometry, Perkin-Elmer Corp. Norwalk Conn.

PERRY, S.C., CRAGLE, R.G. and MILLER, J.K. (1967) J. Nutr. 93 : 283.

PFEFFER, E. and RAHMAN, K.A. (1974) Z. Tierphysiol. Tierernährung Futtermittelk. 33 : 209.

PFEFFER, E., THOMPSON, A. and ARMSTRONG, D.G. (1970) Br. J. Nutr. 24 : 197.

PHILLIPSON, A.T. (1970) In Duke's Physiology of Domestic Animals, 8th ed., p.424 (M.J. Swenson, editor). Ithaca, New York, U.S.A. : Cornell University Press.

PHILLIPSON, A.T. and STORRY, J.E. (1965) J. Physiol. 181 : 130.

PLESS, C.D., FONTENOT, J.P. and WEBB, K.E. (1975) V.P.I. & S.U. Res. Div. Rep. 153 : 104.

PRASAD, A.S., FINK, E.B. and ZINNEMAN, H.H. (1959) J. Lab. Clin. Med. 54 : 357.

PUTNAM, P.A., LOOSLI, J.K. and WARNER, R.G. (1958) J. Dairy Sci. 41 : 1723.

QUIN, J.I., VAN DER WATH, J.G. and MYBURGH, S. (1938) Onderstepoort Journal of Vet. Sci. & Anim. Ind. 11 : 341.

RAYSSIGUIER, Y. (1977) Hormone and Metabolic Res. 9 : 309.

REES, M. (1975) Br. Grassl. Soc. Occ. Symp. No.8. p.11.

RITCHIE, N.S. (1971) Proc. Nutr. Soc. 30 : 39A.

RITCHIE, N.S. and HEMINGWAY, R.G. (1963) J. agric. Sci., Camb. 60 : 305.

RITCHIE, N.S. and HEMINGWAY, R.G. (1968) Vet. Rec. 82 : 87.

RITCHIE, N.S., HEMINGWAY, R.G., INGLIS, J.S.S. and PEACOCK, R.M. (1962) J. agric. Sci., Camb. 58 : 399.

ROBERTSON, A., PAVER, H., BARDEN, P. and MARR, T.G. (1960) Res. Vet. Sci. 1 : 117.

ROGERS, P.A.M. (1979) Irish Vet. J. 33 : 115.

ROGERS, T.A. and MAHAN, P.E. (1959) Proc. Soc. Exp. Biol. Med. 100 : 235.

ROGERS, P.A.M. and VAN'T KLOOSTER, A.Th. (1969) Meded. LandbHoogesch. Wageningen. 11 : 26.

ROOK, J.A.F. (1961) Nature, Lond. 191 : 1019.

ROOK, J.A.F. (1963) J. Comp. Path. 73 : 93.

ROOK, J.A.F. and BALCH, C.C. (1958) J. agric. Sci., Camb. 51 : 199.

ROOK, J.A.F. and CAMPLING, R.C. (1959) J. agric. Sci., Camb. 53 : 330.

ROOK, J.A.F. and CAMPLING, R.C. (1962) J. agric. Sci., Camb. 59 : 225.

ROOK, J.A.F. and STORRY, J.E. (1962a) Nutr. Abs. & Rev. 32 : 1055.

ROOK, J.A.F. and STORRY, J.E. (1962b) Proc. Nutr. Soc. 21 : xl.

ROOK, J.A.F., BALCH, C.C. and LINE, C. (1958) J. agric. Sci., Camb. 51 : 189.

ROOK, J.A.F., CAMPLING, R.C. and JOHNSON, V.W. (1964) J. agric. Sci., Camb. 62 : 273.

ROSS, E.J. and GIBSON, W.W.C. (1969) Vet. Rec. 84 : 520, 525.

SCHMIDT, C.L.A. and GREENBERG, D.M. (1935) Physiol. Rev. 15 : 297.

SCOTT, D. and DOBSON, A. (1965) Quart. J. Exptl. Physiol. 50 : 42.

SCOTT, P.R., KELLY, J.M., WHITAKER, D.A. and CAMERON, N.D. (1980) Vet. Res. Comm. 4 : 225.

