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CALCINED MAGNESITES FOR RUMINANTS

by

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**A thesis submitted for the degree of Doctor of Philosophy
in the Faculty of Veterinary Medicine of the
University of Glasgow.**

**Department of Veterinary
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CONTENTS

	Page
SUMMARY	1
INTRODUCTION	3
LITERATURE REVIEW	4
The distribution and functions of magnesium in the body	
Factors affecting magnesium absorption	10
HYPOMAGNEAEMIA AND HYPOMAGNEAEMIC TETANY	14
Clinical symptoms and incidence of hypomagnesaemic tetany	15
Hypomagnesaemia and voluntary feed intake	18
Magnesium and hypocalcaemia	19
Treatment and prevention of hypomagnesaemia and hypomagnesaemic tetany	21
Availability of dietary magnesium	27
Dietary magnesium requirements for ruminants	28
Magnesium in feedstuffs and pasture	29
Magnesium in supplements	30
Summary of work by Wilson (1981)	33
Commercial magnesium oxide	34

SECTION 1

Availability comparisons of calcined magnesites and other magnesium sources 39

Balance trial techniques 41

Treatment of balance trial results 44

Experiments 1 - 5 45

Experiment 6 The effects of feeding increasing amounts of supplementary magnesium to sheep in cages 58

Discussion Experiments 1 - 6 62

(i) Apparent dietary availability

(ii) Variations in the magnesium concentration of the products

(iii) Particle size distribution

(iv) Determination of surface area using the BET technique

Introduction

Materials and Methods

Results and discussion

SECTION 2

Further availability comparisons of calcined magnesites using techniques to reduce the time-cost of experimentation 74

Experiment 7 A comparison of the availabilities of different calcined magnesites using the chromium marker technique for individually fed sheep 75

Experiment 8 Further availability comparisons of calcined magnesites using a modified faecal marker technique for individually fed sheep 78

Experiment 9 A time-cost study of the routine procedures involved in nutritional balance trials 82

SECTION 3

Attempts to correlate in vivo assessments of the dietary availability of granular calcined magnesites with in vitro laboratory methods 87

Experiment 10 The determination of NH_4^+ exchangeable magnesium in various calcined magnesites 89

Experiment 11 The determination of citric acid reactivity of various calcined magnesites 92

Experiment 12 The determination of pH-static reactivity of various calcined magnesites 94

SECTION 4

Attempts to correlate apparent dietary availability results with solubility tests for calcined magnesites in vivo 97

The Nylon bag technique 99

Experiment 13 An investigation into the solubility of various calcined magnesites in the rumen of cows maintained indoors and at grass 101

General discussion 104

SECTION 5

Attempts to induce hypomagnesaemia in suckler cows and ewes using various diets low in magnesium, and its alleviation by supplementing with various calcined magnesites 106

Experiment 14 An attempt to induce hypomagnesaemia in lactating suckler cows given a low magnesium diet 109

Experiment 15 An attempt to induce hypomagnesaemia in lactating ewes given a low magnesium diet 111

Experiment 16 An attempt to induce hypomagnesaemia in suckler cows and its alleviation with various calcined magnesite supplements 112

Experiment 17 An attempt to induce hypomagnesaemia in ewes during pregnancy and lactation given a low magnesium maize-based diet, and the effects of supplementation with various dietary calcined magnesites 116

Experiment 18 An attempt to induce hypomagnesaemia in ewes during lactation when given a low magnesium draff-based diet, and the effects of supplementation with various dietary calcined magnesites 121

Experiment 19 The induction of hypomagnesaemia in pregnant and lactating suckler cows given a low magnesium draff-based diet, and its alleviation with various calcined magnesites 125

Experiment 20 An attempt to induce hypomagnesaemia in ewes during pregnancy when given a low magnesium draff-based diet, and the effects of supplementation with various calcined magnesites 129

General Discussion 132

SECTION 6

Scanning electron microscopy	135
(i) Calcined magnesite samples	136
(ii) Calcined magnesite samples after 48 h in the rumen	136
(iii) Calcined magnesite samples after 7 days in the rumen	137
 Microanalyses	 137
 Transmission microscopy and electron diffraction	 138
 General Conclusions	 141

SECTION 7

The use of magnesium oxide and sodium bicarbonate to influence the yield and composition of milk by cows	143
---	------------

<u>Experiment 21</u> The separate and combined effects of calcined magnesite and sodium bicarbonate on the yield and composition of milk of cows given grass silage	146
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REFERENCES	151
-------------------	------------

APPENDIX 1	160
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SUMMARY

Calcined magnesite is given to ruminant animals as a preventive measure against hypomagnesaemic tetany resulting from magnesium deficiency. The major objectives of this thesis were to investigate the dietary availability of the four major calcined magnesites available on the UK feed market and to determine the consistency of these results by investigating four different samples of each over a 2 year period using a standard technique in comparison with a standard magnesium hydroxide. Using the bioavailability results obtained it was hoped to determine a quick in vitro test to correlate with apparent availability.

Following a description of normal magnesium physiology and metabolism in ruminants, the symptoms and incidence of hypomagnesaemic tetany, preventive measures and treatments, Section 1 describes the results of a number of balance trials carried out to determine the apparent dietary availability of various calcined magnesites.

Although significant differences in availability occurred between the different products investigated within individual trials, no significant overall difference occurred when the results were combined. However a trend occurred whereby the Spanish Agma product appeared to be of superior availability and the Greek material of poor availability. Within each set of four products tested much greater availability was found for the Greek and Chinese products than for the two sources of Spanish origin. The mean availability results obtained for each calcined magnesite were Agma 27.8% (+ 8.21), Navarras 22.4% (+ 3.30), Chinese 23.5% (+ 10.01), and Greek 19.4% (+ 9.54) compared to the standard magnesium hydroxide 30.6% (+ 3.57). Three other magnesium sources were also investigated, namely magnesium chloride, a fine grade Chinese calcined magnesite and a magnesium chelate, magnesium metalosate. They did not differ markedly from the granular calcined magnesites.

In Section 2 the comparative time-costs and accuracies of different methods to determine bioavailability are investigated. The normal balance trial was more accurate and not appreciably more costly in time than methods involving restricted collection or grab-sample collection of faeces.

In Section 3 it was found that apparent availability results - as determined in Section 1, could not be correlated with three different laboratory extraction techniques - solubility in ammonium acetate,

citric acid or pH static titration at a constant pH 6.5 with hydrochloric acid.

In Section 4 it was established that apparent availability results could be correlated with solubility of calcined magnesite as determined in vivo using the nylon bag technique with fistulated cows given indoor diets. However this correlation did not exist with cows maintained at grass although the products were generally more soluble.

In Section 5 a number of low magnesium diets were investigated with both pregnant and lactating suckler cows and ewes. Differences between calcined magnesite supplements were reported in terms of the blood magnesium response.

The physical characteristics and properties of the different calcined magnesites were investigated in Section 6 using Electron microscopy and other techniques. Differences in gross morphology of crystal blocks, level of impurity ions and surface areas were found between products which appeared to be specific to the country of origin.

Section 7 reviews literature on the effects of sodium bicarbonate alone, or together with magnesium oxide on milk yield and milk composition. Unlike much American work however, these treatments appeared to have no effect almost certainly because the basal diet used was not conducive to the production of milk of low butterfat content.

INTRODUCTION

Calcined magnesite has been and continues to be the principle method used to supplement magnesium in ruminant diets for the prevention of magnesium deficiency particularly in lactating cattle and sheep. However dietary treatments are not always successful in preventing hypomagnesaemic or clinical tetany. One particular factor that may be involved in this apparent failure is the bioavailability of the magnesium in the calcined magnesite. Wilson (1981) carried out an in-depth study into the availabilities of different magnesium supplements which included a large number of different calcined magnesites and emphasised the important effects of particle size and temperature of calcination on the apparent availability to the ruminant.

The work to be described in this thesis follows on from that of Wilson (1981). The thesis aims to study in greater depth the variation in availability of the four major calcined magnesites on the commercial market and investigate the consistency of these products in terms of availability, particle size distribution and magnesium content of the products. In addition the ergonomics of different balance trial techniques are investigated.

Following on from the work carried out by Wilson (1981) the in vivo and in vitro solubilities of the various calcined magnesites are investigated in a further attempt to establish a suitable test to correlate with apparent availability results obtained in feeding trials with the objective of offering a ready laboratory method of availability prediction.

Various low magnesium diets are investigated and the relative efficacies of the different calcined magnesites are assessed in terms of blood magnesium response in pregnant and lactating ewes and suckler cows.

The physical properties of the different calcined magnesites are studied using electron microscopy and diffraction techniques to identify possible differences that may affect the apparent differences in availability between the products investigated.

Sodium bicarbonate and calcined magnesite, both separately and combined in complete diets (ca. 400 g maize silage and 600 g concentrates/kg) have improved feed intakes and milk fat production in dairy cows in a number of American studies. Their separate and combined effects are investigated using a typical British diet for dairy cows with 10% inclusion of cereals.

LITERATURE REVIEW and BACKGROUND INFORMATION

Wilson (1981) has provided an extensive review of literature covering magnesium physiology and metabolism, hypomagnesaemia and hypomagnesaemic tetany, and its treatments and prevention, in a PhD thesis entitled 'Magnesium supplementation of ruminant diets'. The following review aims to provide a background for the topic of this present thesis, namely dietary availability of calcined magnesites when given to ruminants and will summarise the literature and the principal findings of Wilson (1981).

The incidence and symptoms of hypomagnesaemic tetany are described together with factors affecting the condition. Various prophylactic measures employed in the prevention of hypomagnesaemic tetany are given together with a description of the production process involved in the manufacture of calcined magnesite.

The distribution of magnesium in the body

The animal body contains approximately 0.05% by weight of magnesium with approximately 59% in the skeleton, 40% in the cells of soft tissue and about 1% in extracellular fluids (ECF). Thus a 500 kg adult cow would contain approximately 250 g magnesium, of which 150 g would be skeletal, 100 g in the soft tissues and only about 2.5 g in the ECF. Similarly an adult sheep weighing approximately 60 kg would contain 30 g magnesium, 18 g skeletal, 12 g in the soft tissue and as little as 0.3 g magnesium in the ECF.

(i) Skeleton

Magnesium constitutes 0.5-0.7% of bone ash in a Ca : Mg ratio of approximately 55 : 1. It is present largely as Mg^{2+} and $MgOH^+$ ions held by electrostatic attraction within the hydration shell at the apatite crystal surface. Magnesium ions can replace calcium ions in the crystal lattice unless the magnesium concentration in the surrounding extracellular fluid is low when calcium ions replace magnesium ions at the adsorption site. Young animals suffering from magnesium deficiency can mobilise 30% or more of skeletal magnesium (Duckworth & Godden, 1941; Blaxter & Rook, 1954). 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 ECF falls due to increased tissue demand, (e.g. during growth or lactation) or a decrease in dietary magnesium intake. This can occur in milk-fed calves.

The labile portion tends to be highest in the young animal, decreasing markedly with age. As the animal matures a large part of the skeleton becomes metabolically inert and the mobility of skeletal magnesium is restricted due to the progressive diminution of its blood supply. The adult is thus much less able to draw on bone reserves of magnesium at times of increase in demand, or dietary deficiency, and this helps to explain the greater incidence of hypomagnesaemic tetany in older animals.

Field (1960) calculated that from the uptake of ^{28}Mg by the skeleton of a 5-year old sheep only about 2% of the total skeletal magnesium is available for release in response to physiological demands.

The skeletal reserves in depleted animals can be replaced by oral or intravenous administration of magnesium but restoration may be incomplete and less rapid than depletion (Cunningham, 1933; Duckworth & Godden, 1943; Smith 1959).

(ii) Intracellular fluids (ICF)

Most body tissues have a similar value of approximately 15 m mol/l of intracellular water (Wilson, 1960). Isotope tracer studies have shown that a small proportion of the intracellular magnesium is exchangeable (Brandt *et al*, 1958).

Various workers have investigated changes in intracellular magnesium during periods of magnesium deficiency but their results are not conclusive. MacIntyre & Davidson (1958) found a fall of magnesium in muscle occurred during the development of magnesium deficiency in rats and Blaxter *et al*, (1954) reported a fall in the magnesium content of red blood cells of calves. However Blaxter *et al* (1954) and Smith (1957) observed no measurable decline in magnesium concentration in soft tissues of hypomagnesaemic cattle and calves.

(iii) Extracellular fluids (ECF)

The ECF are composed of plasma and the interstitial fluid. The concentration of magnesium in the ECF in ruminant animals is normally

about 1.05 m mol/l and values for the plasma or serum levels for normal healthy animals have been reported to vary from about 0.49 to 1.56 m mol/l (Allcroft & Green, 1934; Duncan et al, 1938; Fisher, 1960).

Allcroft (1947) classified the lower limit of the normal range of plasma magnesium in cattle at 0.70 m mol/l, values below which can properly be referred to as hypomagnesaemia. This value is also known to be close to the renal threshold.

Values between 65-80% of total plasma magnesium have been estimated for the ultrafilterable magnesium (Blaxter et al, 1960). The remaining magnesium is bound reversibly to plasma albumin and globulins. This binding is modified by pH and by the concentration of other ions, notably calcium, which compete for binding sites (Carr, 1955).

There is little evidence that 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).

Functions of magnesium in the body

The basic biochemical role for magnesium in living tissues is as the activator of many enzymes. There are two main areas of magnesium activity; enzyme systems concerned with carbohydrate metabolism and hence energy production and enzyme activities involved in the nervous system.

One of the important reactions in carbohydrate metabolism where magnesium is a specific cofactor is the oxidative decarboxylation of α -ketoglutaric acid to form succinic acid in the Krebs's Cycle. Magnesium is also one of the cofactors in the oxidative ^{de}carboxylation of pyruvic acid to acetylcoenzyme A. All reactions involving the breakdown of adenosine triphosphatase to adenosine diphosphate and the release of energy depend on the presence of magnesium.

The other important area of magnesium activity 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 function.

The lack of magnesium will have significant effects on the nervous system and the body as a whole. Rook & Storry (1962) in their review of

magnesium nutrition stated that a reduction of extracellular magnesium would increase the release of acetylcholine by motor nerve endings, increase the sensitivity of the end plate to liberated acetylcholine and decrease the hydrolysis of liberated acetylcholine with a consequent tendency to delay the recovery from stimulation. An increase in the sensitivity of the muscle membrane to the electrical impulse from the nerve will thus cause a greater twitch and inhibit the relaxing factor of muscle.

Magnesium deficiency in all species manifests itself in the derangement of the nervous system. However the degree of irritability of the neuromuscular system depends not only on the concentration of magnesium ions but also on the concentration of other ions, notably calcium.

Body losses of magnesium

(i) Endogenous faecal losses

Endogenous faecal losses are 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.

Since the contribution of magnesium in the saliva is relatively important and saliva output by the cow is affected by the fibrousness and bulk of the diet (Bailey, 1961), high roughage rations may be associated with high endogenous loss of magnesium. Smith (1961) found that milk-fed calves with access to wood shavings developed hypomagnesaemia more rapidly than muzzled calves and this was attributed to increased salivary output in the unmuzzled calves.

The ARC (1980) have published a table of endogenous faecal magnesium values estimated using isotope dilution methods and measurements of faecal excretion of magnesium of animals given artificial diets extremely low in magnesium. The ARC (1980) have adopted the value of 3.0 mg/kg LW/day for both cattle and sheep, except for milk-fed calves of up to 100 kg when 2.0 mg/kg LW/day has been assumed.

(ii) Urinary excretion

The kidneys play an important role in the regulation of the concentration of magnesium in the ECF 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). Wilson (1960) calculated the threshold concentration in the plasma of ewes to be about 0.82 m mol/l and this agrees with measurements in cattle which give a value of about 0.74–0.82 m mol/l (Storry & Rook, 1962). The ARC (1980) give the renal threshold for serum magnesium concentration of not greater than 0.74–0.83 m mol/l; values in excess of this may be taken as an index of adequate magnesium nutrition.

Urine excretion of magnesium by hypomagnesaemic animals falls virtually to zero and 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 the animal.

Field et al, (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.

Other workers have reported close correlation between dietary magnesium intake and urinary output (Rook & Campling, 1962; Storry & Rook, 1963; O'Kelly & Fontenot, 1969; Chicco et al, 1972; Rahnema & Fontenot, 1983).

(iii) Milk

Magnesium excretion via the milk is considerable. Wernery et al (1973) in an extensive survey of commercial dairy herds reported a mean value of 0.125 g magnesium/kg milk and the ARC (1980) have adopted this value for the calculation of daily magnesium requirements. A cow yielding 20kg milk may thus lose 2.5 g magnesium daily via this route, i.e. the equivalent of the total magnesium in the ECF.

The magnesium content of colostrum is high (0.4 g Mg/kg) but within hours of parturition it falls to a fairly constant level which

is not affected by stage of lactation or milk yield (Guegèn & Salmon-Legagneur, 1959).

There is no fall in the magnesium concentration of milk when the intake of magnesium (Groenwald, 1935) or of feed is reduced (Robertson *et al*, 1960) or even if hypomagnesaemia develops (Cunningham 1936; Robertson *et al*, 1960).

The ARC (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 of milk would be about 0.34 g magnesium, a value approximately equal to the total magnesium content of the ECF.

Magnesium Absorption

Until 1969/70 it was widely believed that the small intestine was the main absorption site of magnesium (Care & Van't Klooster, 1965; Phillipson & Storry, 1965) but improvement of the techniques applied in absorption studies - the use of re-entrant cannulae - have led to a better understanding of magnesium absorption from the gastrointestinal 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 *et al*, 1963). This observation was confirmed by a number of workers whose results indicate that net absorption of magnesium is mainly localised in the digestive tract proximal to the duodenum in sheep (Pfeffer *et al*, 1970; Grace & MacRae, 1972; Axford *et al*, 1975; Ben-Ghedalia *et al*, 1975; Strachan & Rook, 1975; McLean *et al*, 1984) and in cattle (Rogers & Van't Klooster, 1969; Kemp *et al*, 1973; Bertoni *et al*, 1976) and the rumen and reticulum in particular are identified as the main site of magnesium absorption (Tomas & Potter, 1976).

The mechanism of magnesium absorption is currently believed to be an active process (Martens & Rayssiguier, 1979). Martens *et al* (1976) concluded from their observations on ^{28}Mg transfer across isolated rumen epithelium studied *in vitro* that magnesium absorption occurs by an active process. Martens & Rayssiguier (1979) showed that magnesium absorption involved the transport against concentration and electrical potential gradients, saturation kinetics, competitive inhibition and temperature sensitivity and it was inhibited by dinitrophenol.

A.D. Care and colleagues at Leeds University have carried out extensive studies into the mechanism of magnesium absorption from the digestive tract, and Beardsworth et al, (1986) demonstrated the isolated washed rumen technique. Care et al, (1984) showed that the effects of a high K/Na ratio and high ammonium ion concentration within the rumen were additive in causing decreases in net effluxes of both magnesium and sodium and they concluded that the efflux of magnesium across the rumen wall depends at least in part on a functional system for sodium transport.

Factors affecting magnesium absorption

(i) Dietary Na, K, Ca and P

Young grass is low in sodium and, due to intensive use of fertiliser, high in potassium. Fontenot et al, (1973) discussed the inter-relationships of potassium, nitrogen and magnesium in ruminants. Tomas & Potter (1976) showed that potassium supplementation had inhibited magnesium absorption from the rumen. Greene et al, (1983) found that a large depression in magnesium absorption occurred in wether sheep when dietary potassium levels increased from 1.2 - 2.4% with further reductions when increased to 4.8%. This was also in agreement with findings by Suttle & Field (1969) and Poe et al, (1985).

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

(ii) Ammonia

The crude protein of young grass is between 20-30% and thus relatively high (Metson & Saunders, 1978). Since this protein is readily fermented, ammonia concentrations in the rumen fluid may rise to levels between 30-70 m mol/l. Head & Rook (1955) first discussed the possible role of high ammonia levels in the rumen in the development of hypomagnesaemia. A negative relationship between crude protein level and apparent magnesium digestibility was found by Kemp et al, (1961) in cows, and a similar relationship was established between the soluble nitrogen fraction in grass and blood magnesium concentrations in sheep

(Larvor & Guegèn, 1963).

Martens & Rayssiguier (1979) found that magnesium absorption from the rumen decreased when ammonia concentrations increased. However Moore *et al*, (1972) and Fontenot *et al*, (1973) could find no effect following extensive studies on the level of crude protein on magnesium absorption.

It had been suggested that increased ammonia levels in the rumen fluid decreased pH leading to precipitation of magnesium ammonium phosphate (Simesen, 1970; Kemp *et al*, 1961). However this does not agree with findings by Hemingway & Brown (1967) that magnesium ammonium phosphate is in fact a good dietary magnesium and phosphorus source.

Martens & Rayssiguier (1979) suggested that the depression of rumen absorption of magnesium is possibly an ammonium toxicity effect on the mucosa since magnesium absorption is impaired only with acute rises in ammonia levels, and the animal adapts over an extended period of time.

(iii) Energy deficit

An increase in the ratio of nitrogen to total water soluble carbohydrates in grass was shown to coincide with increased occurrences of tetany (Mayland *et al*, 1974). Martens & Rayssiguier (1979) postulated that the imbalance between crude protein and readily available carbohydrate may give rise to an energy deficit. Dishington (1965) found that cows fed a diet of grass with 25-30% crude protein would not satisfy their energy requirements unless they consumed an amount of grass which would exceed their protein requirement by 200%. This would lead to increased rumen ammonia production the adverse effects of which have already been discussed, and reduced volatile fatty acid (VFA) formation (Martens & Rayssiguier, 1979).