SCOTTISH AGRICULTURAL COLLEGES (S.A.C.). Publication No.29. Nutrient allowances for cattle and sheep. February 1978.

SCOTTO, K.C., BOHMAN, V.R. and LESPERANCE, A.L. (1971) J. Anim. Sci. 32 : 354.

SHERWOOD, L.M., POTTS, J.T.Jr., CARE, A.D., MAYER, G.P. and AURBACK, G.D. (1966) Nature 209 : 52.

SIMESEN, M.G. (1968) Medlemsblad Danske Dyrloegeforen 51 : 848.

SIMESEN, M.G. (1970) In "Clinical Biochemistry of Domestic Animals" (Eds. C.E. Cornelius and J.J. Kaneko) Vol.1 p.341 Academic Press, New York.

SIMESEN, M.G. (1977) Nord. Vet. Med. 29 : 284.

SIMESEN, M.G., LUNAAS, T., ROGERS, T.A. and LUICK, J.R. (1962) Acta. Vet. Scand. 3 : 175.

SIMPSON, D.J. (1971) Vet. Rec. 89 : 174.

SIMS, F.H. and CROCKSHANK, H.R. (1956) Texas Agr. Exp. Sta. Bul. 842.

SJOLLEMA, B. (1928) Tijdschr. Diergeneesk. 55 : 22.

SJOLLEMA, B. (1930a) Vet. Rec. 10 : 425.

SJOLLEMA, B. (1930b) Vet. Rec. 10 : 450.

SJOLLEMA, B. (1932) Biedermanns Zentralbl.(B) Tierernaehrung 3 : 507.

SJOLLEMA, B. and SEEKLES, L. (1929) Tijdschr. Diergeneesk 56 : 979.

SMITH, A.M. and REID, J.T. (1955) J. Dairy Sci. 38 : 515.

SMITH, R.H. (1957) Biochem. J. 67 : 472.

SMITH, R.H. (1958) Biochem. J. 70 : 201.

SMITH, R.H. (1959a) Biochem. J. 71 : 306.

SMITH, R.H. (1959b) Biochem. J. 71 : 609.

SMITH, R.H. (1961) J. agric. Sci., Camb. 56 : 343.

SMITH, R.H. (1962) Biochem. J. 83 : 151.

SMITH, R.H. and EDRISE, B.M. (1978) Proc. Nutr. Soc. 37 : 60A.

SMITH, R.H. and HORN, J.P. (1976) In "Nuclear Techniques in Animal Production and Health." International Atomic Energy Agency, Vienna. p.253.

SMITH, W.H., LECHTENBERY, V.L. and HODGES, J.R. (1974) J. Anim. Sci. 39 : 1001.

SMYTH, P.J. (1969) Irish Vet. J. 23 : 4.

SMYTH, P.J., CONWAY, A. and WALSH, M.J. (1958) Vet. Rec. 70 : 846.

STEWART, A.B. and HOLMES, W. (1953) J. Sci. Fd. Agric. 4 : 404.

STEWART, J. and MCCDIE, E.W. (1956) J. Comp. Path & Therapeutics 66 : 10.

STILLINGS, B.R., BRATZLER, J.W., MARRIOTT, L.F. and MILLER, R.C. (1964) J. Anim. Sci. 23 : 1148.

STORRY, J.E. (1961) J. agric. Sci., Camb. 57 : 97.

STORRY, J.E. and ROOK, J.A.F. (1962) J. Sci. Fd. Agric. 13 : 621.

STORRY, J.E. and ROOK, J.A.F. (1963) J. agric. Sci., Camb. 61 : 167.

STOUT, P.R., BROWNELL, J. and BURAU, R. (1967) Agron. J. 59 : 21.

STRACHAN, N.H. and ROOK, J.A.F. (1975) Proc. Nutr. Soc. 34 : 11A.

SUNDSTOL, F. (1974) Meldinger Norges Landskogskole 53 : 50.

SUTTLE, N.F. and FIELD, A.C. (1967) Br. J. Nutr. 21 : 819.