Experiments investigating the possible effects of readily available carbohydrates have given 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 given a semi-purified diet (House & Mayland, 1976b). Madsen *et al*, (1976) found glucose supplementation to hay increased apparent magnesium absorption in sheep but had no effect when added to grass. Giduck & Fontenot (1987) in trials with sheep supplemented with either glucose, sucrose, lactose or starch found that

the dietary magnesium availability was significantly improved ($P < 0.05$).

Martens & Rayssiguier (1979) postulated that raising energy supply not only results in increased VFA production, but also provides more energy for the synthesis of microbial protein which in turn reduces the concentration of ammonia in the rumen and "hence abolishes the inhibitory action on magnesium absorption".

(iv) Lipolysis

In the ruminant it has been shown that hypomagnesaemia may be associated with increased lipolysis (Rayssiguier, 1977a,b). Rayssiguier showed that lipolysis induced by the intravenous infusion of adrenaline caused hypomagnesaemia in ewes and cows. Hypomagnesaemia is increased by treatment with an α -receptor blocking drug (phentolamine) and inhibited by propranolol, a β -receptor blocking drug. Hypomagnesaemia is correlated with stimulation of lipolysis and sodium nicotinate inhibits the increase in non-esterified fatty acids and hypomagnesaemia resulting from infusion of adrenaline.

Various workers have supplemented ruminant diets with lipids but their results are not consistent. Reduced magnesium utilisation in cattle has been associated with dietary additions of animal fat (Kemp *et al*, 1966; Rogers 1979) and peanut oil (Wilson *et al*, 1969). However Grace & Body (1979) could find no effect of corn oil on apparent magnesium absorption or plasma magnesium levels. However, an increased intake of long chain fatty acids increases the precipitation of insoluble soaps largely with magnesium or calcium which are excreted in the faeces, thus a proportion of the dietary magnesium is rendered unavailable.

Increased activities of a liver derived enzyme, (aspartate amino transferase) have been observed by Reid & Collins (1980) in cows with fatty livers, i.e. more than 20% fat, one week after calving. These cows also had significantly lower plasma magnesium concentrations (0.85 m mol Mg/l) than normal cows (0.93 m mol Mg/l). Overfat cows begin to mobilise fat before calving and Treacher *et al*, (1982) have shown that such cows which subsequently develop fatty livers are more susceptible to metabolic diseases including milk fever around the time of calving.

There is other evidence of an association between hypomagnesaemia and the mobilisation of fat. Fasting or exposure to cold induces a drop

in blood magnesium level and an increase in free fatty acids. Allcroft & Burns (1968) showed that grass tetany may result from problems of adaptation to sudden outdoor grazing and Rayssiguier & Larvor (1976) showed that lipolysis induced by exposure to cold caused hypomagnesaemia.

(v) Binding of Mg^{2+} ions by bacterial cells

Fitt et al, (1972) investigated the possible relation between the binding of magnesium ions by rumen bacterial cell walls and the development of hypomagnesaemia. Magnesium uptake by isolated rumen bacteria was shown to increase with increased magnesium concentration and with increased pH. At low magnesium concentrations magnesium uptake is substantial and directly proportional to the magnesium concentration. However it was also found that the relative quantities of magnesium and calcium bound by the rumen bacterial cell walls were not proportional to their molar concentrations and there was a distinct preference for calcium ions (Fitt et al, 1974a).

A requirement for magnesium by the rumen micro-organisms for their own metabolic processes may lead to a reduction in availability of dietary magnesium to the host animal (Fitt et al, 1974b).

(vi) Magnesium and Monensin

The basic role of monensin is to facilitate the passage of ions across cell membranes. Monensin has a strong affinity for sodium and potassium, increasing the potassium concentration outside the cell and increasing the activity of the Na-K pump (Greene et al, 1988). Starnes et al, (1984) reported that monensin increased the apparent absorption and retention of magnesium in steers. Kirk et al, (1985) reported that lambs fed monensin had 52.4% greater ^{Mg} retention than lambs receiving a control diet. Greene et al, (1983) reported that magnesium availability is correlated negatively with high levels of dietary potassium and that the pre-intestinal region is the site of depressed magnesium absorption in ruminants consuming elevated quantities of potassium. In trials with steers, Greene et al, (1988) found that feeding monensin (25 mg/kg) increased ($P < 0.05$) the apparent magnesium availability and the amount of magnesium absorbed in the pre-intestinal region (47.7 vs. 29.8% and 8.5 vs. 5.1 g/d respectively).

(vii) Other factors

Sudden emotional stress has been suggested as playing a part in the trigger mechanism which reduces the blood magnesium to tetany level during a hypomagnesaemic phase. Grass tetany is more frequent during oestrus in cows and hypomagnesaemia has been noticed during transport tetany. Any animal which has a low blood magnesium concentration is a potential case of tetany and may become a clinical case if exposed to undue excitement (Smith & Edrize, 1978).

A number of workers have correlated increased susceptibility to hypomagnesaemia as a result of fasting and cold exposure (see Lipolysis). Terashima *et al*, (1982) found that unless sheep were subjected to the additional stress of fasting, exposure to the cold did not affect plasma magnesium levels. However their results indicated that moderate cold stress may be intensified by fasting. Adverse climatic conditions e.g. cold, wind, rain, snow etc. will result in a decrease in grazing time and thus consumption which will lead to increased susceptibility to hypomagnesaemia.

HYPOMAGNEAEMIA AND HYPOMAGNEAEMIC TETANY

Although magnesium is an essential element for all mammals, disturbances of magnesium metabolism seem to be most common in ruminants. Hypomagnesaemia is an example of a production disease in ruminants due to the input/output imbalance of magnesium.

The discovery that 'grass tetany' was due to hypomagnesaemia was made shortly after recognition that parturient paresis was due to hypocalcaemia. Sjollem & Seekles (1929) showed that tetany followed a sharp fall in plasma magnesium often in combination with a moderate degree of hypocalcaemia.

Although the incidence of hypomagnesaemia is low compared with hypocalcaemia, the financial losses which result are similar because of the high mortality associated with hypomagnesaemia. Subclinical hypomagnesaemia is an economically important disorder of dairy cows because it results in reduced milk yield and total solids, loss of condition and perhaps fertility and an increased incidence of hypocalcaemia.

In general, hypomagnesaemic tetany seems to be a problem found

only in areas of temperate climate with good rainfall conducive to the production of rich pasture grazing. The regions typically affected include North European countries (e.g. Britain, France, Holland, Germany and the Scandinavian countries), New Zealand, Australia and parts of North America.

Clinical symptoms of hypomagnesaemic tetany

The clinical signs of hypomagnesaemic tetany appear as either acute or subacute. In the acute form, the onset of the condition can be very rapid in that a seemingly healthy animal will suddenly collapse in convulsive fits and unless curative treatment is supplied, may die within 15-30 minutes. The main symptom is one of hyperexcitability of the neuro-muscular system. This varies in degree from tremors in the superficial muscles to opisthotonus where the spinal column is arched and the head thrown back. The limb muscles usually show tetanic contractions and the retraction of the upper eyelids gives the appearance of exophthalmus. Other symptoms include gnashing of the teeth, frothing at the mouth, increase in respiratory rate and an elevated body temperature. This period of violent activity is often followed by a comatose condition prior to death or in some cases the convulsive and comatose conditions alternate several times during the course of the attack.

The subacute or chronic form of hypomagnesaemic tetany is essentially similar to the acute form and it differs only in the degree to which the various symptoms are shown. The main symptom is hyperexcitability of the neuromuscular system but in subacute cases this is only evidenced by muscular twitching and tremors. The affected animal usually reacts nervously to an external stimulus e.g. touch, sound. In many cases it walks with an exaggerated gait and may appear to have an exaggerated view of obstacles. Such cases may continue to exhibit these mild symptoms for many days with either eventual recovery or alternatively death. In some cases the subacute condition is merely a prelude to the acute form of the disorder.

Incidence of hypomagnesaemic tetany

Green (1939) and Allcroft (1947) first identified that the incidence of hypomagnesaemic tetany varies from year to year - there

are some years when few cases occur and others when there is a spate of outbreaks. The variation is almost certainly due to climatic differences influencing either the animal itself or the pasture upon which the animal is subsisting and perhaps the amount of other feeds given.

For a given year there is a seasonal variation in the incidence of hypomagnesaemic tetany. This was first noted by Allcroft (1947) who found a peak incidence of clinical cases in April, followed by a sharp decline during the summer and a secondary peak in the autumn (October - November) with other cases reported throughout the winter.

Outbreaks of tetany in the autumn and early winter are normally found in out-wintered beef herds grazing poor pasture with little or no supplementary food. Such cases are usually associated with a chronic form of hypomagnesaemia which has developed over a long period and this is widely believed to be attributed to the low plane of nutrition on which these animals are maintained (Inglis *et al*, (1954) and the severe climatic conditions to which these animals are exposed (Allcroft & Green, 1938; Allcroft, 1947).

The national incidence of hypomagnesaemia in dairy cows has not been authoritatively recorded and thus its economic significance may therefore not be apparent. Hypomagnesaemia results in the loss of health and production, or death in clinical cases. But the more important loss results from the chronic subclinical condition associated with low total food intake in milkers and increased incidence of hypomagnesaemia in calving cows.

Whitaker & Kelly (1982) reviewed the results of the Dairy Herd Health and Productivity Service (run by the Royal (Dick) School of Veterinary Studies, Edinburgh with Dalgetty Spillers Feed Division and cooperating veterinary surgeons). They collected monthly data on disease incidence from 206 farms with an average herd size of 110 cows. In the year up to the end of August 1981 there was a 1% average incidence of clinical hypomagnesaemia, the highest level occurred in May but cases occurred in all months. It appeared that the west side of Britain had a greater problem.

The incidence of subclinical hypomagnesaemia (serum magnesium level $< 0.78 \text{ m mol/l}$) was as high as 7% of milking cows and 15% of dry cows tested in some months. The high incidence of subclinical hypomagnesaemia in dry cows may be attributed to relatively low or non-existent compound feed inputs.

Cattle and hypomagnesaemic tetany

Lactating cattle are the most susceptible to hypomagnesaemic tetany and the disease is usually associated with cows since the economic considerations are greatest, but it can occur in all types of cattle (Burns & Allcroft, 1967). It has been suggested that there is a difference in susceptibility between breeds in Britain with Ayrshire cows recorded as the most susceptible and Jersey cows the least. Older cows are more susceptible than heifers, and cows which have had more than 6 calves are fourteen times more likely to develop hypomagnesaemic tetany than heifers in their first lactation (Blaxter & McGill, 1956) and cows in their third and fourth lactations are 6-10 times more susceptible. The increased susceptibility of older animals is probably attributable to the lower labile reserves of body magnesium and not higher milk production. 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.

Sheep and hypomagnesaemic tetany

In sheep the condition is mainly confined to lactating ewes with lambs at foot, generally 2-6 weeks after lambing. With sheep hypomagnesaemia occurs mainly in the spring and is closely associated with the flush of grass especially where improved pasture is being grazed to enhance milk production for the lambs. It is also associated 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 gimmers or those with singles and there is a higher mortality rate from tetany in sheep than cattle (Kelly, 1979).

Calves and hypomagnesaemic tetany

Hypomagnesaemia in calves is normally associated with the feeding of milk for an abnormal length of time (Duncan et al, 1935). Blaxter & Sharman (1955) reported hypomagnesaemic tetany in suckled calves on beef rearing farms in Invernesshire with fasted calves showing the greatest decrease in serum magnesium (0.125 g/kg on a FM 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 and Smith (1959) observed depletion of bone magnesium by as much as 30% with reduced plasma levels.

In most practical dairy calf rearing systems, however, some supplementary dry food is available from a relatively early age and the incidence is not as widespread as it is in adult ruminants. Suckler calves in beef herds may experience the condition.

Hypomagnesaemia and voluntary feed intake

There have been various suggestions that low magnesium intakes may reduce voluntary feed consumption e.g. Martin *et al*, (1964) recorded that the extent of cellulose digestion by steers given a low magnesium semi-synthetic diet was increased from 45 to 68% by addition of magnesium to the diet. The voluntary intake by lambs fell from 0.8 to 0.3 kg within 4 days of removal of supplementary magnesium but increased progressively to 0.75 kg within 4 days of magnesium supplementation.

Ammerman *et al*, (1971) reported reduced voluntary intake in sheep fed diets essentially devoid of magnesium to 32% of the control sheep. A close correlation was found between voluntary feed intake and cellulose digestion, both in vitro and in vivo; acetic and proprionic acid was reduced with an increase in pH and reduced feed intake.

Payne (1977) has indicated that there are "reports" (unspecified) that "mild hypomagnesaemia induces not only irritability but also an inappetance as shown by a decline in dry matter intake Such animals give poor milk yields which dramatically improve as soon as supplements of magnesium are fed".

Scott *et al*, (1980) reported preliminary conclusions from over 4,000 cows obtained over one year from 300 commercial dairy herds. There was evidence that below marginal concentrations of blood magnesium ($< 0.78 \text{ m mol/l}$) were associated with below mean concentrations of blood urea. This they took to be indicative of reduced voluntary feed intake.

Magnesium and hypocalcaemia

Cattle rarely become hypocalcaemic except at calving when they are unable to mobilise calcium rapidly enough to compensate for the sudden increase in the output of calcium from the blood into the milk as lactation begins (Sansom, 1978). About 8% of cows become severely hypocalcaemic and suffer from milk fever or parturient paresis (Mullen, 1975). There is evidence that subclinical hypomagnesaemia may increase the susceptibility of cows to milk fever and reduce the prophylactic efficiency of Vitamin D and its analogues (Davies et al, 1978).

Allcroft noted in 1947 that cows showing clinical hypomagnesaemic tetany were also hypocalcaemic - 76% of animals having hypomagnesaemia (< 0.70 m mol Mg/l) had a concomitant hypocalcaemia. Later, Hemingway et al, (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 m mol Ca/l) as well as hypomagnesaemia (0.21 m mol Mg/l). However tetany did not occur in the high proportion of ewes which showed low plasma magnesium but maintained normal calcium levels. These findings are in agreement with Burns & Allcroft (1967) and Forbes (1972). Hemingway & Ritchie (1965) discussing the changes in plasma calcium and magnesium during the development of clinical tetany identified a repeated occurrence. Low plasma magnesium levels in lactating ewes were found to exist for several days before the development of clinical tetany but the pronounced drop in plasma calcium concentration only occurred during the 24 hours immediately prior to the appearance of the clinical tetany. This perhaps "indicates that the actual onset of clinical signs of tetany is in some way associated with the rapid fall in plasma concentration superimposed on an existing state of hypomagnesaemia".

During the last 15 years and since 1982 in particular, a large amount of evidence has been accumulating that a low dietary magnesium intake in the dry period before calving may predispose cows towards milk fever. Payne et al, (1973) reviewed the results of metabolic profiles from 75 herds and found there was a tendency for abnormally low blood magnesium concentrations to occur in dry cows.

Allen & Davies (1981) indicated that "some herds in which chronic hypomagnesaemia is a problem seem to be specially prone to severe outbreaks of milk fever with the incidence approaching 80-90% after calving".

Whitaker & Kelly (1982) surveyed the incidence of clinical and subclinical hypomagnesaemia in dairy cows in England and Wales reviewing the results of 200-2000 blood samples taken each month from 206 dairy herds averaging 110 cows/herd. They recorded subclinical hypomagnesaemia (0.78 m mol/l) in some months in up to 7% of milking cows and in up to 15% of dry cows particularly during the autumn; a secondary peak occurred during the spring. They concluded that the general trend for a higher percentage of dry cows to be affected each month is probably a reflection of relatively low or non-existent cake feeding levels. This is most important in the autumn when dry cows are often maintained for long periods on grass alone and suffer an incidence of hypocalcaemia at calving up to 85% in some herds.

Barber et al, (1983) found that of 23 cases of milk fever in the West of Scotland, 4 had blood magnesium concentrations below 0.41 m mol/l and a further 5 had values below 0.82 m mol/l. For 35 cases occurring in the East of Scotland, 6 cows had values between 0.41-0.82 m mol/l and they concluded that "failure to offer magnesium supplementation to dry cows will exacerbate the problem (of milk fever)".

Contreras et al, (1982) and Van de Braak et al, (1986) both suggest that cows with moderate hypomagnesaemia may be less able to release calcium from bone after calving. They both gave cattle either a low magnesium or a magnesium supplemented diet for a period and the cows were then administered continuously with EDTA solution with the effect of increasing the rate of mobilisation from bone. In every situation a reduced mean blood magnesium concentration in the order of 0.54-0.76 m mol/l resulted in markedly reduced rates of release of calcium. Contreras et al, (1982) concluded that "dry pregnant cows should receive sufficient magnesium to maintain their blood magnesium concentrations near the normal mean during the last weeks of pregnancy".

Sansom et al, (1982) recorded that older cows irrespective of magnesium status were less able to mobilise calcium in response to artificially induced hypocalcaemia than were younger animals. Hypomagnesaemia also reduced calcium mobilisation rate more in old than in young cows; thus for a given fall in plasma magnesium, older cows are at greater risk.

Van de Braak et al, (1986) found that of 7 cows with an induced hypocalcaemia the only one that did not require calcium/magnesium

therapy was a heifer.

Published experimental data thus seems to confirm both survey and field studies that even mild hypomagnesaemia may so reduce calcium mobilisation rate as to increase the risk of milk fever and the data also substantiates the common finding of association between increasing age and susceptibility to milk fever.

Treatment of hypomagnesaemic tetany

Treatment for an animal showing clinical signs of hypomagnesaemic tetany must be prompt. It involves the administration subcutaneously of a solution containing a magnesium salt, normally magnesium sulphate and usually an additional injection of calcium as calcium borogluconate. The rapid introduction of ionic magnesium into the blood is liable to be acutely toxic and fatal. The subcutaneous injection of magnesium sulphate is the normal method employed but Bell *et al*, (1978) has reported magnesium chloride enemas as an effective treatment for cows in tetany. The administration of tranquilisers e.g. chlorpromazine or barbiturate can ease the animal's condition but extreme care is necessary when handling animals in tetany due to their hyperexcitable state and death can result from any kind of stress, even the prick of a needle.

A confounding fact in the treatment of tetany is that most cases occur during the night (see Prevention) and often an animal that was apparently healthy the previous day is found dead the following morning. Treatment has to be immediate if it is to be successful and thus animals are vulnerable during the unsupervised hours of night-time.

Black's Veterinary Dictionary (1979) states that only approximately 25% of treated cases of tetany are successful thus the emphasis must be placed on prevention.

Prevention of hypomagnesaemia and hypomagnesaemic tetany

Prevention or control of hypomagnesaemia and hypomagnesaemic tetany generally involves increasing the intake of magnesium by the animal.

Husbandry methods can play a useful part in prevention, much of the problem of hypomagnesaemia is man-made and the result of intensive

grassland management. Practical measures include the provision of shelter for out-wintered animals and the gradual transfer of animals from winter stall diets to spring pasture. Obviously alterations to fertiliser programmes will conflict with attempts towards intensive grassland management but essentially a minimum amount of nitrogen should be applied in the spring and potassic fertilisers should be applied later. Clover, which contains a higher concentration of magnesium than grass species should also be encouraged in the sward. It may however contribute little to the first spring growth.

The following measures are examples of practical methods of magnesium supplementation:

(i) Magnesium Fertilisers

A number of workers have shown that tetany can be prevented by increasing the pasture magnesium content by applications of magnesium fertilisers to the soil (Parr & Allcroft, 1957; Todd, 1965; Campbell, 1972). Applications of magnesium oxide usually as calcined magnesite, dolomite or magnesium sulphate have been shown to be effective. MAFF (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 to be dressed. However, this treatment is very expensive and as a long term measure, the use of magnesium limestone as a routine liming agent, where needed, can be recommended as a cheaper control treatment. The beneficial effects of magnesium fertilisers however, only occur on light, relatively acid soils; dressings applied to medium loam or heavy loam with a pH above 6.5 are not effective (Burns & Allcroft, 1967).

(ii) Pasture Dusting

Pasture dusting is a common practice, particularly in Northern Ireland where fine calcined magnesite powder (< 75 μ m) is spread as a surface dust which adheres to the grass especially if the leaves are damp with dew or rain, immediately prior to grazing. Magnesium oxide applied at 25-30 kg/ha has been shown to be effective in preventing hypomagnesaemic tetany in grazing cows (McConaghy *et al*, 1963; Todd & Morrison, 1964).

17 kg/ha has been recommended for use with paddock grazing

systems. The paddocks are dusted as the cows are put to pasture or a weeks supply of pasture can be dusted ahead of the cattle. On a set-stocked system or where cows graze one area for more than 10 days, 34 kg/ha is required. The dust should be spread as evenly as possible over the pasture preferably when there is some dew or light rain on the grass. Each treatment provides protection lasting approximately one week and routine treatments on intensive pasture 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 (Rogers, 1979). For success the method obviously depends on weather conditions, e.g. heavy rain will wash the compound off the leaves or the wind can blow it away. The method is not effective if the grass is less than 10 cm long or if granular calcined magnesite is used. It is an unsuitable method for control of seasonal hypomagnesaemia and tetany in beef cows at grass in winter and only suits a system of intensive grazing in the spring. Dusting can represent an economic saving when pasture is plentiful and concentrates are only fed as a carrier for calcined magnesite.

(iii) 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 Mg/day in cattle uniformly over a 28-50 day period when at grass and 0.3-0.5 g/d over 20-40 days in sheep at grass and for calves given milk. The recommended treatment for a cow is 2 bullets given 1-2 days prior to going out to pasture in the spring and Ritchie & Hemingway (1968) have reported the effectiveness of this method of control. However, rumen bullets do not always afford complete protection against hypomagnesaemia especially in areas where tetany is a problem (Kemp & Todd, 1970; Stuedemann *et al*, 1984).