SUTTLE, N.F. and FIELD, A.C. (1969) Br. J. Nutr. 23 : 81.

SWAN, J.B. and JAMIESON, N.D. (1956) N.Z. J. Sci. Technol.(A). 38 : 363.

TAYLOR, T.G. (1959) J. agric. Sci., Camb. 52 : 207.

t'HART, M.L. (1960) B.V.A. Conference on Hypomagnesaemia, London. p.88.

t'HART, M.L. and KEMP, A. (1956) Tijdschr. Diergeneesk 81 : 84.

t'HART, M.L. and KEMP, A. (1957) E.P.A. Proj. Utrecht No.204, p.193. (European Productivity Agency).

THE FERTILISER AND FEEDING STUFFS (AMENDMENT) REGULATIONS (1976). Statutory Instruments 1976 No.840 H.M.S.O., London.

THOMAS, J.W. (1959) Symposium on Magnesium and Agriculture, West Virginia University. p.131.

THOMAS, B., THOMPSON, H., OYENUGA, V.A. and ARMSTRONG, R.H. (1952) Emp. J. Exp. Agric. 20 : 10.

THOMPSON, A. (1960) E.V.A. Conference on Hypomagnesaemia, London. p.75.

THOMPSON, N.A., YOUNG, P.W. and RYS, G.J. (1974/1975) Ann. Rep. Res. Divn. Agric. Res. (New Zealand) p.141.

TODD, J.R. (1961) J. agric. Sci., Camb. 56 : 411.

TODD, J.R. (1965) Br. Vet. J. 212 : 371.

TODD, J.R. (1967) Vet. Rec. 81, Clinical Supplement No.12, V1.

TODD, J.R. (1976) In "Nuclear Techniques in Animal Production and Health," International Atomic Energy Agency, Vienna. p.227.

TODD, J.R. and HORVATH, D.J. (1970) Br. Vet. J. 126 : 333.

TODD, J.R. and MORRISON, N.E. (1964) J. Br. Grassl. Soc. 19 : 179.

TODD, J.R. and RANKIN, J.E.F. (1959) Vet. Rec. 71 : 256.

TODD, J.R. and THOMPSON, R.H. (1960) Br. Vet. J. 116 : 437.

TODD, J.R., HORVATH, D.J. and ANIDO, V. (1969) Vet. Rec. 84 : 176.

TODD, J.R., SCALLY, W.C.P. and INGRAM, J.M. (1966) Vet. Rec. 78 : 888.

TOMAS, F.M. and POTTER, B.J. (1976a) Br. J. Nutr. 36 : 37.

TOMAS, F.M. and POTTER, B.J. (1976b) Aust. J. agric. Res. 27 : 873.

VAN KEUREN, R.W. and HEINEMANN, W.W. (1962) J. Anim. Sci. 21 : 340.

VANSCHOUBROEK, F.K. (1959) Nederlands Melk Zuiveltÿdschr. 13 : 113.

VERDEYEN, J. (1953) Compt. Rend. Rech. IRSIA 9 : 87.

WALSER, M. (1961) J. Clin. Invest. 40 : 723.

WERNERY, H., HELSCHEN, W., REICHMUTH, J. and TOLLE, A. (1973) Wien. tierarztl. Mschr. 60 : 55.

WHITE, J.B. (1953) B.V.A. Publ. No.23.

WILKINSON, J.M. and PRESCOTT, J.H.D. (1970) Anim. Prod. 12 : 71.

WILLIAMS, C.H., DAVID, D.F. and IISMAA, O. (1962) J. agric. Sci., Camb. 59 : 381.

WILSON, A.A. (1960) Vet. Rev. & Annot. 6 : 39.

WILSON, G.F. (1980) Anim. Prod. 31 : 153.

WILSON, G.F. and GRACE, N.D. (1978) N.Z. J. Exp. Agric. 6 : 267.

WILSON, G.F., REID, C.S.W., KOLLOY, L.F., METSON, A.J. and BUTLER, G.W. (1969) N.Z. J. agric. Res. 12 : 467.

WILSON, R.K. (1963) Vet. Rec. 75 : 698.