The recommended treatment for sheep (Egan, 1969) and calves (Hemingway & Ritchie, 1969) is 1 bullet/animal administered to ewes with young lambs 2 days prior to a change of grazing or cessation of

supplementary food. However, Kelly (1979) has reported that bullets have been regurgitated and lost at pasture.

Bullets are effective in preventing tetany in some situations but less able to prevent subclinical hypomagnesaemia.

(iv) Direct Feeding

Most animal feed compounders produce magnesium enriched compound feeds that can be fed during high risk periods for different classes of stock, and when fed at the recommended level are an efficient method of supplying the required amount of magnesium. Calcined magnesite can be incorporated in the feed to supply an extra 30 g Mg/cow/day. If concentrates are fed for milk yield at grass, magnesium oxide as calcined magnesite can be added directly to the concentrates in the parlour.

For cows given silage and where no concentrates are used, calcined magnesite can simply be sprinkled onto the silage at the time of feeding. Other conserved forages have been treated with magnesium salts for the prevention of hypomagnesaemic tetany. Campbell (1972) reported the successful feeding to sheep of hay to which magnesium oxide was added prior to baling, and for beef cows, magnesium oxide in aqueous solution was sprayed onto hay at the time of feeding (Herd *et al*, 1965).

To control tetany in milk fed calves, an extra 7-14 g calcined magnesite or equivalent amounts of soluble magnesium salts to supply 4-8 g Mg/calf/day may be mixed with the milk. Milk powder contains approximately 0.12% Mg (DM) and an increase of 0.25-0.35% Mg would prevent hypomagnesaemia in most calves (Rogers, 1979).

(vi) Water supplementation

Water supplementation is recommended where pasture dusting or direct magnesium feeding cannot be used. There are two main approaches to water supplementation. In both, a soluble magnesium salt (acetate, sulphate or chloride) is added to the drinking water in a concentrated solution containing about 5% magnesium. The first method uses a proportioner device to keep the magnesium content of the trough water at a constant concentration throughout the day. However, the devices are expensive and require extensive plumbing around the paddocks, are

liable to breakdown and have to be adjusted frequently. The second, and more effective method, adds a pre-determined amount of magnesium to the trough each day regardless of water intake. For most milking herds the average daily intake, depending on weather conditions, is between 4-40 l/cow/day representing a possible 10 times variation (Rogers, 1979) largely depending on rainfall and temperature. For this approach to be successful it is essential that no other sources of drinking water are available e.g streams, ponds. Calcined magnesite, which is the cheapest source of magnesium, is insoluble and thus cannot be used for water supplementation.

(vii) Free access minerals and molasses based licks

The methods of supplementing magnesium discussed so far are only suitable for use with intensive farming systems. Animals maintained on extensive systems e.g. hill grazing of sheep and suckler herds only receive minimal, if any, concentrate feedstuffs. In these situations mineral mixtures containing magnesium compounds are made available either as simple containers full of a mineral mixture, liquid feeds dispensed from a ball feeder or minerals included as a component of feed blocks.

A homemade lick containing equal parts by weight of molasses and calcined magnesite can be quite effective although the method has disadvantages in that intake can vary widely between individual animals; animals may either eat it well for some weeks and then refuse it, and the tubs require mixing at least twice daily or the supplement will settle to the bottom and not be eaten (Rogers, 1979).

Various commercial magnesium licks based on molasses or syrup are available using magnesium chloride or acetate. Generally they contain about 3.5% magnesium and about 40-50% molasses. With cattle the recommendation is to provide 1 feeder per 10 cows aiming to supply 0.5 l/cow/day to supply 22 g magnesium. Disadvantages include large animal/animal and farm/farm variations with the inability to predict mean intake.

Other commercial products include blocks or dry mineral mixtures containing magnesium. The self-help blocks are consumed by teeth scraping and generally contain about 5-6% magnesium as magnesium oxide. The aim is to supply 500-600 g/day for cattle and 100 g/day for sheep providing 30 g and 5.6 g magnesium respectively. As with magnesium

licks disadvantages include the large between animal and farm variations and the inability to predict mean intake. An increase in intake occurs when raining and decreases in frosty or windy conditions or if the block is too hard. The magnesium from dehydrated molasses based feedblocks, consumed by licking are generally more palatable than compressed blocks and generally contain approximately 10.5% magnesium. Frye *et al*, (1977) discuss the relative acceptabilities of supplemental magnesium oxide mixtures by beef cows.

The current standard preventive recommendation is still 2 oz (56g) calcined magnesite per day for cattle and 1/4 - 1/2 oz (7-14 g) per day for sheep which provides about 30 g and 4-8 g magnesium respectively (at about 87% MgO).

There is no experimental evidence to provide the most effective dose for sheep and these values were probably derived from the recommendation for cattle. Traditionally the aim of supplementation is to provide the quoted levels of calcined magnesite or equivalent if another salt is used. The possible use of smaller quantities of calcined magnesite has seldom been investigated and Wilson (1981) identified significant differences in bioavailability of the various calcined magnesites on the U.K. market.

Allcroft (1953) recorded the failure of 2 oz calcined magnesite to maintain normal plasma magnesium and to prevent deaths from hypomagnesaemic tetany in some herds. The fact that a supplement of 2oz does not afford complete protection in cattle would suggest that any less a quantity would inevitably provide less protection although there is no scientific evidence to support this.

Irrespective of the quantity it is essential that it is given daily since there are no carryover protective effects from day to day. Allcroft (1960) reports that under conditions conducive to hypomagnesaemia, serum magnesium levels in cows fell to dangerously low levels within 48 h of ceasing oral supplementation even though a high intake of magnesium had been given daily for several weeks previously.

Response of serum magnesium levels to supplementary magnesium varies amongst individuals. Ritchie & Hemingway (1963) reported 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.

The diurnal variation in blood magnesium concentration is an important factor in the consideration of supplementation. Ritchie & Hemingway (1963) and Wilson (1981) have repeatedly shown that once per day oral administration of calcined magnesite gives a peak blood magnesium concentration after about 4 hours which declines rapidly to the base level after a further 4 hours. Once a day consumption of magnesium e.g. as may be received in parlour concentrates, does not give 24 h protection. This agrees well with practical observations that cows exhibit clinical tetany typically before milking and not within a few hours of receiving supplementary feed.

Availability of dietary magnesium

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 or utilised.

The most direct measurements involve the use of radioactive tracer techniques but in the case of magnesium the only available tracer is ^{28}Mg which has a short half life of 21.3 h and is also very expensive. The use of ^{28}Mg has therefore been limited (Field, 1959; Care, 1960; McAleese *et al.*, 1961; Simeson *et al.*, 1962; Larvor, 1976).

Most magnesium availability figures have been obtained using complete balance trial data i.e. measurements of total dietary inputs, total faecal and urinary outputs. However some workers have used urinary output expressed as a percentage of intake as apparent availability (Rook & Campling, 1962) or the output in milk and urine as a percentage intake (Rook *et al.*, 1964).

However the most direct expression of apparent availability is 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) and is the method used in the work described in this thesis.

In 1965 the ARC 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. In 1980 the ARC rejected the term 'availability' to replace it with 'coefficient of absorption'. They define 'absorption' as the amount of mineral supplied in the diet that enters the body from the gut and 'apparent absorption'

is the absorption less the net endogenous secretion in the gut. The 'coefficient of absorption' is the amount absorbed divided by the amount ingested. However most of the literature refers to the same coefficient, usually expressed as a percentage, as availability.

The coefficient of absorption of dietary magnesium has been investigated in a wide range of dietary constituents and composition.

The ARC (1980) gives an extensive list of calculated values for the coefficient of absorption of the magnesium for a range of mixed diets and classes of stock. The ARC (1980) calculated the overall mean dietary availability of feeds using a total of 270 measurements in cows (lactating and non-lactating, although few were estimated at grass), steers, 3 month-old calves, lactating ewes and wethers from a total of 28 various references. The greatest number of values are for lactating cows with a total of 108 measurements with an overall mean value of 0.267. The ARC estimated the mean dietary availability of feeds to be 29.4% but the error was very large (s.dev. \pm 13.5%). For the calculation of allowances which provide a safety margin, the lower decile value of 0.17 is recommended.

Calves and lambs receiving milk diets are considered separately from other ruminant animals. The coefficient of absorption is very high in the young animal but falls away rapidly with age.

Dietary magnesium requirements for ruminants

The ARC (1980) provides the most recent assessment of the nutrient requirements of ruminant livestock. The ARC publication gives tables of the estimates of average requirement and recommended dietary allowances for maintenance, growth, pregnancy and lactation for both cattle and sheep, with separate estimates for milk-fed calves. In the published tables the dietary requirements for magnesium are given using the coefficient of absorption of 0.294 with the recommended dietary allowances (given in parenthesis) using the coefficient of absorption of 0.17 i.e. the lower decile range (a similar description will be given in this discussion).

The relative requirements are similar for cattle and sheep i.e. gMg/kg liveweight and the major net requirements are those for maintenance and lactation.

The greatly increased demands imposed by lactation are illustrated thus - the daily requirement (g/day) (for recommended dietary

allowance) for a non-pregnant dry 600 kg Friesian cow is 5.1 (8.8) but when yielding 30 kg milk/day, the requirement and allowance is raised to 18.9 (32.6). A 500 kg steer gaining 1.0 kg/day requires only 6.5 (11.3).

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 5-60kg liveweight and 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 per day.

Requirements for pregnant cows gradually increase throughout gestation from 6.2 (10.8) up to 8.9 (15.5) g Mg/day. Similarly there is an increase for pregnant ewes from 0.77 (1.32) to 1.13 (1.90) g Mg/day for a 75 kg ewe and 0.41 (0.71) to 0.77 (1.33) g Mg/day for a 40 kg ewe.

Magnesium in feedstuffs and pasture

Magnesium is abundant in most common feedstuffs relative to the apparent requirements by animals. Normal indoor diets for cattle and sheep are rarely deficient in magnesium. Indeed it is quite difficult to devise a magnesium deficient diet without using artificial dietary ingredients (see Section 5).

The concentrates used in animal feeding vary widely in magnesium content. Cereal and protein concentrate feeds have varying contents of magnesium (ADAS, 1976); cereal grains generally contain 1.1-1.7 g Mg/kg and the oil seed meals contain approximately twice these concentrations. Good food sources of magnesium are wheat bran (5 g/kg DM) and most vegetable protein concentrates e.g. linseed and cottonseed cake (6 g/kg DM).

Animal products used as protein supplements vary in magnesium content with the proportion of bone they contain. Pure 'meat' meal may only contain 0.4% magnesium while commercial meat and bone meals contain 1% or more depending on the proportion of bone included in the meal.

Hypomagnesaemic tetany however, is typically found in lactating cattle where grazed herbage is the dominant dietary component, thus most attention is focussed on the magnesium content of pastures. The maximum intake of a 600 kg cow giving 30 kg milk/day is about 17 kg DM

and thus in order to supply sufficient dietary magnesium it must contain 2 g Mg/kg DM. However grazed herbage rarely contains 2 g Mg/kg DM (1-1.5 g is more typical) and in Britain both hay and grass silage contain about 1.5 g Mg/kg DM (+0.5). Allcroft & Burns (1968) reported that hypomagnesaemia did not occur in pastures containing over 2 g Mg/kg DM and this value has since been adopted by many workers as the desired magnesium concentration for control of hypomagnesaemia.

Seasonal variations in the magnesium concentration in herbage and its availability are wide. The magnesium content of pastures in temperate regions is lowest in the spring, increases during the summer (Todd, 1961) and tends to fall during the winter. Thomas *et al*, (1955) found the magnesium content of a series of hay crops to range from 0.06 to 0.15%.

In addition to the seasonal variation, the magnesium content of pastures can be affected by the species composition of the sward. Leguminous species are usually substantially richer than grasses in magnesium (Thomas *et al*, 1952; Turner *et al*, 1978) but legumes begin growth later in the season and thus make a minimal contribution to the magnesium content of the early spring pastures.

Thomas *et al*, (1952) and Parr & Allcroft (1957) identified differences between species and found that Timothy (*Phleum pratense*) generally had lower magnesium levels than other grasses.

Fertiliser treatments of pasture can affect the magnesium content and the use of magnesium containing fertilisers has been mentioned earlier. The use of nitrogenous and potassic fertilisers can influence the mineral composition of pasture. Nitrogen fertilisers can reduce the proportion of clover in a sward, and potassium applications generally decrease pasture magnesium levels since most plants absorb potassium from the soil in preference to magnesium (Blaxter *et al*, 1960; Hemingway, 1961).

Magnesium in supplements

The recommended values for the coefficients of absorption of magnesium (ARC, 1980) have been derived for natural diets and do not necessarily apply to supplementary magnesium offered in mineral form.

There is relatively little published work on the availability of inorganic magnesium in supplements. The availability of magnesium supplements to be described in this thesis are expressed as that

percentage of supplementary magnesium not present in the faeces. Many workers studying dietary magnesium availability have also used this method, others have expressed availability as the percentage loss in the urine (Storry & Rook, 1963). Much of the published work reports supplementation of semi-synthetic low magnesium diets (Storry & Rook, 1963) which cannot reasonably be compared with natural diets e.g. grass, where a different rumen fermentation occurs.

There is remarkably little information on magnesium availability to animals at pasture although Wilson (1981) has investigated magnesium supplementation in outdoor situations using the indigestible faecal marker technique for availability assessments.

There is also notably little published information on feed grade magnesium oxide availability, a magnesium source extensively used by the animal feed industry although Wilson (1981) has carried out an extensive study into the availability of various feed grade magnesium oxides (see Review of work by Wilson, 1981). A number of workers however have used reagent grade magnesium oxide, a fine white powder, containing 99.9% magnesium oxide compared with feed grade magnesium oxide which can vary from powder to coarse granules and generally contains 85-87% magnesium oxide.

Table X shows the apparent availabilities of different magnesium sources estimated by various workers. In some references however, although availabilities of different magnesium sources were compared no actual figures were stated (Moore et al, 1971; House & Mayland, 1976).

Reagent grade magnesium oxide was often used as a reference standard and although it has a relatively high availability (32-75%) it is unlikely to be of importance as a supplement in practical situations.

It can be clearly seen from Table X that dolomitic limestone and the raw magnesite ore (magnesium carbonate) are both poorly available (13-18%). However some workers have shown carbonate to be an effective source of magnesium (Huffman et al, 1941; Thomas, 1959) and Moore et al, (1971) found similar availabilities for magnesium oxide and carbonate although dolomitic limestone was poorly available. Magnesium in magnesium phosphate was found to be less available than the reagent grade magnesium oxide but relatively high availabilities were found (26-48%) (Fishwick & Hemingway, 1973; Fishwick, 1978; Hemingway & McLaughlin, 1979; Wilson, 1981).

Storry & Rook (1963) investigated the availability of magnesium

Table X. The apparent availability of magnesium from different sources estimated by different workers.

Magnesium source	Apparent Availability %	Reference		
Reagent grade MgO	32	Storry & Rook	1963	dry cows
	51	Gerken & Fontenot	1967	bullocks
	73	Ammerman <u>et al</u>	1972	sheep
	75	Chicco <u>et al</u>	1972	sheep
	47	Fishwick & Hemingway	1973	sheep
	48	Fishwick	1978	sheep
	42	Hemingway & McLaughlin	1979	sheep
	42	Wilson	1981	sheep
	53	Rahnema & Fontenot	1983	sheep
Reagent grade MgCO ₃	72	Ammerman <u>et al</u>	1972	sheep
MgCO ₃ (magnesite ore)	14	Ammerman <u>et al</u>	1972	sheep
	16	Wilson	1981	sheep
Dolomitic limestone	14	Gerken & Fontenot	1967	bullocks
	18	Wilson	1981	sheep
	13	Rahnema & Fontenot	1983	sheep
Mg phosphate	33	Fishwick & Hemingway	1973	sheep
	26-44	Fishwick	1978	sheep
	33	Hemingway & McLaughlin	1979	sheep
	48	Wilson	1981	sheep
Mg ammonium phosphate	66	Hemingway & Brown	1967	sheep
Ca magnesium phosphate	42	Fishwick & Hemingway	1973	sheep
Mg sulphate	19	Storry & Rook	1963	dry cows
	78	Ammerman <u>et al</u>	1972	sheep
Mg chloride	30	Storry & Rook	1963	dry cows
Mg nitrate	31	"	"	"
Mg acetate	34	"	"	"
Mg citrate	47	"	"	"
Mg lactate	32	"	"	"
Mg silicate	22	"	"	"

from a diverse range of magnesium salts. These differ from the other results quoted in that they are expressed as the percentage of supplementary intake excreted in the urine.

Although differences in availability between various magnesium salts have been clearly identified these results were further compounded by work by Jesse et al, (1979, 1981) who reported that the availability of the magnesium was not simply due to differences between magnesium compounds. They reported a decrease in availability to cows of magnesium from magnesium oxide with increasing particle size of the oxide. Perry & Busse (1981) investigated the effects of particle size on availability of magnesium oxide given to steers on magnesium depleted diets. Wilson (1981) substantiated these findings in an extensive study of the effect of particle size of various calcined magnesites on availability and also the effect of temperature of calcination (see Review of work by Wilson, 1981). Finally, the ARC (1980) states that the magnesium supplement that is given to animals at risk of hypomagnesaemia "should be readily soluble in rumen liquor".

Summary of work by Wilson (1981)

The most extensive research associated with magnesium supplementation of ruminant diets was published in 1981 by Dr C L Wilson. Three major findings can be drawn from this work which relate to the dietary availability of the magnesium in calcined magnesites, these included (i) the country of origin, (ii) temperature of calcination and (iii) particle size.

(i) Country of origin

Wide differences were detected in the dietary availabilities of material from different sources i.e. different countries of origin. For example, a Chinese granular product produced an apparent availability (determined by balance trials) of 2.0%, a Greek granular product, 14.6% and a Spanish product (Agma), 18.0%. (All products were purchased in 1979).

(ii) Temperature of calcination

The temperature of calcination of the product was important in so far as complete conversion of the carbonate to the oxide was desirable and severe over-burning was undesirable. 'Raw' magnesite or under-calcined magnesite produced at temperatures up to 650°C (for 0.75 h) was apparently poorly available (-19.3 to 15.7%) whereas magnesites calcined over the range 800 - 1,000°C (for 0.75 h) were highly available (34.7-41.5%). Severe heat treatment of calcined magnesite at 1,300°C for 3 hours appeared to adversely affect availability (see Figure X).

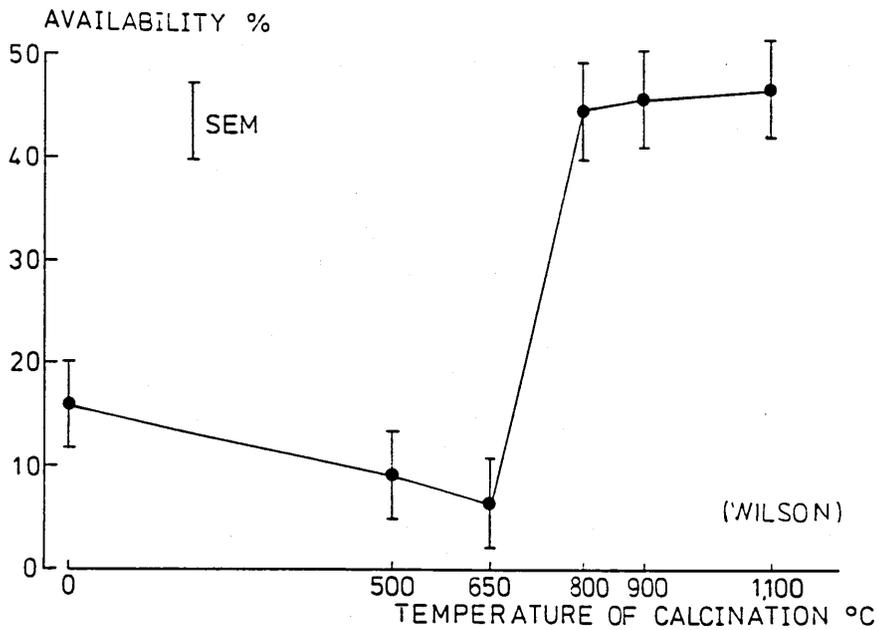
(iii) Particle size

The most marked differences in availability were observed between calcined magnesites of different particle sizes, fine particle grades being generally more available than coarse. This was a consistent finding in different experimental situations i.e. with sheep indoors, with hypomagnesaemic ewes and grazing cattle outdoors. The mean availability for 11 powdered magnesium oxides of various origins (< 75 µm particle size) was 38.8% (SEM=3.15) and that of 26 granular materials (ranging in size from > 75 µm up to 2 mm) and included commercial products of equally diverse origin which contained some fine particles, was 17.7% (SEM=2.23) (see Figure Y).

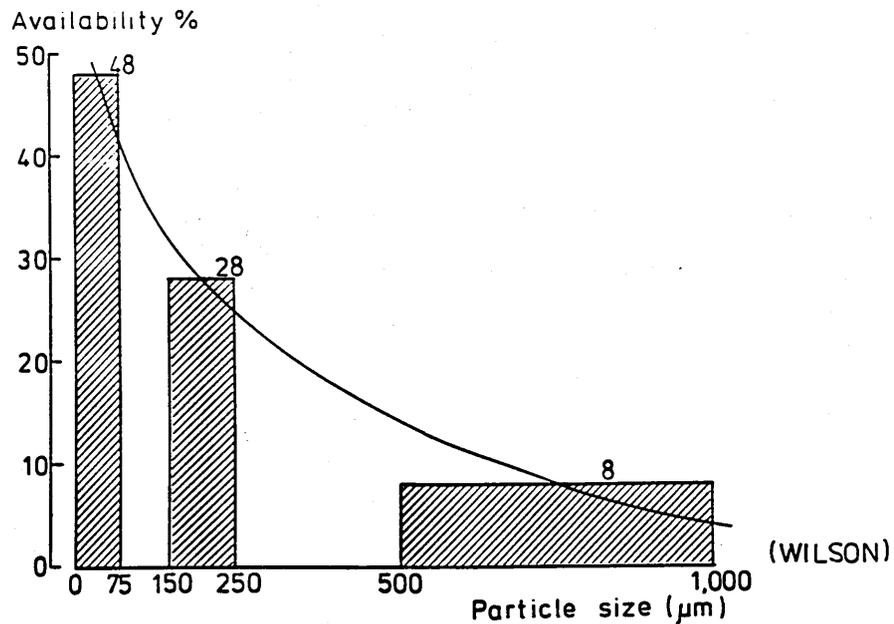
Figure X Effects of temperature of calcination on availability (Wilson, 1981).

Figure Y Particle size and availability (Wilson, 1981).

Availability of magnesites calcined at different temperatures determined by balance trial



Availability of different particle size fractions of a granular MgO



COMMERCIAL MAGNESIUM OXIDE

The main suppliers of calcined magnesite produced from natural magnesites are Spain, Greece, China, Turkey, North Korea, Austria and Czechoslovakia. Calcined magnesites originating from magnesium hydroxide derived from the chloride present in sea water and brines are also produced in the U.K., Italy, the Netherlands, Ireland and Israel. Sea water magnesites are generally more consistent in composition and quality than natural magnesites and can be made within very close chemical and physical tolerances and thus are in demand from industries requiring magnesia of specifically high purity, e.g. refractory and steel industries, ceramics, chemical processing, synthetic rubbers and paper.

The relative importance of the countries producing magnesia from natural magnesites has changed considerably since the end of the 1970's. China, and to a lesser extent North Korea and Turkey, have increased dramatically in importance at the notable expense of the EEC producers Greece and Spain, e.g. in terms of calcined magnesites the EEC imports in 1979 from Greece and Spain was 223,000 tonnes, China 47,000 tonnes. In 1987 the imports from Greece and Spain had fallen to 135,000 and those from China increased to 77,000 tonnes (personal communication, I.C.I. Nutrition, 1989).

Over 90% of the U.K. animal feed market is for granular products, powders being almost exclusively used for pasture dusting. Virtually all the calcined magnesite is sold to animal feed compounders and mineral supplement manufacturers, with under 2% going for direct sale to farmers. Calcined magnesite with essentially 0-2 mm particle size with a minimum of 85% MgO has become the industry standard.

The three major calcined magnesites used in the U.K. are the Spanish (Magnesitas de Rubian SA (Agma) and Navarras), Chinese and Greek granular materials. In 1985 the estimated Chinese calcined magnesites market share in the U.K. for fertiliser and feed amounted to 14.2%, Greek 18.5%, Navarras 27.8% and Agma (MGR) 34.6%. In 1988 this became 26.3%, 15.9%, 22.3% and 24.8% respectively. (I.C.I. Nutrition, Personal communication, 1989).

There are distinct differences in appearance and magnesium content of the different products, although all are produced by essentially the same process, due to differences in the nature of the original magnesite deposits. The magnesite ore varies in colour and appearance

due to various impurities e.g. silica, mica chips and ferrous iron (Fe II) (white colour) and ferric iron (Fe III) (red colour) which are present as a mixed iron magnesium carbonate mineral (breunnerite). There are also large differences in their particle size distributions and for a detailed particle size analysis of these products see Table 15.

The calcined magnesite production process

Traditionally the calcining (or burning) process has been carried out in a variety of shaft, multiple hearth and small rotary kilns. Older types of shaft kiln must be fed with lump material (+ 50 mm) but multiple hearth and rotary kilns can be used for finer material. More recently gas suspension calciners have been developed which can handle fine flotation material (of $< 200 \mu\text{m}$) in an energy efficient process (e.g Navarras material).

The calcining of mineral magnesite by a particular producer in Spain (Magnesitas de Rubian SA) will be described as an example of the stages in the production of natural calcined magnesite.

Magnesitas de Rubian (MGR) possess only a small concession of an enormous area of magnesite deposits in Galicia, North West Spain. The existing vein of magnesite ore covers an area of 4,000 ha and is 8 km long, although this is not all pure magnesite. Until 1986 the magnesite had been open-cast mined but from August 1986 underground mining commenced. The present working area is estimated at 4 m tonnes with an estimated 3,000 m tonnes in reserve. MGR produce calcined magnesite for agricultural purposes alone although small quantities are used in tanneries, there is little industrial demand due to the presence of an iron impurity. Production is primarily governed by demand and sales.

The quarry face from open cast mining is 100 m from top to bottom with three main seams of magnesite running along it. Advantages of underground mining as opposed to open-cast include a saving in extraction costs since this no longer involves the cost of removal of the overburden; for every 1 tonne of ore there was 15 tonne waste material. Underground mining reduces contamination with material other than pure magnesite resulting in a cleaner end product.

The magnesite seam is 12-14 m thick with an incline of 18° to the south, but in practice the extraction is kept at a 10° incline since this was the easiest for the machinery. The system of mining is known

as a 'room and pillar' method whereby each 'room' is 8 m wide by 5 m high and allows for 60% extraction, the remainder forming the pillars. The ore is transported from the quarry in large trucks to a factory situated 1 1/2 km away. Some 125,000 tonnes/annum of raw magnesite is extracted.

The ore is first fed through two crushers to reduce the size of the material entering the kiln (see Figure Z). The ore first enters a primary crusher ('jaw' type) and the smaller material then enters the secondary crusher ('cone' or 'rotary' type) from whence the crushed material is stored in hoppers.

The ore is screened to below 10 mm particle size before being fed at a constant rate into a large rotary kiln for calcination.

The kilns are fired with a mixture of coke (75%) and oil (25%). The finely ground coke (10% above 70 μm) and oil (heated to change its viscosity) are stored separately and then mixed. Two kilns are in operation and the main kiln is 68 m long with an internal diameter of 0.6 m.

The kiln has a maximum revolution speed of 1 rev/min, the speed of revolutions are changed according to the size of the material entering the kiln. Rotating at 0.75 rev/min the magnesite takes on average about 3 hours to pass through.

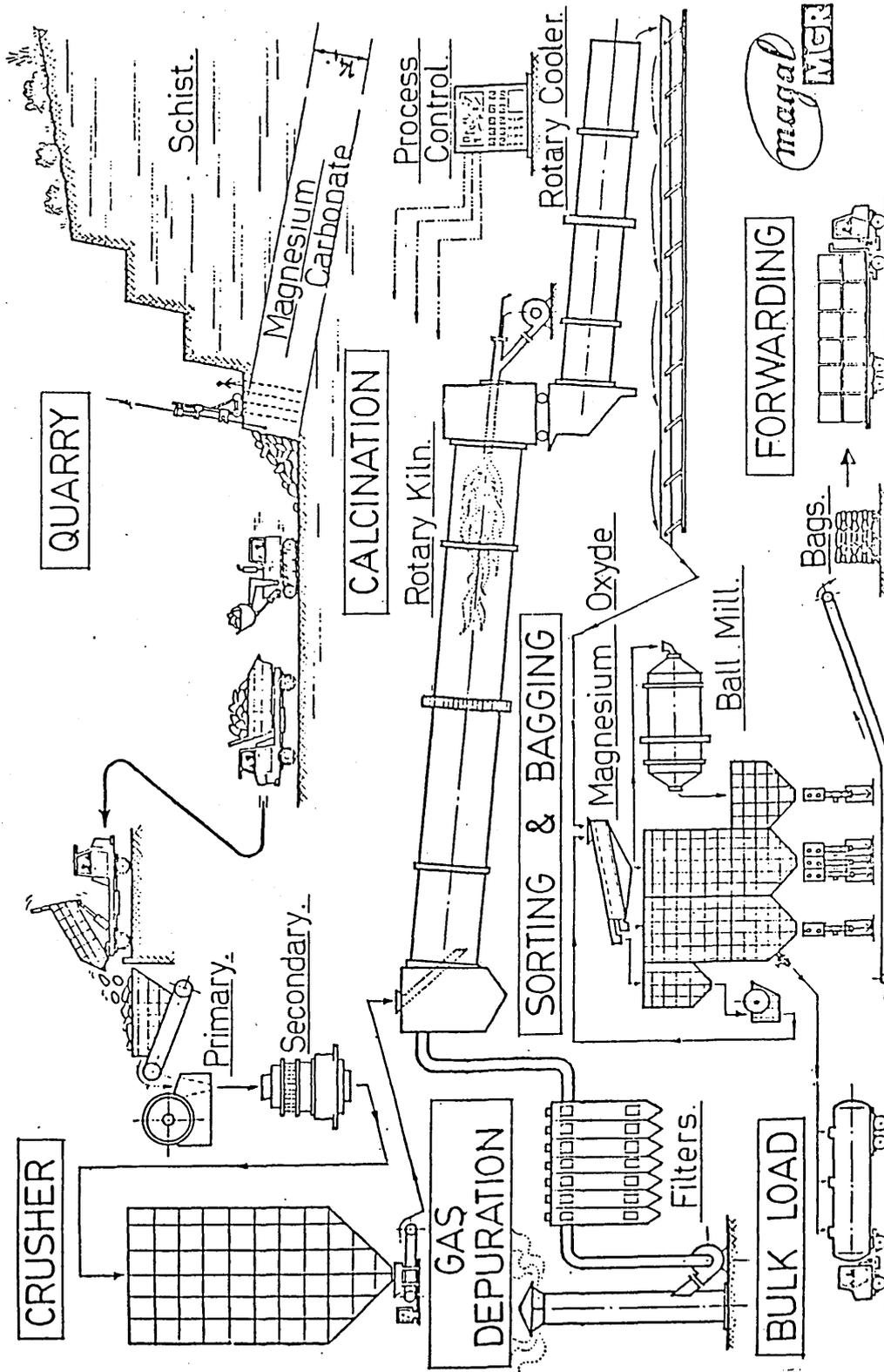
The flame is approximately 15 m long and the temperature of the kiln is carefully monitored using an IR pyrophotometer; the actual temperature is dependent upon the intended use of the magnesite i.e. higher temperatures are required for 'dead-burnt' magnesite. The temperature is normally registered between 800-900°C. The calcination reaction commences only above about 400°C but the speed and extent of calcination increases with temperature to an optimum between 800-900°C when the most chemically reactive oxide is produced. Some material will inevitably pass through the flame itself and thus experience much higher temperatures - such material is rendered relatively inactive and becomes what is known as 'dead-burnt'.

The kiln is in operation for 365 days every year less two weeks for repairs, but a smaller kiln with 40% capacity of the main kiln is available for emergencies or when increased capacity is required.

The calcined magnesite leaves the kiln and enters the cooler, of similar dimensions, through which cold air is forced. The cooled calcined magnesite is then screened before entering hoppers. Material above 4 mm is rejected, that between 2-4 mm is used for fertiliser and

Figure Z Production process of calcined magnesite.

MAGNÉSITAS DE RUBIAN S.A. - Production Process.



that below 2 mm as animal feed grade. Provided the material is not rejected as the result of frequent random analyses the materials are packaged into 50 kg sacks for sale. Loss on ignition (LOI) is determined by burning at 960°C to constant weight and this gives a measure of the extent of calcination.

Throughout the screening and packaging a very fine powder or cyclone dust is produced. Research (Wilson, 1981) has shown that it is very available (43.1%) to ruminants and at present a small amount is used for pasture dusting.

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

The primary producer of calcined magnesite in Greece is Grecian Magnesite SA. The magnesite ore is open-cast mined from deposits at Yerakini and Kastri in Chalkidiki (North Greece). The companies assets also include deposits on the island of Euboea. Hand-sorting of the magnesite ore is a common occurrence in Greece and Turkey where comparatively cheap labour combined with the nature of the ore (white magnesite, dark serpentine) makes it a viable consideration. Grecian Magnesite SA also use more sophisticated versions of optical sorting such as photometric ore sorters, particularly for the finer fractions.

Magnesitas de Navarras use a processing method of flotation to remove the impurities from magnesite ores. Introducing a flotation circuit to a natural magnesite plant normally has a dual purpose - to improve the purity and to increase the recovery by processing fines that would otherwise be discarded.

In China, two magnesite quarries of similar composition occur at Shantung and Tallien, although a number of ores occur across the country. The calcination of the Chinese magnesite is typically carried out using shaft kilns fired by coal or coke. A major modernisation programme is in progress to upgrade the quality of its magnesite product (B. Coope, 1987). In the past the natural advantages possessed by the companies huge high purity magnesite deposits have been nullified by old-fashioned coke-fired sintering systems which were

effectively pumping impurities into the product. A more sophisticated plant involving gas suspension calcining and pressurised shaft kiln technology is now being installed which should increase the Chinese product's appeal to foreign buyers.

SECTION 1

APPARENT DIETARY AVAILABILITY OF VARIOUS CALCINED MAGNESITES

APPARENT DIETARY AVAILABILITY OF VARIOUS CALCINED MAGNESITES

INTRODUCTION

The most common commercial source of magnesium used in the animal feed industry is calcined magnesite. There are four major sources of granular calcined magnesites used in the UK animal feed industry namely Spanish Agma, Navarras, Chinese and Greek.

The following series of experiments are concerned with the investigation of the aforementioned calcined magnesites. The four main balance trials to be described were carried out over a period of 2 years to investigate the consistency of each product in terms of bioavailability (Experiment 1, Dec 1986 - March 1987; Experiment 2, May 1987 - Aug 1987; Experiment 3, Oct 1987 - Dec 1987; Experiment 4, Sept 1988 - Nov 1988). Earlier work, (Wilson, 1981) has shown particle size to be an important factor determining the availability of calcined magnesites. Thus the consistency of particle size distribution and magnesium concentration were also investigated. Other sources of magnesium were also examined but only to a minor degree.

To avoid bias, all supplements were purchased separately by ourselves on the open market prior to the start of each trial. A single purchase of magnesium hydroxide manufactured from sea water (Stetley Minerals Ltd) was used in all four trials as a standard comparator.

A standard diet was adopted consisting of 80% dried grass and 20% barley, except for Experiment 1 which consisted entirely of dried grass. 20 tonnes of dried grass nuts was purchased in 1986, sufficient to supply all four trials. The diet provided energy and protein for maintenance and was not lacking in dietary magnesium.

The same wether lambs were used in Experiments 1 and 2 and a further group of comparable lambs were used in Experiments 3 and 4.

The apparent availability of the different magnesium supplements was calculated using the magnesium output in the faeces for each individual treatment and is expressed as the percentage of supplementary magnesium not present in the faeces. A relatively high faecal magnesium value represents a relatively low apparent dietary availability.

The effects of feeding increasing levels of magnesium as calcined magnesite is also investigated.

A Time-Cost study is detailed giving the ergonomics of balance trials and an alternative method to reduce the time cost is described.

BALANCE TRIAL TECHNIQUES

The principle behind the balance trial technique in nutritional studies is to obtain accurate measurements of total inputs and total outputs of particular feed components or supplements. Where an element like magnesium is concerned the comparative value of different magnesium-containing supplements can only be assessed by difference from a constant background input and output of magnesium from the basal diet.

To ensure as great a uniformity as possible in the series of balance trials conducted in this thesis three major decisions were made. Firstly, 20 tonnes of dried grass pellets were purchased in one consignment prior to the first trial and intended to last throughout. The diet for the first trial was composed entirely of grass pellets but for the following three balance trials a standard diet of 80% grass and 20% barley was adopted. The barley was added to facilitate the complete consumption of the various magnesium supplements. These supplied approximately 113 and 67 g DCP, and 10.8 and 9.0 MJ ME per kg DM per head per day (Experiment 1 and Experiments 2, 3 and 4 respectively). (Values for Experiment 1 refer to MAFF, 1987; values for Experiments 2, 3 and 4 refer to calculated digestibility figures). A note of analyses specific to the different experiments can be found under the description of materials used. These feeds were not intended to provide diets inadequate in magnesium. Indeed the magnesium contents were about 1.94 g/kg DM for the dried grass and 1.10 g/kg DM for the barley.

The second decision concerned the experimental animals. Care was taken in the selection of the sheep used in the experiments. These were well-matched Suffolk x Greyface wethers aged initially about 8 months. The same animals were used in Experiments 1 and 2. A further group of similar sheep (initially aged about 5 months) were used in Experiments 3 and 4.

Thirdly, the amount of supplementary magnesium given for each source investigated was invariably such as to give 2.0 g Mg/day.

The equipment and routine procedures for balance trials for sheep housed in metabolism cages.

Animals

The maximum number of sheep that could be housed in the metabolism building were used for each trial. Wether sheep are used since this facilitates separate collections of faeces and urine. Their hindquarters were clipped to allow cleaner collections of faeces, reducing the chance of faeces adhering to the wool. The sheep were introduced into the metabolism cages a few days prior to the start of the balance trial in order to acclimatise them to the diet, environment and routine before the experiment commenced. These animals always settled quickly and become relatively calm to handle.

Metabolism cages

These were made from galvanised metal to a standard size and design. Each had a wire mesh floor beneath which was a sloping metal tray or chute designed to collect and channel urine towards an aperture at the back. The cages were arranged side by side in two long rows facing one another in the well ventilated metabolism house.

Daily quantities of the basal diet were carefully weighed out in advance into strong paper bags for each 14-day period. Representative samples for each period were taken for general and magnesium analyses.

The diet was given in two approximately equal portions at 07.30 h and 16.00 h each day. Spillage of food by the sheep whilst eating was carefully collected and replaced in front of the animal for consumption as it is essential that precise food intakes were maintained. Any rare and small refusals were weighed and noted.

Sufficient daily magnesium supplements were weighed into small polythene tubs with lids in advance for each 14-day period. Several samples were taken, and each analysed in duplicate. The supplements were added to the surface of the morning feed. The bruised grass nuts given in Experiment 1 and barley in the other three experiments facilitated total consumption of the supplements.

Throughout the experiments the sheep were supplied with fresh water ad libitum.

The sheep were fitted with a standard type of leather harness.

This consists of one long strap that runs along the back and in front of the chest, and two straps encircling the body, one just behind the forelegs and the other in front of the hind legs. Four spring-loaded quick-release scissor-grip metal clips were attached by string at four well-spaced points on this rear strap. To collect the faeces a semi-rigid rubberised bag (Avon Rubber Co. Ltd) was held closely positioned over the rear of the sheep by means of the scissor-grip hooks. The contents of the faecal bags were emptied twice a day. During a collection period the faeces were emptied into a large numbered polypropylene bin with lid behind the cage. The total faeces from each sheep collected over seven days were weighed and then thoroughly mixed in a wheelbarrow and a representative sub-sample taken. The samples were dried (to give the DM content and hence the daily faeces DM output), ground and a subsequent sub-sample submitted for magnesium analysis.

Urine was collected directly from the sloping metal tray beneath the cage floor into a numbered polypropylene jug. The urine passed through a glass wool filter placed in the funnel to prevent particulate matter entering the jug (e.g spilled faeces and traces of feed). Each morning the chute was rinsed using a small amount of water and the washings were also filtered into the jug, picking up any urine which had not drained completely from the chute. The amount of added water is not important.

During a collection period the jug was emptied daily, or as required, into a 25 litre numbered polypropylene container situated behind the cage containing dilute hydrochloric acid to prevent precipitation of salts from the urine during storage. After the 7 days collection the total urine plus acid and chute washings from each sheep was weighed, well shaken and subsampled for magnesium analysis.

A pale brown precipitate was occasionally found deposited on the chute of the cage or in the urine collection jug. The deposit in most instances dissolved in dilute HCl and was added to the bulk urine. The degree of deposition appeared to be unrelated to the magnesium supplementation of the diet (and on analysis contained negligible magnesium, 2.78 mg/g) but would appear to be specific to individual sheep.

Each treatment period was of 14 days duration and the first 7 days accustomed the sheep to the new treatment and allowed the residues of the previous supplement to clear from the digestive tract. During the

changeover week urine and cage washings were allowed to run to waste and the faeces collected in the bags was discarded.

Venous blood samples were taken at the start and end of the trial, by jugular puncture using evacuated heparinised tubes and the plasma magnesium concentrations were determined.

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 urine, over each 7 day collection period for each individual sheep. Retention is a figure calculated from the difference between total magnesium input and total magnesium output (faeces plus urine for each sheep).

Apparent availability of a supplement was calculated for each sheep according to :

$$\frac{\text{supplement Mg intake} - \text{faecal Mg derived from supplement}}{\text{supplement Mg intake}}$$

This method of calculation assumes a constant availability for basal diet magnesium whether supplements were being given or not. The basal diet magnesium availability was calculated as 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 given. This figure was then used to calculate the amount of faecal magnesium presumed to be derived from the basal diet during periods when magnesium supplements were being fed and hence the quantity of faecal magnesium derived from each supplement.

EXPERIMENT 1

Introduction

Earlier work (Wilson, 1981) has suggested that particle size of calcined magnesites influences bioavailability to the animal. Wilson (1981) found that finer particles have increased availability when compared with coarse particles of the same product and that a relationship exists between particle size and availability. Any differences detected among different products may thus be a reflection of differences in particle size distribution.

Calculation of the surface area of a cube or sphere shows that a small reduction in size will result in a considerably increased surface area. If it is assumed that a particle of calcined magnesite roughly resembles a cube or sphere, ignoring the possibilities of an uneven surface or a porous nature, particles in a size range of 150 μm to 1 mm will have a theoretical surface area from 6-42 mm^2 . However particles ranging in size from 75-150 μm will have a surface area from 42-90 mm^2 . From such calculations it was decided to separate the whole products into three fractions according to these very considerable differences in surface areas, the size of the particle being determined by sieve mesh size. The whole of each product was sieved into the following fractions : coarse, all particles above 125 μm ; medium, 75-125 μm ; fine, under 75 μm . The separation was carried out by means of a series of brass laboratory test sieves (Endecotts Ltd. London) until complete segregation was achieved.