WIND, J., DEIJS, W.B. and KEMP, A. (1966) Jaarb. I.B.S. Wageningen. 91.

WOLTON, K.M. (1960) Proc. 8th Internat. Grassland Congress, p.544.

YOUNG, P.W. and RYS, G. (1977) Proc. 29th Ruakura Farmers' Conference, Hamilton, N.Z. p.30.

YOUNG, P.W., O'CONNOR, M.B. and FEYTER, C. (1979) Proc. 31st Ruakura Farmers' Conference, Hamilton, N.Z. p.110.

APPENDIX 1

Particle size distributions of some commercial granular calcined magnesites

Table 1 gives the mean particle size distributions for seven products used in the thesis. The values given are the mean results obtained from ten 300 g samples of each product shaken for 20 minutes (Allen, 1980) in a "nest" of brass laboratory test sieves (Endecotts Ltd., London) in a shaking machine (Ridsdale & Co. Ltd., Harkington, Harrogate).

Table 1 Appendix 1. Mean particle size (μm) distributions (%) for different calcined magnesites (C.M.s).

Calcined Magnesite	Used (or fractions used) in Expt. numbers	<75	75-150	150-250	250-500	500-1,000	1,000-2,000	>2,000
Spanish (Agma FG85) (1979)	1.1,2.1,5.1,5.2,7.1,7.2	10	18	23	28	21	-	-
" " (1980)	2.3,3.4	15	22	25	19	13	6	-
Chinese "granular, 0.3-2.0 mm" (1979)	2.1,2.2,7.2	7	4	6	12	33	37	1
" " " (1980)	2.3,3.4	4	2	2	5	28	45	14(< 2,800 μm)
Chinese "gritty, 30 mesh"	2.3,3.4	14	11	18	46	11	-	-
Greek granular (1979)	2.1,2.2,5.1,7.2	11	8	11	23	35	12	-
" " (1980)	2.3,3.4	8	5	7	12	22	35	11(< 3,500 μm)

APPENDIX 2

1. Analytical Methods

(i) Dry Matter

The dry matter (DM) in the food and faecal samples was determined by heating 0.5 to 1.0 kg quantities in a hot air oven at 90°C for 36 to 48 h until a constant weight was attained.

(ii) General analysis of food samples

The crude protein (CP) was measured by an automated Kjeldahl technique (Kjel-Foss Automatic 16210), and the ether extract (EE), crude fibre (CF) and ash contents of food samples were determined by the standard methods (The Fertiliser and Feeding Stuffs Regulations, 1976).

(iii) Magnesium

The magnesium content of blood, food, calcined magnesites, faeces and urine samples was determined by atomic absorption spectrophotometry (Perkin-Elmer, 1976). Prior to analysis, samples of faeces, food and calcined magnesite were dissolved in digestion acid (a 3 : 2 : 1 mixture of nitric, perchloric and sulphuric acids) and diluted as appropriate.

(iv) Calcium

The calcium content of blood and food samples was determined by atomic absorption spectrophotometry (Perkin-Elmer, 1976). Prior to analysis, food samples were ashed and dissolved in dilute hydrochloric acid.

(v) Chromium

The chromium content of food and faecal samples was determined by atomic absorption spectrophotometry according to the method of Williams, David and Iismaa (1962). The samples were initially dry ashed.

(vi) Sodium and Potassium

Sodium and potassium contents of food and rumen liquor samples were determined by flame photometry. Food samples were initially ashed and dissolved in dilute hydrochloric acid, and rumen liquor samples were filtered through gauze and centrifuged prior to analysis.

(vii) Phosphorus

The phosphorus content in food samples was determined by a modification of the colorimetric method of Cavell (1955). Phosphorus in blood samples was determined by the colorimetric method of Fiske and Subbarow (1925).

(viii) pH

The pH of rumen liquor samples was determined using a Pye Unican Ltd. pH meter. The combined glass and reference electrode was immersed directly into the fluid.

2. Blood Sampling

Venous blood samples were taken in heparinized vacutainer tubes by jugular puncture from sheep, and by jugular puncture or from the tail vessel of cows. Whole blood samples were centrifuged immediately and the plasma removed for analysis.