Materials and Methods

Twenty-four wether sheep, principally Suffolk x Greyface of mean initial liveweight 44 kg were maintained in metabolism cages over the 12 week experimental period. The sheep received 1.0 kg pelleted grass nuts plus 200 g crushed grass nuts per day given in two approximately equal portions at about 07.30 and 16.00 h. This provided 88 g DCP and 16.4 MJ ME. Fresh water was available ad lib.

The objective of the experiment was to obtain the apparent availability of magnesium in the first consignment of each of the four products, namely Agma, Navarras, Chinese and Greek. For each product estimations were made of (a) the whole, (b) the coarse, (c) the medium

Table 1. Experiment 1. Particle size distributions and magnesium contents of the magnesium supplements.

	Diam. um	AGMA		NAVARRAS		CHINESE		GREEK	
		%	g/kg	%	g/kg	%	g/kg	%	g/kg
Whole		100	513	100	506	100	491	100	495
Fine	<75	15.1	514	3.3	487	2.4	526	1.3	436
Medium	75-125	16.2	519	5.1	502	3.1	518	1.5	452
Coarse	>125	68.7	526	91.7	521	94.5	470	97.1	512

Coarse fraction includes:

	%	%	%	%
125 - 250	24.1	34.2	4.8	4.9
250 - 500	21.3	55.5	10.6	14.3
500 - 1000	10.0	1.5	34.6	26.5
1000 - 2000	13.3	0.5	41.3	37.0
>2000	-	-	3.2	14.4

Table 2. Experiment 1. Daily faecal Mg (g).
 (Mean value of 6 sheep per treatment SEM = 0.084).

	Whole	Coarse	Medium	Fine
Nil	1.628			
H Mg(OH) ₂	2.947			
A Agma	2.862	3.037	3.082	3.118
N Navarras	3.265	3.052	3.075	3.047
C Chinese	2.915	3.147	2.958	2.822
G Greek	2.963	2.943	2.907	2.940
SEM	0.0878	0.0712	0.070	0.079

Significance

P < 0.001 A,N,C,G,H>Nil A,N,C,G,H>Nil A,N,C,G,H>Nil A,N,C,G,H>Nil
 P < 0.01 N>A,C
 P < 0.05 N>G,H A>C

Table 3. Experiment 1. Daily urinary Mg (g) (Mean value of 6 sheep per treatment SEM = 0.073).

	Whole	Coarse	Medium	Fine
Nil	0.568			
H Mg(OH) ₂	0.778			
A Ayma	0.887	0.767	0.755	0.833
N Navarras	0.737	0.863	0.867	0.782
C Chinese	0.798	0.853	0.960	0.958
G Greek	0.777	0.847	0.957	0.828
SEM	0.061	0.073	0.065	0.067

Significance

P < 0.001	A > Nil		C, G, > Nil	C > Nil
P < 0.01		N, C, G > Nil	N > Nil	A > Nil
P < 0.05	C, G, H > Nil		C, G > A	N, G > Nil

and (d) the fine particle size divisions (as described earlier), i.e. giving a total of 16 treatments. Additionally the sheep were given magnesium hydroxide (Steetley Ltd) and a nil treatment.

The design of the experiment was an incomplete Latin square involving 6 consecutive periods each of 14 days. There were 8 blocks of 3 sheep in each period four of which were assigned to the classification of whole product, coarse, medium and fine fractions (as defined above) and four of which were assigned to the classification of Agma, Navarras, Chinese and Greek source materials. These were compared to sheep receiving magnesium hydroxide and those receiving no supplement.

Over the course of the experiment (a) each sheep received each of the four treatment supplements assigned to that block plus the magnesium hydroxide and a nil supplement and (b) each treatment was tested in six different sheep. The design of the experiment allowed the interpretation to concentrate on the two main factors of comparison between a) whole products and b) different particle sizes of each separate product.

The sheep received 2.0 g supplemental magnesium with their morning feed. The first 7 days of each period was a changeover period and during the following 7 days total collections were made of both faeces and urine which were sampled and analysed for magnesium.

Table 1 details the particle size distribution and the magnesium concentration within each of the products used. The magnesium hydroxide contained 404.4 g Mg/kg.

Results

The diet was well consumed throughout, the crushed grass nuts facilitated total clean up of the supplement. The diet provided sufficient energy for maintenance, the sheep gained a mean of 0.5 kg liveweight per week for the duration of the trial.

The basal diet provided 2.17 g Mg/day. The mean daily amounts present in the urine and faeces of sheep receiving the basal diet alone were 0.57 and 1.63 g Mg respectively. The overall mean blood magnesium concentration was about 0.9 mmol/l.

The main results are shown in Table 2 (faeces) and Table 3 (urine). Table 4 includes data for apparent magnesium retention, calculated apparent availability and blood magnesium concentrations.

As might have been expected, unsupplemented sheep had significantly less ($P < 0.001$) magnesium in their faeces than those receiving any one of the different supplements (Table 2). Differences in faecal magnesium output were shown to exist between products and the various fractions. Relatively high faecal magnesium values represent relatively low apparent dietary availability.

The Navarras whole product gave the highest faecal magnesium of all treatments and Agma the least (Table 2). The faeces of sheep receiving the Navarras whole product contained significantly higher amounts of magnesium than both Agma and Chinese ($P < 0.01$) and the Greek and magnesium hydroxide ($P < 0.05$). No other significant statistical difference existed between the other whole products.

Within the different sized fractions there were no significant differences between the various coarse fractions or between the medium fractions.

However within the fine fractions the Agma product produced significantly higher faecal magnesium than the Chinese product ($P < 0.05$).

When comparing the faecal outputs of all 18 treatments together, the Navarras whole product gave the highest faecal magnesium content. The Chinese coarse fraction produced the second highest faecal magnesium concentration and gave significantly higher faecal magnesium values than the Chinese fine ($P < 0.01$), and Agma whole and Greek medium ($P < 0.05$).

After the nil treatment, the sheep supplemented with the Chinese fine fraction gave the lowest faecal magnesium output and was significantly less than the Agma fine, Chinese coarse ($P < 0.01$) and Agma and Navarras medium fractions ($P < 0.05$).

For urinary magnesium outputs (Table 3) relatively high values would be associated with relative superior dietary availability. All whole product treatments, except for the Navarras, produced significantly higher urinary magnesium outputs. Agma whole product produced the highest urinary output compared to the remaining three calcined magnesites and the hydroxide.

All treatments within the fine fractions produced significantly higher urinary magnesium than the nil treatment, but within the medium and coarse fractions all except for Agma were significantly higher. Within the medium fractions both the Chinese and Greek products produced significantly higher urinary magnesium than the Agma ($P < 0.05$).

Table 4. Experiment 1. Mean daily magnesium intakes, outputs in faeces and urine and apparent retentions, apparent dietary availability and mean blood magnesium concentrations (6 sheep/treatment).

Supplement		Nil	Mg(OH) ₂		
Intake	g	2.17	4.17		
Urine	g	0.57	0.78		
Faeces	g	1.63	2.95		
Retention	g	-0.03	0.44		
Availability	%	-	34.0		
Blood Mg	m mol/l	0.89	0.97		
Agma		Whole	Coarse	Medium	Fine
Intake	g	4.17	4.17	4.17	4.17
Urine	g	0.89	0.77	0.76	0.83
Faeces	g	2.86	3.04	3.08	3.12
Retention	g	0.42	0.36	0.33	0.22
Availability	%	38.5	29.5	27.5	25.5
Blood Mg	m mol/l	0.96	0.95	0.95	0.94
Navarras		Whole	Coarse	Medium	Fine
Intake	g	4.17	4.17	4.17	4.17
Urine	g	0.74	0.86	0.87	0.78
Faeces	g	3.27	3.05	3.08	3.05
Retention	g	0.16	0.26	0.22	0.34
Availability	%	18	29	27.5	29
Blood Mg	m mol/l	0.93	0.97	0.91	0.93
Chinese		Whole	Coarse	Medium	Fine
Intake	g	4.17	4.17	4.17	4.17
Urine	g	0.8	0.85	0.96	0.96
Faeces	g	2.92	3.15	2.96	2.82
Retention	g	0.45	0.17	0.25	0.39
Availability	%	35.5	24	33.5	40.5
Blood Mg	m mol/l	0.93	0.96	0.99	0.98
Greek		Whole	Coarse	Medium	Fine
Intake	g	4.17	4.17	4.17	4.17
Urine	g	0.78	0.85	0.96	0.83
Faeces	g	2.96	2.94	2.91	2.94
Retention	g	0.43	0.38	0.30	0.40
Availability	%	33.5	34.5	36.0	34.5
Blood Mg	m mol/l	0.90	0.90	0.92	0.95

Comparatively, the Agma coarse and medium fractions together with the Navarras whole product (which all produced a low magnesium urinary output) gave relatively high faecal magnesium outputs when compared to the other treatments.

Comparing the 18 treatments together, the Chinese medium and fine fractions together with the Greek medium fraction, produced significantly more magnesium in the urine ($P < 0.05$) than the Navarras whole product which in turn correlates with comparable lower (significantly) faecal magnesium outputs.

Apparent retention is calculated as the total magnesium intake less the sum of the faecal magnesium and the urinary magnesium outputs (Table 4). The nil treatment (i.e. the retention obtained from the dietary magnesium alone) gave a value of -0.03 g/day. For the whole products and the hydroxide, all treatments except for the Navarras gave similar apparent retention values ($0.42 - 0.45$ g/day). The Navarras produced an apparent retention of 0.16 g/day but this lower value would be expected due to the higher faecal magnesium output.

Within any particular fraction size similar retention figures were obtained but a greater variation existed within the coarse fractions.

No significant differences occurred between blood magnesium values (Table 4). The nil treatment produced a blood magnesium concentration of 0.89 m mol/l and the various supplements produced blood magnesium levels between 0.90 and 0.99 m mol/l. No correlations could be made between blood magnesium concentrations and apparent availabilities.

The apparent dietary availability of the magnesium was 34% for the hydroxide (Table 4). For the whole products the Navarras (18%) was least available but that value seems to be anomalous as the values for the separate fractions were in the range 27.5 - 29.0%. Only for the Chinese product was there an apparent increase in availability with a decrease in particle size. Further description of comparative availabilities is deferred until the General Discussion at the end of this Section.

EXPERIMENT 2

Introduction

Experiment 1 investigated the bioavailability of four granular calcined magnesites and their different particle size fractions. In this experiment a second batch of the same granular magnesites was investigated. A Turkish granular calcined magnesite was also investigated.

Materials and Methods

Fourteen wether sheep used previously in Experiment 1 of mean liveweight 49 kg were maintained in metabolism cages under the same conditions as described in Experiment 1 for a 12 week feeding trial i.e. 6 periods each of two weeks. The sheep received a basal diet of 1.0 kg dried grass nuts (1.94 g Mg/kg DM) and 200 g barley (1.15 g Mg/kg DM) per day providing more than adequate magnesium at 1.95 g daily, 126 g crude protein (72 g DCP) and 19.4 MJ gross energy (9.7 MJ ME).

From data obtained in Experiment 1 it was possible to block the sheep into two groups of seven as uniformly as possible according to their faecal dry matter output. The experiment was thus conducted in the form of two 7 x 6 incomplete Latin Squares.

The six main treatments within each block were the whole products Agma, Navarras, Chinese, Greek and Turkish calcined magnesites, the standard magnesium hydroxide and a nil treatment. The magnesium contents of these supplements (g/kg) were Agma 481, Navarras 523, Chinese 537, Greek 477, magnesium hydroxide 404 and finally Turkish 453.

The magnesium supplements were added to the morning feed so as to provide 2.0 g of supplementary magnesium for 14 consecutive days. Each sheep received a different supplement in each of the 14-day periods including one period during which no supplement was received. Within each block each treatment was replicated 6 times during the 84 day trial giving a total of 12 sheep per treatment.

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 and sampled for magnesium analyses.

Table 5. Experiment 2. The mean daily intakes, outputs in faeces and urine and apparent retentions of magnesium (g) and the calculated apparent availabilities (12 sheep/treatment).

	Intake	Faeces	Urine	Apparent Retention	Apparent Availability %
Nil	A 1.95	1.47	0.53	-0.05	-
Mg hydroxide	B 3.95	2.95	0.88	0.12	26.0
Agma	C 3.95	3.10	0.73	0.12	18.5
Navarras	D 3.95	3.02	0.78	0.15	22.5
Chinese	E 3.95	3.00	0.80	0.15	23.5
Greek	F 3.95	3.15	0.69	0.11	16.0
Turkish	G 3.95	2.90	0.65	0.40	28.5
SEM		0.078	0.054		
Significance					
Faeces	P < 0.001	A < B,C,D,E,F,G			
	P < 0.05	F > G			
Urine	P < 0.001	A < B,E			
	P < 0.01	A < D; B > G			
	P < 0.05	A < C; B > F; E > G			

Results

The diet and supplements were well consumed throughout the trial. The sheep gained 2.5 kg liveweight during the 84 day trial period.

The principal results are shown in Table 5. The basal diet provided a mean of 1.95 g magnesium per day. The mean daily amounts in the urine and faeces of sheep receiving the basal diet alone were 0.53 and 1.47 g magnesium respectively.

All supplements significantly increased faecal magnesium outputs. The Greek material produced the largest amount of magnesium in the faeces compared to the other treatments and was significantly greater than the Turkish ($P < 0.05$). The Turkish produced the least amount of faecal magnesium and consequently the highest apparent availability at 28.5% compared to the Greek at 16.0%. The hydroxide also produced a superior apparent availability at 26.0% and little differences occurred between the remaining three magnesites.

All treatments except for the Greek and Turkish significantly increased urinary outputs. The hydroxide produced the highest urinary magnesium which was significantly greater than that produced by the Greek ($P < 0.05$) and (surprisingly) the Turkish material ($P < 0.01$). The Chinese material also produced significantly greater urinary magnesium than the Turkish ($P < 0.05$). The lower urinary magnesium produced by the Turkish resulted in a much higher apparent retention of magnesium (0.4 g/day) compared to the remaining treatments which varied between 0.11 and 0.15 g/day.

EXPERIMENT 3

Introduction

Third consignments of the principal calcined magnesites used in Experiments 1 and 2 were investigated.

Materials and Methods

Twenty four growing wether sheep (Suffolk x Greyface) with initial mean liveweight 35 kg were maintained in metabolism cages over the six week experiment. Each sheep received a basal diet of 0.8 kg dried grass nuts plus 0.2 kg rolled barley (0.1 kg of which contained chromic oxide in a nut) providing 1.73 g Mg, 107 g crude protein (60.2 g DCP) and 16 MJ gross energy (8.1 MJ ME) i.e. above maintenance. Water was available ad lib.

After the initial one week acclimatisation period the experiment was conducted in the form of four balanced 6 x 3 randomised blocks. The balanced replicated block design allowed for a total of twelve replications of each supplement or no addition. The six treatments were Agma, Navarras, Chinese and Greek calcined magnesites, magnesium hydroxide and a nil treatment. The calcined magnesites were added to the morning feed so as to provide 2.0 g magnesium per day.

Total faeces and urine collections were made, subsampled and analysed for magnesium. Chromium analyses were also undertaken on the faeces for parallel studies in association with Experiment 7 to be described.

The magnesium contents of the supplements (g Mg/kg) were Agma, 537; Navarras, 531; Chinese, 495; Greek, 527; Hydroxide, 404.

Results

The diets and supplements were well consumed throughout the trial and the principle results are detailed in Table 6. The sheep gained 0.73 kg liveweight during the 6 week trial. The basal diet supplied 1.73 g magnesium per day with corresponding daily urinary and faecal outputs of 0.44 and 1.0 g respectively giving an apparent retention of 0.2 g per day.

All the supplements increased urinary and faecal magnesium

Table 6. Experiment 3. The mean daily intakes, outputs in urine and faeces apparent retentions of magnesium (g) and the calculated apparent availabilities (%). (12 sheep/treatment).

	Intake	Faeces	Urine	Apparent Retention	Apparent Availability
Nil	A 1.73	1.00	0.44	0.29	-
Mg Hydroxide	B 2.73	2.41	0.73	0.59	29.5
Agma	C 2.73	2.46	0.66	0.61	27.0
Navarras	D 2.73	2.48	0.66	0.59	26.0
Chinese	E 2.73	2.78	0.68	0.27	11.0
Greek	F 2.73	2.69	0.60	0.44	15.5
SEM		0.058	0.034		
Significance					
Faeces	P < 0.001	A < B,C,D,E,F E > B,C,D			
	P < 0.01	F > B,C			
	P < 0.05	F > D			
Urine	P < 0.001	A < B,C,D,E			
	P < 0.01	B > F			

concentrations ($P < 0.001$). Differences were apparent in faecal magnesium outputs between supplements. The sheep supplemented with the Greek product gave significantly higher levels of magnesium in their faeces than those supplemented with Agma and magnesium hydroxide ($P < 0.01$) and Navarras ($P < 0.05$).

The sheep supplemented with magnesium hydroxide gave higher urinary magnesium levels than the other supplements; the difference between the hydroxide and the Greek product was significant at $P < 0.01$. Surprisingly high apparent retentions of magnesium occurred with up to 0.61 g magnesium apparently retained when sheep were supplemented with the Agma material. Similar apparent retentions occurred with both the hydroxide (0.58 g) and Navarras (0.59 g).

These apparent retention figures which are somewhat higher than those obtained in previous but comparable experiments may be due in part to the age of the animals. The wether lambs used in this experiment were at least 5 months younger than those used in Experiment 2.

The apparent dietary availabilities of the Chinese and Greek products (11.0 and 15.5%) were markedly less than for the Agma (27.0%) and Navarras (26.0%) and for the hydroxide (29.5%). This will be described in more detail in the General Discussion of this section.

EXPERIMENT 4

Introduction

The comparative availability of a fourth consignment of the four major granular calcined magnesites used in Experiments 1, 2 and 3 were investigated. Magnesium hydroxide obtained from Steetley Minerals Ltd. and used in Experiments 1, 2 and 3 was again used as a standard comparator.

Materials and Methods

Twenty four 6 month old wether lambs, mean liveweight 40 kg were maintained in metabolism cages and received the standard diet used in the earlier trials (Experiments 1, 2 and 3) of 1.00 kg dried grass nuts and 200 g barley per head per day. 100 g of the 200 g barley allocation was given as a barley/chromic oxide cube to be used for comparative studies with Experiment 8 where other sheep given the same treatments were maintained on slatted floors. The basal diet provided 128 g CP (72 g DCP), 19.7 MJ GE (9.7 MJ ME) and 1.73 g magnesium. Fresh water was available ad lib.

The experimental design consisted of four 6 x 3 incomplete Latin Squares. The lambs were randomly allocated to one of four blocks. Within each block each sheep received one of the 6 original treatments as in Experiments 1, 2 and 3, namely the four calcined magnesites, Agma, Navarras, Chinese and Greek, the standard magnesium hydroxide and a nil treatment.

The magnesium concentrations of the supplements (g Mg/kg) were : Agma, 525; Navarras, 516; Chinese, 583; Greek, 484 and Hydroxide 404.

All the products were added to the morning feed such as to give an additional 2.0 g Mg/day.

Each of the feeding periods involved 7 days run-in and 7 days collection of faeces and urine.

Results

The feed and supplements were well consumed and the sheep achieved a mean liveweight gain of 4.3 kg during the course of the trial. The mean faecal and urinary magnesium outputs are presented in Table 7.

Table 7. Experiment 4. The mean daily intakes and outputs of magnesium in the faeces and urine and the apparent retentions and availabilities (%) (12 sheep/treatment).

		Intake	Faeces	Urine	Apparent Retention	Apparent Availability
						%
Nil	A	1.73	1.19	0.58	-0.04	-
Mg hydroxide	B	3.73	2.536	1.01	0.19	32.7
Agma	C	3.73	2.65	0.97	0.11	27.0
Navarras	D	3.73	2.73	0.85	0.15	23.0
Chinese	E	3.73	2.71	0.91	0.11	24.0
Greek	F	3.73	2.94	0.88	-0.10	12.5
SEM			0.062	0.042		

Significance

Faeces	P < 0.001	A < B,C,D,E,F
		F > B,C
	P < 0.01	F > E
	P < 0.05	F > D; B < D,E
Urine	P < 0.001	A < B,C,D,E,F
	P < 0.05	B > D,F

The basal diet provided a mean of 1.73 g Mg/day. The mean daily amounts of magnesium present in the urine and faeces of sheep receiving the basal diet alone were 0.58 and 1.19 g respectively, and the retention, calculated by the difference, was -0.04 g/day.

All the supplements significantly increased both faecal and urinary magnesium outputs.

For the granular calcined magnesites, the Greek product appeared to be of markedly lower availability. Sheep receiving the Greek product had significantly more magnesium in their faeces than the Agma ($P < 0.001$) and the Chinese product ($P < 0.01$). The availability value for Agma at 27.0% approached that for magnesium hydroxide (32.7%). Both Navarras and Chinese calcined magnesites had availability values somewhat less than Agma (i.e. higher faecal magnesium outputs) but not significantly so.

Sheep receiving magnesium hydroxide produced significantly higher urinary magnesium outputs than both the Navarras and Greek supplements ($P < 0.05$) corresponding to the superior apparent availability value.

Apparent retention values for magnesium varied between 0.1 and 0.2g per day. The Greek material produced an apparent negative retention value (-0.1g) presumably due to the high faecal magnesium output.

EXPERIMENT 5**Introduction**

It was apparent from the results of Experiments 1, 2 and 3 that the apparent dietary availability of granular calcined magnesites was very variable and could, on occasion, be quite low. It was therefore decided that a small experiment would be conducted simultaneously with Experiment 4 to investigate the apparent dietary availability of three contrasting magnesium-containing materials other than the four calcined magnesites.

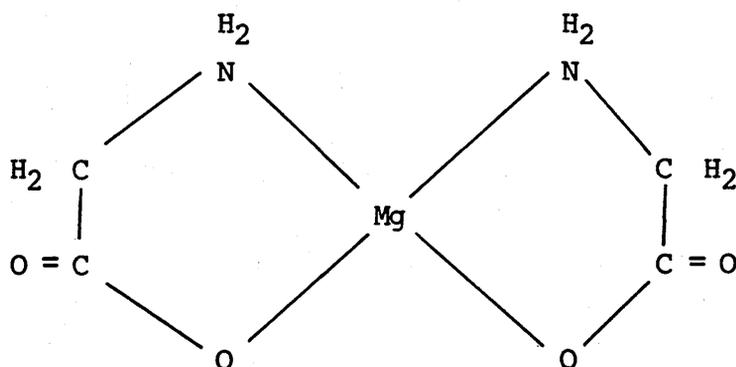
The sheep used were entirely comparable to those used in Experiment 4. The sheep were housed in the same building and the feeding periods were the same. It was considered that the mean values determined for the sheep given the nil and magnesium hydroxide supplements in Experiment 4 could be regarded as also being part of Experiment 5.

The magnesium sources investigated were magnesium chloride, magnesium metalosate (Thomson & Joseph Ltd) and a fine grade Chinese calcined magnesite (Colin Stewart Minerals Ltd).

Due to its hygroscopic properties, a concentrated solution of magnesium chloride was prepared using reagent-grade magnesium chloride hexahydrate (BDH). The solution was carefully measured and added to the feed using a pipette each day.

The fine grade Chinese calcined magnesite was considered by Colin Stewart Minerals Ltd to be a very reactive material according to their citric acid reactivity tests.

The magnesium metalosate, commercially known as Magnesium Chelazome, contained approximately 11% magnesium by weight. The magnesium is supplied in an amino acid dipeptide chelated form.



The manufacturer claims that a mineral in a chelated state is potentially more available to an animal than when supplied by an inorganic salt.

Materials and Methods

Six 6 month old wether lambs were maintained in metabolism cages and received the standard diet used in Experiment 4 of 1.00 kg dried grass nuts and 200 g barley per head per day. The basal diet provided 72 g DCP, 9.7 MJ ME and 1.73 g magnesium with fresh water available ad lib.

The three treatments, magnesium chloride, magnesium metalosate and the fine grade Chinese calcined magnesite were investigated as two 3 x 3 Latin Squares. The magnesium input/output data for the unsupplemented diet were derived from 12 comparable sheep maintained at the same time for the granular calcined magnesite investigations in Experiment 4 (Table 7).

All the products were added to the morning feed as to provide 2.0g additional magnesium per day.

Each of the three periods involved 7 days run-in and 7 days collection of both faeces and urine.

Results

The feed and supplements were well consumed with no apparent palatability problems.

The mean faecal and urinary magnesium outputs are presented in Table 8.

The basal diet provided a mean of 1.73 g Mg/day and the resulting mean daily amounts of magnesium present in the urine and faeces were 0.58 and 1.19 g respectively with an apparent retention of -0.04 g per day. All the supplements significantly increased both faecal and urinary magnesium outputs.

All the supplements produced comparably low faecal magnesium outputs and thus correspondingly superior apparent availabilities when compared to the granular calcined magnesite results of Experiment 4.

In earlier work it has consistently been shown that fine particle calcined magnesites have higher dietary availabilities than coarse materials. Wilson (1981) found a Greek powdered product to be almost

Table 8. Experiment 5. Mean daily intakes and outputs in faeces and urine and apparent retentions of magnesium (g/day) and apparent availabilities. (6 sheep/treatment).

	Intake	Faeces	Urine	Apparent Retention	Apparent Availability %
Nil					
Mg hydroxide	A 1.73	1.19	0.58	-0.04	-
MgCl ₂	B 3.73	2.536	1.01	0.19	32.7
Mg metalosate	G 3.73	2.63	1.02	0.08	28.0
Fine grade Chinese calcined magnesite	H 3.73	2.68	1.00	0.05	25.5
	I 3.73	2.64	1.00	0.09	27.5
SEM		0.076	0.067		
Significance					
Faeces		P < 0.001		A < B,G,H,I	
Urine		P < 0.001		A < B,G,H,I	

Both Nil and hydroxide values refer to means of 12 sheep per treatment in Experiment 4 (Table 7).

twice as available as its granular counterpart (24.2% and 12.7% respectively). It was therefore surprising that the powdered Chinese product did not produce a higher apparent availability value than the 27.5% when compared to 24.0% or 27.0% for the granular Chinese and Agma products, respectively.

It was also somewhat surprising that neither the fully soluble magnesium chloride and the hygroscopic magnesium metalosate produced availability values which were superior to that of the water insoluble Agma calcined magnesite.

All the supplements produced relatively high urinary magnesium values, again corresponding to apparent availability results. This in turn was reflected in comparably low apparent retention values which varied between 0.05 and 0.09 g Mg/day for the three supplements. These were rather less than the apparent retentions of about 0.11 - 0.19 g Mg/day recorded for the four calcined magnesites in Experiment 4 (Table 7).

EXPERIMENT 6

The effects of feeding increasing amounts of supplementary magnesium to sheep in cages

Introduction

A high dietary magnesium intake is often used to combat hypomagnesaemia. When cattle are given magnesium-containing supplements on a free-access basis it is difficult to regulate the amount of magnesium consumed by individuals. The effect of excess magnesium has not been well defined in cattle although Care (1960) has reported scouring in bullocks given daily 170 g or more calcined magnesite. Work has been carried out to show the effects of high levels of dietary magnesium to calves (Gentry *et al*, 1978; Quillian *et al*, 1980). Gentry *et al* feeding 1, 2 and 4% supplementary magnesium as magnesium oxide (undefined source) to bull calves found that diarrhoea was the most obvious effect of high intake of magnesium. The extent and intensity of the diarrhoea was proportional to the amount of supplemental magnesium consumed. Likewise, Quillian *et al* fed calves diets containing 0.7 or 1.15% magnesium from supplemental magnesium oxide for 28 days. Faecal dry matter percentage was reduced slightly with 0.7% and substantially with 1.15% magnesium. Pierce (1959) investigating the tolerance of sheep for mixtures of sodium chloride and magnesium chloride in the drinking water found that diarrhoea occurred frequently when animals received 0.5% $MgCl_2$ in their ration.

In the experiment to be described the effects of feeding increasing levels of supplementary magnesium as different calcined magnesite sources was investigated, using sheep in metabolism cages.

Materials and Methods

Three wether lambs of mean liveweight 48.5 kg were maintained in metabolism cages simultaneously to Experiment 2. Each sheep received 1.0 kg dried grass nuts and 200 g barley per day supplying approximately 72 g DCP, 9.7 MJ ME and 1.95 g magnesium. Water was available *ad lib*.

The aim of the experiment initially was to investigate the effects of feeding Agma in increasing amounts to sheep. Daily amounts of 4 g,

Table 9. Experiment 6. Mean magnesium output in faeces and urine (g Mg/d).

		No. of sheep	Faecal Mg output	Urinary Mg output	Apparent Retention	Apparent Availability %
Nil		12	1.469	0.532	-0.051	N A
Agma	2g	12	3.10	0.735	0.123	18.5
	4g	3	4.71	1.13	0.11	19.0
	8g	3	7.44	1.29	1.22	25.4
	12g	3	10.45	1.60	1.90	25.2
Navarras	2g	12	3.02	0.78	0.15	22.5
	4g	1	5.82	1.00	-0.87	-9.0
	8g	1	7.49	0.78	1.68	24.8
	12g	1	12.30	0.07	1.58	9.8
Chinese	2g	12	3.00	0.80	0.15	23.5
	4g	1	4.56	0.79	0.60	22.8
	8g	1	8.13	1.30	0.52	16.7
	12g	1	10.17	1.93	1.85	27.5
Greek	2g	12	3.15	0.69	0.11	16.0
	4g	1	4.82	0.96	0.17	16.3
	8g	1	9.24	1.08	-0.37	3.0
	12g	1	10.44	1.55	1.96	25.2

8g and 12 g Agma (as used in Experiment 2) were given to the three sheep in a 3 x 3 Latin Square design. It was then decided to feed the other calcined magnesites, namely Navarras, Chinese and Greek (identical products to those used in Experiment 2) for comparative purposes. The magnesites were added to the basic diet at the three levels but for one collection period only, i.e. one sheep per treatment.

From the first day of supplementation the daily faecal output from each sheep was assessed and graded according to the texture of the faeces as a visual determination of faecal consistency.

The faeces were graded on a scale 1 - 4 as follows:

- 1 - faeces were in the form of solid, compact pellets.
- 2 - some pellets were less well defined and the faeces was beginning to clump together with increasing water content.
- 3 - no individual pellets were visible in the faeces which was in the form of one solid clump within the collection bag.
- 4 - the faeces were in the form of an extremely watery slurry.

Each experimental period consisted of a 7 day run-in period and a 7 day collection period of both faeces and urine.

Results and Discussion

All the feedstuffs and supplements were well consumed, the higher levels of magnesium supplementation appeared to have no adverse effect on feed intake. The mean magnesium output in the faeces and urine for all treatments are described in Table 9 together with calculated apparent retention and availability figures.

Similar apparent availability figures were obtained for both Agma and Chinese at all levels of supplementation. Anomalous availability figures probably as a result of severe scouring occurred for both the Navarras (e.g. - 9.0% at 4 g Mg) and the Greek (e.g. 3.0% at 8 g Mg). The Agma appeared to be the most consistent in terms of availability across the different levels of supplementation and Navarras the most irregular. The Greek material appeared to be of poorer availability than the other supplements, although at 12 g Mg the availability figure was calculated to be 25.2%, similar to that of the Agma and Chinese. It should be remembered however that values for all supplements, except Agma, given at levels of 4 g or more refer to single sheep.

As might have been expected, the urinary magnesium output increased with increasing amounts of supplemental magnesium. The only contradictory results occurred with the Navarras product; very little difference in urinary magnesium output was obtained between the 2, 4 and 8 g levels of supplementation (0.78, 1.00 and 0.78 g respectively) however with a daily intake of 12 g the mean daily urinary magnesium output was as little as 0.07 g.

The apparent retention figures were similarly greater with increased supplemental magnesium. The Navarras and Greek produced apparently abnormal retention figures at the 4 g and 8 g levels of supplementation (-0.87 and -0.37 g respectively) which reflected the anomalous availability figures mentioned above. Retention figures of nearly 2 g per day were obtained with 12 g Agma and 12 g Greek product (1.90 and 1.96 g respectively). Too much reliance should not be placed on values for only one sheep.

The faecal texture observations are detailed in Table 10 and Figure 1. Diarrhoea was the most noticeable visual observation when the sheep were given high supplemental magnesium (8 g and 12 g) in agreement with earlier work (Gentry *et al*, 1978; Quillian *et al*, 1979). The Agma appeared to have the greatest effect on faecal consistency, Chinese the least. Four days of supplementation with Agma providing 12g magnesium produced an extremely watery slurry which persisted for the 14 day period. Both the 4 g and 8 g levels of magnesium supplementation also caused the faeces to have higher water contents than normal ('Normal' faeces are equivalent to Grade 1 on the scale). The Greek and Navarras were the most reactive at the highest level of supplementation.

The Chinese material on the other hand appeared to have little effect on the faecal consistency even at the highest level of supplementation. Supplementation with Chinese at 12 g Mg for 14 days had little or no effect on faecal consistency.

Unfortunately, due to carryover effects from the previous levels of supplementation it was difficult to make accurate assessments on the actual effects of the individual treatments. No time interval was allowed within the experimental design where no supplement was received in order for the faeces to recover from previous supplementations. Thus the faecal texture recorded may have been due in part to the previous level of supplementaion (see Figure 1) e.g. the faecal texture after supplementation with 4 g Mg as Navarras, following 12 g Mg as Greek

Table 10. Experiment 6. The faecal texture of sheep supplemented with increasing levels of magnesium as calcined magnesite.

		DAYS								
		1	2	3	4	6	8	10	12	14
Agma	4g	1	1	1	2	2	2	2	2	2
	8g	1	1	2	3	3	3	3	3	3
	12g	1	1	3	4	4	4	4	4	4
Navarros	4g+	3	3	3	3	3	3	3	3	3
	8g	1	1	1	1	1	1	1	1	1
	12g	1	1	1	1	3	3	3	3	3
Chinese	4g	2+	2	1	1	1	1	1	1	1
	8g	3+	3	1	1	1	1	1	1	1
	12g	1	1	1	1	1	1	1	1	1
Greek	4g	4+	3	1	1	1	1	1	1	1
	8g	1	1	1	1	1	1	1	1	1
	12g	1	1	1	1	2	2	3	3	3

Faecal Texture Scale:

- 1 : Solid compact pellets
- 2 : Some undefined pellets; some clumping
- 3 : No visible pellets, high moisture content causing one compacted clumping with faecal bag
- 4 : Extremely watery slurry

+ faeces showing 'carryover effect' from previous treatment (see Fig. 1)

Figure 1 Expt. 6 Faecal texture of sheep given different levels of supplementary magnesium (g) as various calcined magnesites. (Graded on scale 1-4 : 1, faeces in form of solid compact pellets; 2, some pellets less well defined and faeces beginning to clump together; 3, no individual pellets visible in faeces which was in form of one solid clump; 4, faeces in form of extremely watery slurry).

(● — ● 4 g Mg; ▲ — ▲ 8 g Mg; □ — □ 12 g Mg)

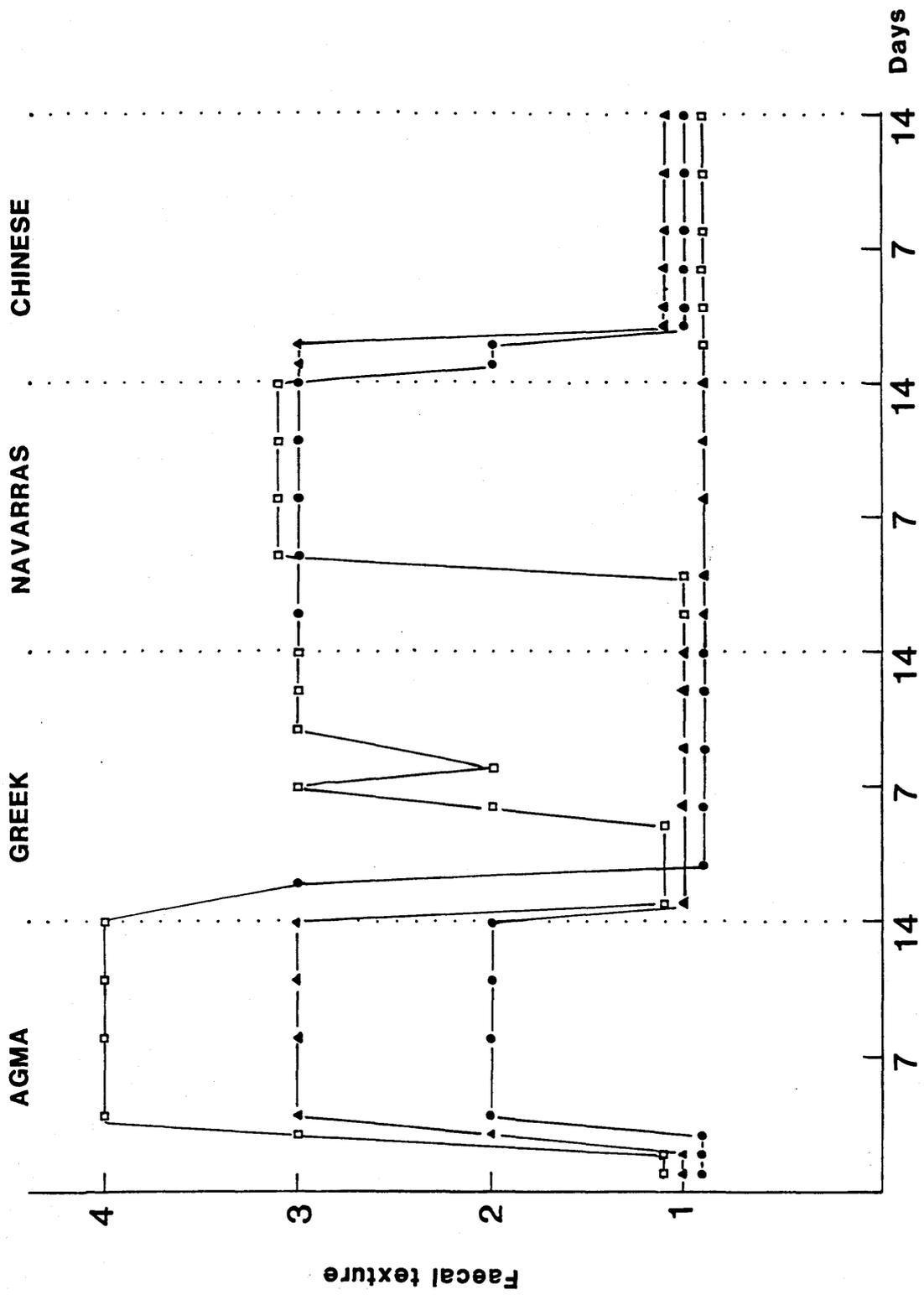


Figure 2 Expt. 6 Faecal dry matter (%) of sheep given different levels of supplementary magnesium as various calcined magnesites.

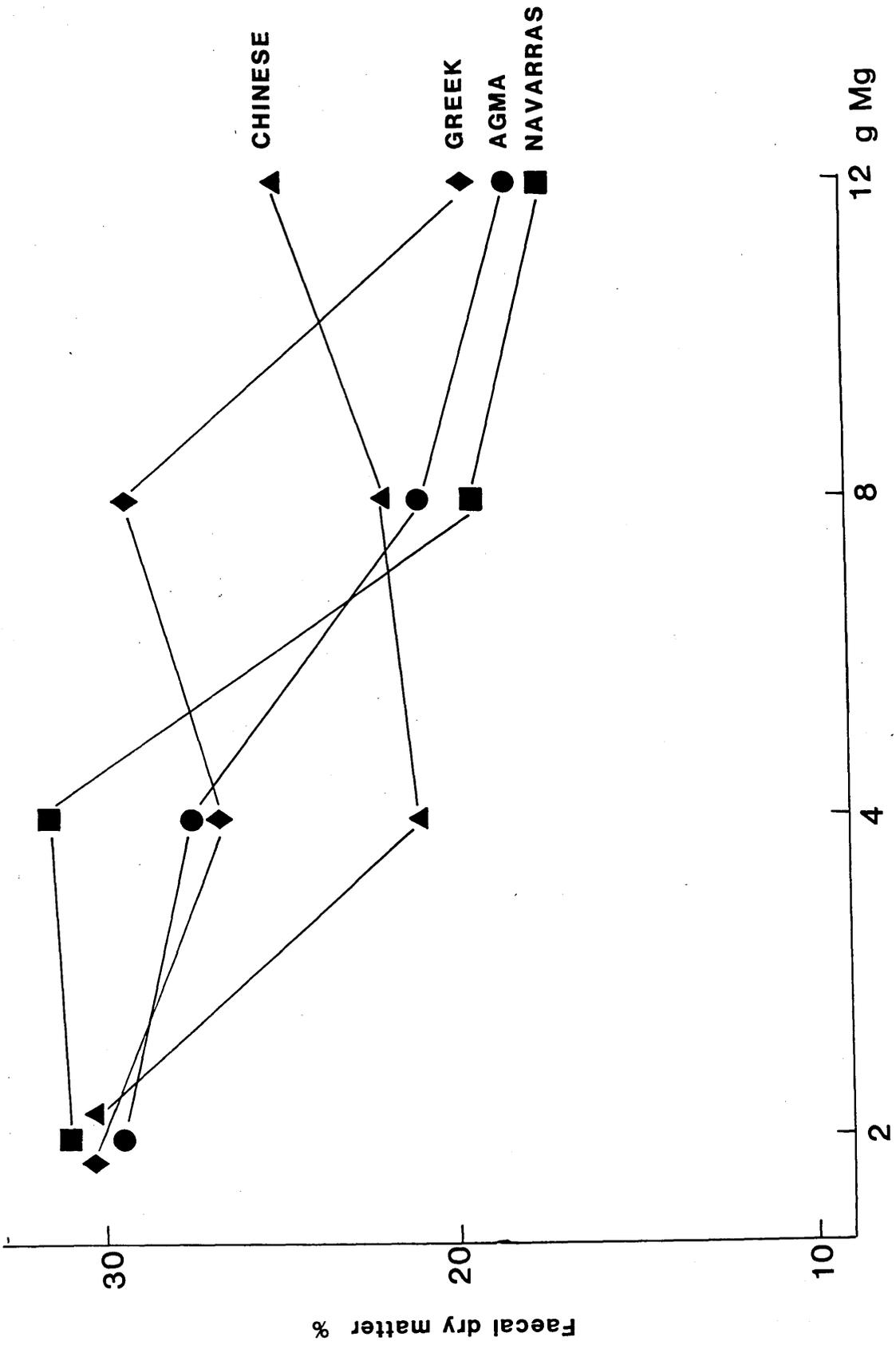


Table 11. Experiment 6. Mean faecal DM (g/kg) of sheep supplemented with different levels of magnesium.

Supplement	2g ^o	4g	8g	12g
Agma +	295	281	216	184
Navarros x	310	318	198	178
Chinese x	305	206	222	249
Greek x	305	274	297	193

+ mean of 3 values

x one value only

o mean of 12 values (from Experiment 2)

remained at Grade 3 for the entire 14 day period. The Agma results are possibly the most accurate in terms of observation since this was the first treatment and therefore there were no carryover effects. However as the results stand, they give some idea of the time involved for the faeces to 'recover' e.g. the faeces of one individual sheep took 3 days to recover from a watery diarrhoea (12 g Mg as Agma) to 'normal' (4 g as Greek).

Table 11 gives the mean faecal dry matter concentrations of the sheep supplemented at the different levels of magnesium and Figure 2 describes the effect of increasing levels of supplemental magnesium on the faecal dry matter. The results reflect the visual observations described in Figure 1 and Table 10 to some extent, e.g. the Chinese supplement of 12 g Mg has little or no effect on dry matter content of the faeces. However they do not suggest the extreme diarrhoea observed with Agma at 12 g Mg, which appears to have similar mean faecal dry matter concentrations to those of the Greek and Navarras at the same level of supplementation.

The general conclusions that can be made from this experiment are that differences exist between the different supplements and different levels of supplementation in their bioavailability and ability to affect faecal consistency. This will have practical importance where calcined magnesites at higher levels of supplementation are used to combat actual or potential incidences of hypomagnesaemia or where individuals consume large amounts on a free-access basis. At the same time it would be appropriate to assume that any effect on faecal consistency, to the extent of producing extreme diarrhoea must be due to a reaction occurring within the digestive tract as a result of an 'active' ingredient.

DISCUSSION. EXPERIMENTS 1 - 6**(i) Apparent dietary availability**

In all the experiments discussed in this Section, dietary magnesium availability has been expressed as that percentage of the supplemental magnesium, given as calcined magnesites, not recovered in the faeces. However other workers (Storry and Rook, 1963; Jesse *et al*, 1981) have expressed availability of the supplements as the increased 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. In all cases described here total collections of urine were meticulously made and thus total magnesium output calculated. Although supplementation significantly increased urinary magnesium no correlation existed between apparent availability and urinary magnesium output. However supplements of higher apparent availability (except for Turkish calcined magnesite) produced comparatively higher amounts of magnesium in the urine.

Unlike work by Wilson (1981) the results obtained in Experiment 1 did not suggest any difference in bioavailability as a result of particle size. The Agma fine fraction was less available than the Agma coarse fraction (25.5% and 29.5% respectively), and the coarse and fine fractions of both the Navarras and Greek products produced identical availabilities (29.0% and 34.5% respectively). The Chinese calcined magnesite was the only product where there was an increase in availability with decreasing particle size. However it must be noted that the parameters for particle size used in Experiment 1 were very different to those used by Wilson (1981) where fine material was described as particles below 75 μm and coarse material as that between 500 - 1000 μm . In this present study (Experiment 1) fine material was defined as particles below 75 μm but coarse material as everything above 125 μm . Thus it was not necessarily expected to reproduce similar results.

Of the calcined magnesites investigated in each of the separate experiments, the Greek product appears inferior to the Spanish Agma, Navarras and the Chinese granular products. As was expected, the fine powdered magnesium hydroxide reproduced similar availability values for each of the four experiments with an overall mean availability (mean of

Table 12. The mean faecal apparent availability figures calculated from the mean faecal output results obtained in Experiments 1 - 5.

Experiment	1	2	3	4	Mean	S.Dev
Nil	Faecal Mg g	1.63	1.47	1.00	1.19	-
Mg(OH) ₂	Faecal Mg	2.95	2.95	2.41	2.536	-
	Avail'y	34.00	26.00	29.50	32.70	30.6
Agma	Faecal Mg	2.86	3.10	2.46	2.65	-
	Avail'y	38.50	18.50	27.00	27.00	27.8
Navarras	Faecal Mg	3.27	3.02	2.48	2.73	-
	Avail'y	18.00	22.50	26.00	23.00	22.4
Chinese	Faecal Mg	2.92	3.00	2.78	2.71	-
	Avail'y	35.50	23.50	11.00	24.00	23.5
Greek	Faecal Mg	2.96	3.15	2.69	2.94	-
	Avail'y	33.50	16.00	15.50	12.50	19.4
Turkish	Faecal Mg		2.90			
	Avail'y		28.5			
Chinese fine grade	Faecal Mg				2.64	
	Avail'y				27.5	
Mg metalosate	Faecal Mg				2.68	
	Avail'y				25.5	
Mg(Cl) ₂	Faecal Mg				2.63	
	Avail'y				28.0	

the means) of 30.6%. The good reproducibility of results between the four experiments using identical material gives some weight to the quite wide range of apparent availability results obtained for the other supplements in the various experiments.

Each apparent availability figure for one experiment refers to the mean of a specific number of values; 6 values for Experiment 1, and 12 values for Experiments 2, 3 and 4. In Experiment 5 the means were for 6 sheep. Thus the mean apparent availability figure in Table 12 refers to the mean of the mean apparent availability values. The standard error of the mean for dietary availability in Table 12 is 3.75 i.e. the least significant difference ($P = 0.05$) is 11.31.

As described earlier significant differences exist between the availabilities of the different calcined magnesites used within the different trials. However, although a wide difference exists between the mean of the mean apparent availabilities, no significant difference actually exists e.g. Greek 19.4%, Agma 27.8%, $Mg(OH)_2$ 30.6%. Considering the trend of the four trials together, of the calcined magnesites investigated the Agma appears to be of higher availability, the Navarras and Chinese of similar availability (22.4 and 23.5% respectively) with the Greek consistently of poorer availability.

An important objective of this thesis has been to assess the consistency in dietary availability of each of the four sources of granular calcined magnesite when each was obtained at similar times on the open market. Table 13 presents a summary of the data in Table 12.

Table 13. The coefficients of variation of the apparent mean dietary magnesium availabilities determined in Experiments 1 - 4. (42 sheep/product).

Product	Availability %	S. Dev.	S. Dev. as % mean. †
Hydroxide	30.6	3.57	11.7
Agma	27.8	8.21	29.5
Navarras	22.4	3.30	14.8
Chinese	23.5	10.01	42.5
Greek	19.4	9.54	49.5

† i.e. Coefficient of variation

The mean availability for the magnesium in magnesium hydroxide was the most consistent (c.v. = 11.7%). This is only to be expected as material from the same 25 kg bag was used in all experiments. The Navarras product was also very uniform from batch to batch (c.v. = 14.8%). This may be related to the facts that it originates from a single source and manufacture involves separation by flotation. This eliminates larger particles and this will be discussed in more detail later. Whilst the Navarras products appeared to be more uniform than the Agma products, the overall availability of the latter was higher at 27.8% with a c.v. of 29.5%.

In marked contrast both the Chinese and Greek products were very variable from batch to batch (c.v.s = 42.5 and 49.5% respectively). This may reflect diverse origins and/or product manufacture and specifications.

It can be seen from Table 12 that the apparent availability figures obtained in Experiment 1 are much higher than those found in the remaining three trials. The mean availability figure for the five major supplements (i.e. Agma, Navarras, Chinese, Greek and magnesium hydroxide) in Experiment 1 are significantly greater than those of Experiments 2 and 3 ($P < 0.05$). All four trials however were essentially similar, i.e. the same diets, housing, comparable sheep. There was, however, a particular circumstance specific to Experiment 1, i.e. the particularly cold conditions under which the trial was carried out. However, if indeed climatic conditions contributed to producing higher apparent availabilities this does not agree with findings by Martens & Rassiguier (1979) nor the general field experience that cold, wet and windy conditions often precipitate the onset of clinical tetany.

In Experiment 3 and 5 further magnesium sources were investigated for their apparent and comparative availabilities.

The Turkish calcined magnesite investigated in Experiment 3 produced a relatively higher apparent availability (faecal magnesium output was less than the Greek, $P < 0.05$) at 28.5% which compares well with the mean availabilities for the range of Agma products. However this result was based on only 12 values compared with 42 for the other calcined magnesites.

It is important to appreciate however that the samples of these products used in the trials may not be truly representative of the calcined magnesites from these particular countries. It is known that the Agma comes from one particular quarry and calcining plant and

likewise for Navarras. But the Grecian Magnesite SA company produces calcined magnesite from two different quarries and both the Turkish and Chinese calcined magnesite may come from any one of a number of different quarries.

A fine grade Chinese calcined magnesite was also investigated (Experiment 5). The apparent availability obtained (27.5%) differed little from a granular counterpart at 24.0%, or any other granular calcined magnesite investigated suggesting again that particle size has not been an issue to achieve better availabilities.

It might be assumed that a totally soluble source of magnesium would be potentially more available than an insoluble one with the advantage of being in solution and thus readily absorbed. However two contrasting and very soluble sources were investigated (Experiment 5) namely magnesium chloride solution and a hygroscopic dipeptide material, magnesium metalosate. They produced apparent availability values that suggested that they were not superior to the water insoluble calcined magnesites (28.0 and 25.5% respectively).

The effects of feeding increasing amounts of supplementary magnesium to sheep in cages was also investigated. This is obviously an important issue when it is impossible to monitor individual intake when minerals are available on a free access basis. The findings in terms of faecal consistency were in agreement with those of other workers (Gentry et al, 1978; Quillian et al, 1980). Sheep receiving 12 g magnesium as calcined magnesite (as used in Experiment 2) experienced severe diarrhoea. However it was interesting to note that the various calcined magnesites differed in their ability to cause diarrhoea.

The Agma product appeared to be the most effective with diarrhoea occurring within 3 days of supplementation at 12 g but the Chinese had absolutely no effect even after 14 days of supplementation. Similar dietary availability results were obtained at the different levels of supplementation for both the Agma and Chinese products.

It is very apparent from all the availability data presented here that a threshold to apparent availability appears to exist. The highest availability figures obtained did not surpass 40% and this would appear to be the maximum for all sources. It may be possible that other factors are involved in producing this threshold. The magnesium ions may be complexed within the rumen by other substances rendering them unavailable.

Fitt et al (1972) postulated that the intervention of the rumen

microbial population is responsible for reduced availability of magnesium owing to binding of magnesium by bacterial cells in the reticulum. The rumen micro-organisms utilise the magnesium for their own metabolic processes thus leading to a reduction in availability of dietary magnesium to the host animal.

It has also been reported that plant constituents, e.g. crude protein and potassium may affect the absorption of magnesium from the digestive tract of ruminants (Kemp et al, 1961). The apparent availability of magnesium decreases as the crude protein and potassium concentrations of herbage increase. However despite this phenomena it can be concluded from these trials that the source of the calcined magnesite is important in determining the availability of the magnesium.

In Section 3 a number of laboratory extraction tests are described using the same calcined magnesites in an attempt to correlate the apparent availability data obtained here with the in vitro findings.

(ii) Variations in the magnesium concentration of the products

Table 14 describes the mean magnesium concentration (g/kg) values for each consignment of the different calcined magnesites used in Experiments 1 - 4 and 5). Each value refers to a mean of at least four samples analysed during the course of each separate experiment.

Little difference exists between the different calcined magnesites in the percentage of magnesium in the product, varying between a mean of 49.5% (Greek) and 52.8% (Chinese). The single sample of Turkish granular material appeared to contain rather less and the single sample of Chinese powdered material rather more than the other products.

Between different consignments, the Navarras is the most consistent with the least variation in magnesium concentrations (c.v. = 2.1%). The Chinese product has the greatest variation between different consignments (c.v. = 8.0%) caused primarily by the fourth consignment which has a magnesium concentration of 583.4 g/kg compared to a mean of 509.1 g/kg for the remaining three.

Table 14. The mean concentrations (\pm standard deviations) of the magnesium contents of the four consignments of each calcined magnesite.

	Expt.		Mean	S.Dev.	S.Dev. as % of mean †
Agma	1	513.5			
	2	481.2			
	3	537.2			
	4	524.8	514.2	24.02	4.7
Navarras	1	506.2			
	2	523.4			
	3	531.3			
	4	516.1	519.3	10.68	2.1
Chinese	1	495.4			
	2	536.9			
	3	494.9			
	4	583.4	527.7	42.05	8.0
Greek	1	491.2			
	2	477.2			
	3	526.7			
	4	484.4	494.9	21.97	4.4
Mg(OH) ₂ ^x	1 - 4	404.4			
Turkish ^x	2	453.0			
Chinese x fine grade	5	582.4			

† Coefficient of variation

x Single source

(iii) Particle size distribution

Earlier work by Wilson (1981) has shown that particle size greatly influences the bioavailability of calcined magnesites; the finer the material the greater the availability, the coarser the material the poorer the availability. The results obtained in Experiment 1 however did not agree with these findings, but this may perhaps be explained by the different particle size parameters used. Despite the present findings it was decided to look more closely at the overall particle size distribution of each consignment to determine how consistent each product was and then attempt to correlate the apparent availability results obtained in Experiments 1, 2, 3 and 4 with specific particle size ranges.

Initial observation of the four major calcined magnesites appear to fit into two broad groups - the finer granuled Spanish magnesites and the coarser grained Chinese and Greek calcined magnesites.

Each consignment of the four different products was sieved into different particle size fractions. The sieves were in a vertical series and shaken in a regular manner via an electric motor. One kg of whole product was sieved out through a series of brass laboratory sieves (Endecotts Ltd, London) and repeated four times. The mean proportions, by weight, of particles within each size range were recorded and expressed as a percentage (Table 15). The results confirmed the initial observation that both the Chinese and Greek materials contained a greater proportion of larger particles than either of the Spanish products.

Neither the Agma or the Navarras products contained particles greater than 2 mm and the Navarras had no material greater than 1 mm. These findings were consistent for each of the four consignments. The Chinese and Greek materials on the other hand were not consistent from one consignment to another. The first two consignments of the Greek product contained approximately 50% by weight of particles greater than 1 mm including 15% above 2 mm. The third and fourth consignments however contained only approximately 30% by weight of material above 1mm and this included only 7% above 2 mm.

The fourth consignment of Chinese product was very distinctly different to the remaining three across the different particle size ranges. The first three Chinese products contained up to 48% of material above 1 mm and included up to 11% above 2 mm. The fourth

Table 16. Percentage of particles above 1 mm and under 150 mm diameter in association with determined apparent dietary magnesium availability.

Experiment		% over 1000 um	% under 150 um	% availability
AGMA	1	13	34	38.5
	2	8	28	18.5
	3	15	24	27.0
	4	8	43	27.0
NAVARRAS	1	0	11	18.0
	2	0	12	22.5
	3	0	22	26.0
	4	0	22	23.0
CHINESE	1	45	6	35.5
	2	43	10	23.5
	3	48	10	11.0
	4	25	4	24.0
GREEK	1	51	3	33.5
	2	48	2	16.0
	3	28	4	15.5
	4	34	8	12.5

consignment contained 25% between 1 and 2 mm with nothing above 2 mm. Both the Greek and Chinese materials contained generally 25-35% between 500 and 1000 μm diameter.

Conversely in the smaller particle size bands the Agma contained up to 34% material by weight which was less than 125 μm , Navarras 18%, Chinese 8% and the Greek up to 5% but as little as 2%.

Broadly speaking, the Agma products tend to contain material across the whole size ranges with nothing above 2 mm. The Navarras products on the other hand tend to contain much the greater proportion of material between 125-500 μm with little or nothing above this size range. It contains less material below 125 μm than Agma. The Chinese and Greek materials tend to contain at least 60% of material above 500 μm , and between 10 and 25% within the 250-500 μm range. The Chinese products have generally less than 5% below 75 μm diameter and less than 5% in the range 75-125 μm . Generally the fresh product has less than 3% below 125 μm diameter.

Of the four calcined magnesites, the Navarras appears to be the most consistent product in terms of particle size distribution across the four different consignments. This is probably due to the precise Flotation Process employed as a technique to separate out different particle sizes.

The Turkish is a further example of a calcined magnesite available in Britain. The Turkish material tends to resemble the Chinese and Greek products with over 60% of the particles, by weight, above 500 μm but with very little above 2 mm.

It appeared from the results presented in Table 15 that the four different calcined magnesites differed most in their proportion of material at both ends of the particle size ranges. Thus attempts were made to correlate the apparent availability results obtained for each consignment with (a) the percentage of material over 1 mm and (b) the percentage below 150 μm (Table 16). In both instances however, no relationship existed (Figures 3 and 4) as evidenced by the lack of significant regressions.

Other workers have investigated the effect of particle size on the availability of magnesium reporting similar findings to that of Wilson (1981) who investigated a Spanish Agma calcined magnesite purchased in 1979. The Agma was separated into 5 different particle size ranges (μm): A, under 75 (6% of whole product); B, 75-150 (21%); C, 150-250 (26%); D, 250-500 (31%); E, 500-1000 (16%). (Up until 1980 Agma whole

Figure 3 Regression between apparent availability (%) and % of particles over 1000 um in various calcined magnesites.

$$y = 23.9 - 0.029 x, r = 0.07, \text{ NS.}$$

(A,B,C,D, denote different consignments of magnesites;

● - Agma, ■ - Navarras, ▲ - Chinese and ◆ - Greek)

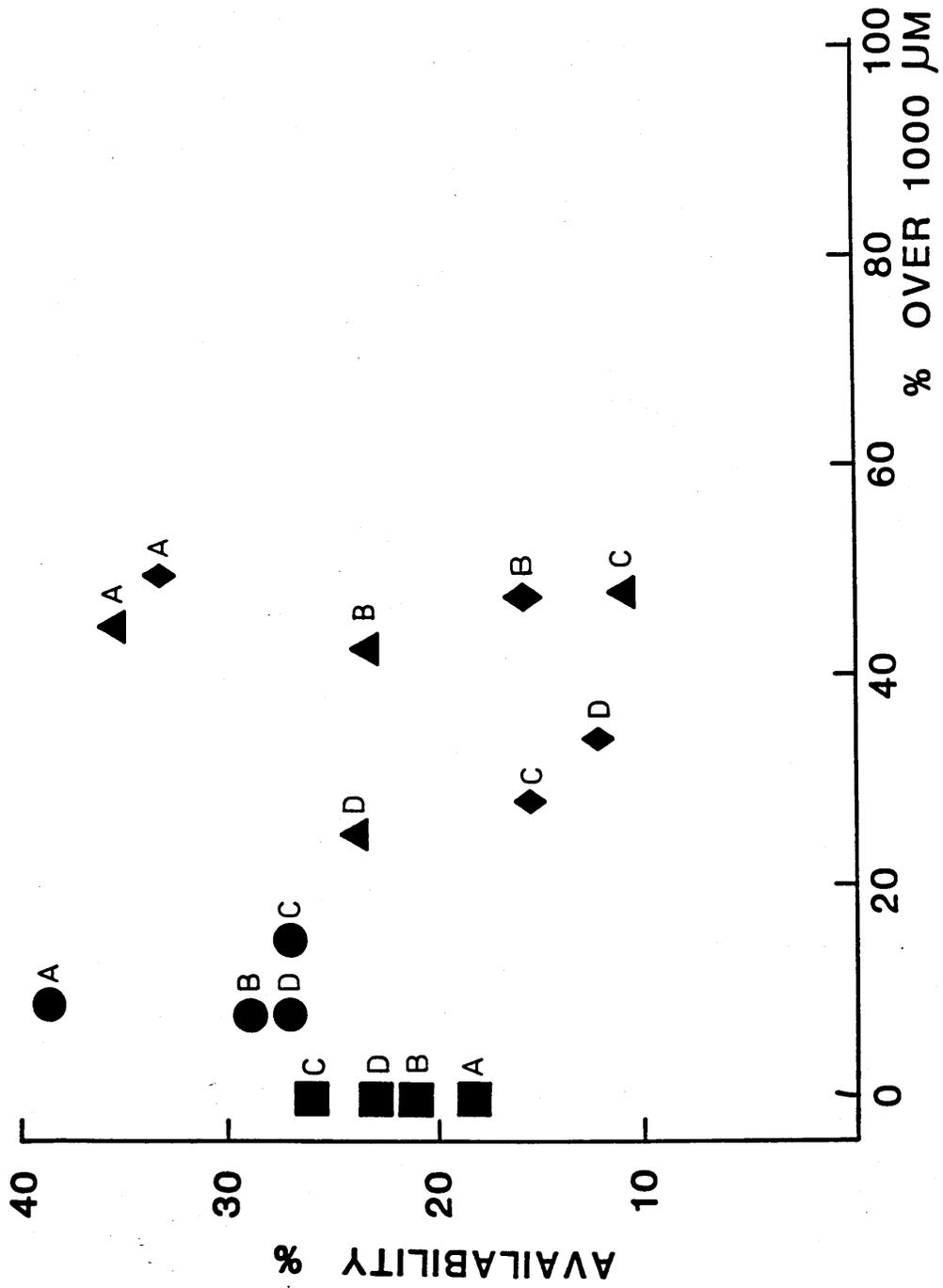
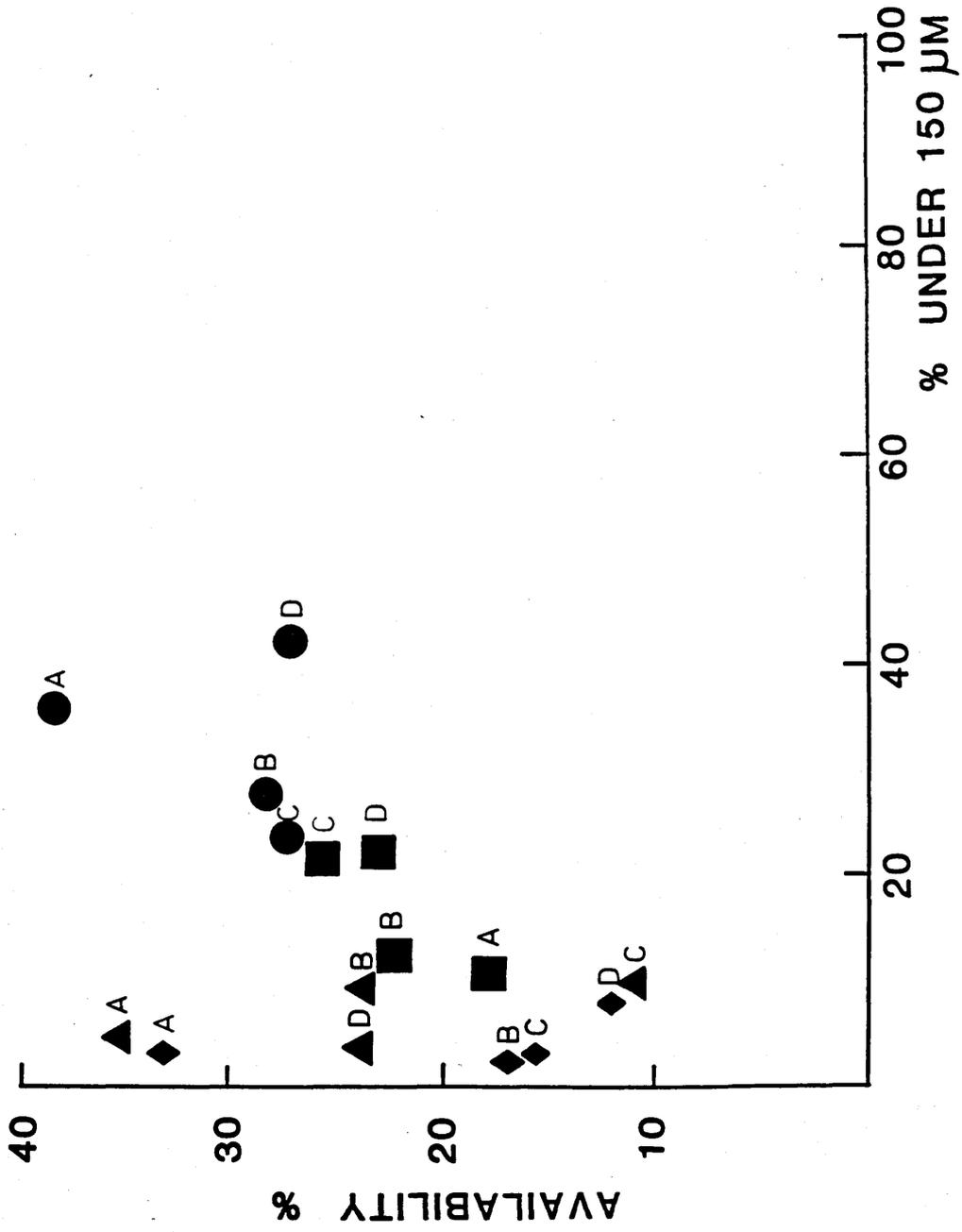


Figure 4 Regression between apparent availability (%) and % of particles below 150 um in various calcined magnesites.

$$y = 20.2 + 0.213 x, r = 0.33, \text{ NS.}$$



product contained no material above 1 mm). A significant negative relationship existed between availability and increasing particle size. Jesse et al, (1981) also investigated the magnesium availability from magnesium oxide particles of differing sizes. However, the particle size ranges investigated included a large degree of overlap in particle size. The fraction containing the finest material (75-850 μm) produced significant increases in urinary magnesium excretion when compared to the 425-1700 μm and 150-600 μm size ranges.

Xin et al, (1989) investigating availability of magnesium oxide with fistulated Holstein cows, also on the basis of urinary excretion of magnesium, reported that magnesium oxide sources (of American origin) with smaller particles (238 μm) are more available to cattle than larger particles (324 and 426 μm).

(iv) Determination of surface area using the BET technique

Introduction

Consideration regarding the apparent differences in the bioavailability of various calcined magnesites investigated in Experiments 1, 2, 3 and 4, led to the hypothesis that the surface area of the calcined magnesite particles may be important. In a mixture of particles of different sizes, the small particles contribute a greater share of surface area and thus would be expected to be more reactive on a weight basis. The calcined magnesites investigated differed greatly in the proportion of different particle size fractions (see Table 15). Both Agma and Navarras have a markedly higher proportion of finer particles in the whole product than either the Chinese or Greek product, and thus would be expected to have a higher surface area on a weight basis.

Surface area is a measurement of both the external and the internal area where cracks and pores within the particle open out onto the surface. The Agma product has often been promoted as being a material that is "very porous with a honey-comb type structure". Thus it might follow that Agma should have a comparably higher surface area than the other products.

Four different batches of the four main calcined magnesites and the standard magnesium hydroxide were analysed for surface area. The surface area measurements were carried out by ICI (Chemicals and Polymers Group) and Boliden-Kemi (Sweden).

The BET technique is based on adsorption of liquid nitrogen (or alternatively krypton or argon). The surface area of a material is the area of a solid on a molecular scale which is exposed to a liquid or gas. The BET technique measurement includes open pores and cracks within the particles.

Materials and Methods

The sample is first heated and evacuated to free the solid from gases and vapours that are acquired from exposure to the atmosphere. The solid is then cooled to a constant temperature, at or near the boiling point of the adsorbate gas, and evacuated. The solid is then exposed to the adsorbate gas in a series of controlled pressure

Table 17. Surface areas of various calcined magnesites determined using the BET technique (m^2/g).

Experiment	1	2	3	4
Calcined magnesite				
AGMA	2	3	2	2
NAVARROS	1.5	1	2	2
CHINESE	18	14	15	16
GREEK	25	8	20	19
Mg(OH) ₂	22	-	-	-

increases. The equilibrium quantities of gas adsorbed are determined for each pressure. The gas volume adsorbed at each pressure defines an isotherm from which it is possible to determine the quantity of gas needed to cover the solid surface both externally and internally. The area of the solid on a molecular scale is calculated using the projected area of each adsorbed gas molecule. The surface area is described in terms of metres squared per gram of material.

Results and Discussion

The surface area measurements are detailed in Table 17.

The surface area values for the different products were very consistent from batch to batch (except for the Greek product from Experiment 2 which has a surface area of 8 m²/g compared to 25, 20 and 19 m²/g for the other three batches).

A very significant difference in size of surface area exists between the Agma and Navarras products on the one hand and the Chinese and Greek materials on the other. The surface areas of the latter are approximately up to ten times the size of the Agma and Navarras products. The surface area of magnesium hydroxide (only one value) was comparable to that of the Chinese and Greek materials.

These results might suggest that the Agma product is not as porous as has been claimed, or that the "honey-comb structure" that may exist internally does not actually open out onto the external surface and therefore be included within the measurements. On the other hand, if these physical characteristics do exist within the Agma particles, they may be present to a greater extent within the Chinese and Greek particles and hence the higher surface areas. The theory that a higher proportion of fine particles, on a weight basis, results in a greater surface area does not express itself in these results. The magnesium hydroxide which is a fine powdered material (all the particles below 175 µm) has a surface area which is similar to the Greek product which typically contains 78% of particles greater than 500 µm.

As part of the electronmicroscopy study into the properties of the calcined magnesites (see Section 6) Dr S. Solomon (Anatomy Department, Glasgow University Veterinary School) carried out a series of thermal gravimetric analyses on the products and LOI was also carried out. Both the Greek and Chinese products suffered weight loss due to the presence of carbonates and hydroxides which suggests that the Agma and Navarras

have been better calcined to give a cleaner magnesium oxide product. Dr Solomon (personal communications, 1989) postulated that the presence of these impurity compounds may account for the greater surface areas found within the Chinese and Greek materials.

This however does not agree with findings by Dr G. Langestrom (Boliden-Kemi, Sweden) who in working with silicates found that an increase in temperature results in increased surface areas due to the breakdown of molecular bonds within particles, thus creating fissures (personal communication, 1989).

Kimyongur and Scott (1986) investigated the calcination characteristics of a magnesite of Turkish origin in the production of a high grade chemically pure product, and a medium grade calcined product for animal feed use. Their findings included evidence to suggest that if a medium quality magnesite was subjected to an increase in temperature beyond 800°C this was likely to lead to a deterioration in surface area (i.e. reduced surface area) and hence product quality.

Comparison between surface area and comparable bioavailability values finds no correlation between increased surface area and reactivity in terms of availability to the animal. Excluding the hydroxide and taking only the calcined magnesites into account, a higher bioavailability value appears to be correlated (not significantly) to a smaller surface area.

SECTION 2

**FURTHER AVAILABILITY COMPARISONS OF CALCINED MAGNESITES USING
TECHNIQUES TO REDUCE THE TIME-COST OF EXPERIMENTATION**

**FURTHER AVAILABILITY COMPARISONS OF CALCINED MAGNESITES USING
TECHNIQUES TO REDUCE THE TIME-COST OF EXPERIMENTATION**

INTRODUCTION

The normal method employed for balance trials is to maintain individual sheep in metabolism cages as described in Experiments 1, 2, 3 and 4. This is a costly procedure both in terms of equipment but especially labour and length of experimental time. Each feeding period requires a 7 day run-in period and then 7 days collection/recording for each determination for each sheep. It is not possible to reduce this time requirement. Accurate collections of both faeces and urine are time consuming. However it is only necessary to collect the faeces when determining digestible energy, digestible crude protein or assessing comparative amounts of magnesium in faeces.

Maintenance of sheep in metabolism cages is a controversial subject in terms of animal welfare. The author's views on this subject are mentioned in the Discussion at the end of this section.

A possible alternative to metabolism cages has been investigated. The faecal marker technique has been extensively used in nutritional studies as a relatively accurate alternative method to the standard balance trial techniques. It avoids the complete collection of faeces and only adequate grab samples are required. Chromic oxide is perhaps the most well known inert faecal marker giving recoveries of 100% in many experiments with ruminants (e.g. Kane et al, 1950; Smith & Reid, 1955; Bloom et al, 1959; Macrae & Armstrong, 1969; Wilson, 1981). Sheep are maintained in individual pens on slats (complying to Welfare Codes laid down by the Agricultural Act, 1968) and the chromic oxide is added to the feed as an indigestible faecal marker. Two experiments will be described, one (Experiment 7) where faecal samples are obtained through grab sampling, the other (Experiment 8) where a total days output is collected in a faecal bag. Both techniques reduce the time period involved for the experiments. It thus seemed useful to compare these two techniques with the standard balance trial involving sheep in cages, with regard to both experimental reliability and the experimental time involved (Experiment 9).

EXPERIMENT 7

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

Introduction

The aim of this experiment was to produce more availability data using chromic oxide in the feed as an inert faecal marker. The faecal samples were obtained by grab-sampling. Experiment 3, with other sheep in cages, was carried out simultaneously and the sheep received identical supplements and feedstuffs for comparative purposes.

Materials and Methods

Two separate groups of sheep were used in this trial; 12 wether lambs of initial liveweight 42.3 kg and 12 older wethers of initial liveweight 69.1 kg. All the sheep were maintained in individual pens (1 m x 2 m) on slatted floors.

The lambs received a basal diet of 0.8 kg dried grass, 0.1 kg barley and 0.1 kg barley/chromic oxide cube, providing 1.73 g magnesium, 60.2 g DCP and 8.1 MJ ME. The older wethers received an increased allocation of the same basal diet of 1.2 kg dried grass, 0.15 kg barley and 0.15 kg barley/chromic oxide cube, providing 2.60 g magnesium, 90.3 g DCP and 12.2 MJ ME. Water was available ad lib.

The trial was conducted as two separate but identical sets of two balanced 6 x 3 randomised blocks. The six treatments were Agma, Navarras, Chinese and Greek calcined magnesites, magnesium hydroxide and a nil treatment, identical products to those used in Experiment 3 which was itself conducted at the same time.

The supplements were added to the morning feed so as to provide 2g supplemental magnesium for the lambs and 3 g supplemental magnesium for the older wethers. The feedstuffs and supplements were increased for the older wethers in such a way that the chromium:magnesium ratio was identical for both groups of sheep.

The mean magnesium concentrations of the supplements and the mean composition of the feedstuffs are identical to those described in Experiment 3.

Each experimental period consisted of a 7 day run-in period, and 3

grab samples per day were taken on day 8 and 9 at exactly the same times each day to reduce potential errors from diurnal variation in chromium excretion.

The three grab samples of faeces were amalgamated, dried, ground and analysed for magnesium and chromium. The availability of the magnesium was calculated thus:

$$\text{Faecal DM output (g)} = \frac{\text{Total chromium fed (g)}}{\text{Chromium concentration in faeces (g/kg)}}$$

$$\text{Total magnesium in faeces (g)} = \text{Faecal DM output (g)} \times \text{magnesium concentration in faeces (g/kg)}$$

$$\text{Apparent availability} = \frac{\text{Suppl. Mg intake} - \text{faecal Mg derived from suppl.}}{\text{supplement Mg intake}}$$

Results

All feedstuffs and supplements were well consumed throughout the trial. The total chromium intake from the feed for the lambs and wethers was 0.117 g and 0.1676 g respectively.

The mean daily faecal dry matter output calculated for the lambs (n = 36) using the chromium as a faecal marker was 365 g (40.4). This compares with comparable lambs in cages, fed identical materials (Experiment 3) with total faecal collections, of 373 g (13.6) (n = 72). With such results it was considered justifiable to use the calculated faecal DM outputs to assess total faecal magnesium outputs and hence the dietary availability of the products.

Table 18 describes the availability data for both lambs and wethers together with the comparable values obtained in Experiment 3 using 7 day total faeces collections from sheep in cages.

From the availability results, obtained using the chromium marker, a similar trend in the results would seem to appear, i.e magnesium hydroxide was the most available with the Chinese and Greek products the least. However when comparing the actual values between those obtained in Experiment 3 and those obtained here, large differences in magnitude occur, especially for the Chinese and Greek materials, e.g Chinese, 11.0% (Experiment 3) with 17.5 and 20.7%; Greek 15.5 (Experiment 3) with 19.5 and 20.3%. This may be due in part to the size

Table 18. Experiment 7. Mean daily outputs in faeces of magnesium (g/day) and apparent availabilities (8) (4 sheep/treatment) calculated using chromium marker technique.

	Lambs supplemented with 2 g Mg		Wethers supplemented with 3 g Mg	
	Faeces	Apparent Availability	Faeces	Apparent Availability
Nil	A 1.09	-	1.76	-
Mg hydroxide	B 2.26	41.5	3.74	34.0
Agma	C 2.59	25.0	3.85	30.3
Navarras	D 2.64	22.5	3.90	28.7
Chinese	E 2.74	17.5	4.14	20.7
Greek	F 2.70	19.5	4.15	20.3
SEM	0.12		0.167	
Significance				
	P < 0.001 A < B,C,D,E,F		P < 0.001 A < B,C,D,E,F	
	P < 0.05 E > B			

of the particles in the Greek and Chinese materials. As will also be mentioned in Experiment 8, Wilson (1981) actually recovered the larger particles of calcined magnesite from faecal samples. Thus when the small faecal grab samples are taken the chances are that they may contain a more variable number of large particles, as opposed to small, of calcined magnesite, or vice versa, i.e. the quite small grab samples totalling about 55 g DM are not adequately representative of the total faecal output and will thus give aberrant availability results. In order to reduce this potential error it is suggested that a 24 hour collection of faeces, by means of a faecal collection bag may be more representative of faecal output. Such an approach is described in Experiment 8.

EXPERIMENT 8

Further availability comparisons of calcined magnesites using a modified faecal marker technique for individually fed sheep

Introduction

In Experiment 7 apparent availability data was calculated from faecal samples obtained by grab sampling. It was considered however, that the differences that existed between availability data obtained by this method and those obtained in Experiment 3 using sheep in cages, may have been due to diurnal variation in excretion patterns and particle excretion in the faeces. Thus it was considered that grab samples were not representative of the total excreta. Hence the aim of this experiment was to modify the technique by obtaining one whole days output (more or less) collected in a faecal bag. Experiment 4 was carried out simultaneously with other sheep in cages receiving identical supplements and feedstuffs.

Unfortunately, however, on analysis during the course of the experiment the chromic oxide cube (consisting of bruised barley with a known added quantity of chromic oxide also received in Experiment 4) which was given to supply the faecal marker in what was hoped to be consistent even quantities, proved to contain a wide variation in chromium content between different samples of the cube. Thus if the sheep were receiving an unknown amount of chromium it would be impossible to calculate accurate faecal dry matter outputs and thus faecal magnesium outputs. Hence an alternative faecal marker to chromium, present in the diet had to be found.

The ARC (1980) have concluded that the coefficient of absorption for copper by cattle was 0.04, i.e. 96% of the dietary input of copper is recovered in the faeces. McEleney (1985) in trials with cows receiving constant inputs of hay and compound feed achieved relatively low coefficients of variation for mean faecal copper concentrations in bulked grab samples of faeces (5.5 - 7.5% respectively) suggesting fairly uniform excretion of copper.

To test the efficacy and accuracy of using copper as an indigestible faecal marker the feedstuffs and faeces from Experiment 4 were analysed for total copper in order to calculate the percentage recovery. From a total of 72 faecal samples of the faeces collected

quantitatively over 7 days a mean 98.5% recovery of copper was obtained. As a result it was decided to use copper as a suitable alternative marker to chromium.

Materials and Methods

Twenty wether sheep, principally Suffolk x Greyface, mean 48 kg liveweight, were maintained in individual pens (2 m x 1 m) with wooden slatted floors. Each sheep received 1.00 kg dried grass nuts, 100 g barley and 100 g chromic oxide/barley cube. (The chromic oxide had been added to the feed to be used as an inert faecal marker, but as was described earlier, the copper content of the feed was used instead). The diet provided 72 g DCP, 9.7 MJ ME and 1.73 g magnesium, and was divided into two approximately equal feeds at about 07.30 and 16.00 h. Water was available ad lib.

Each experimental period was 8 days long of which 7 days was allocated to a run-in period and the total faecal output was collected on day 8. A faecal bag composed of a coarse heavy nylon mesh material (approximately 3 mm) was securely attached to the rear of the sheep by means of a harness, as earlier described for use in metabolism cages. This type of faecal collection bag was used in preference to the rigid rubber bag used with sheep in cages, in order to reduce potential spillage of faeces in a pen where the animal has freedom to move. It could also be used if need be, for female sheep. The bag remained on the sheep for 24 hours to allow a good, but not necessarily complete faecal output. It was assumed that 100% of the feed intake was excreted in a regular manner and some minor spillage of faeces from the bags was not critical. However faeces collection via bags was considered better than grab samples for obtaining samples of reasonable size. The whole faecal sample representing substantially one whole days output was dried, ground then subsampled and analysed for magnesium and copper.

Eighteen of the twenty sheep were involved in a 6 x 3 incomplete Latin Square experimental design. The six treatments were magnesium supplementation to provide 2.0 g magnesium per day in the form of Agma, Navarras, Chinese and Greek calcined magnesites, magnesium hydroxide and a nil treatment, and were identical to the treatments used in Experiment 4. The two remaining sheep in turn received 2.0 g magnesium per day in the form of magnesium chloride, a fine grade Chinese calcined magnesite and magnesium metalosate (Thompson & Joseph Ltd.)

i.e. as given in Experiment 5.

Results

The feed and supplements were well consumed throughout the trial. The feed bucket was of adequate size to prevent spillage of feedstuffs through the slats. Careful observation suggested that very little if any faeces was not collected in the nylon bag.

The total copper intake from the feed was 10.31 mg./day. The faecal concentration was used to determine faecal dry matter outputs and thus total faecal magnesium and hence magnesium availability. The dry matter output was calculated thus:

$$\text{Faeces dry matter (g)} = \text{DM input (g)} \times \frac{\text{Total Cu input (g)}}{\text{Faecal Cu (g/kg)}}$$

The mean daily faecal dry matter output for the whole trial, calculated thus was 456 g (36.6 g) (n = 54). This compares extremely well with the mean faecal dry matter output for the whole of Experiment 4, which was 442 g (19.2 g) (n = 72) and on this basis it was considered justified to use the results to calculate the total daily output of magnesium in the faeces and hence the apparent magnesium availability.

The availability data are presented in Table 19 together with the comparable values obtained in Experiment 4 using sheep in cages.

The difficulty experienced in producing a chromic oxide cube to supply a consistent amount of chromium was unexpected since every action was taken to ensure that the chromic oxide was carefully mixed with the barley before being placed in the mixer and thence the cuber. It is possible that the light fine powdered chromic oxide separated out during the mixing process.

The apparent availability results obtained using copper as a faecal marker (Table 19) were very close to those values obtained in the balance trial, except for the Greek product, i.e. 29.8% and 12.6% respectively. The magnesium hydroxide again produced a similar superior apparent availability to that obtained in previous balance trials. The availability figure obtained for the Greek product was somewhat surprising since it had consistently produced poor availability results with sheep in cages (Experiments 2, 3, and 4).

Table 19. Experiment 8. Dietary magnesium availability of supplements as determined by the faecal marker technique (mean, 9 sheep/treatment).

Supplement	Apparent availability %	Experiment 4 Balance Trial availability %
Mg(OH) ₂	36.5	32.9
Agma	22.7	27.1
Navarros	24.0	23.2
Chinese	24.7	24.1
Greek	29.8	12.6
Fine grade Chinese +	35.0	27.7
Mg(Cl) ₂ +	33.0	28.0
Mg metalosate +	21.5	25.8

+ 2 Sheep per treatment only.

However this disparity between results may be due to particle size. The Greek (and Chinese) material contain large particles of calcined magnesite which Wilson (1981) has actually identified within faecal samples. Thus passage of particles, possibly with diurnal variation in excretion could affect magnesium contents of specific faecal samples. It is thus possible that with a faeces collection of only one day that representative amounts of undigested magnesium from the particularly large particles of calcined magnesite were not present in the subsample taken for analysis.

Such potential errors are reduced with total faecal collections, as with sheep in cages.

It would be interesting to investigate these supplements further using this technique to elucidate the possible problems.

The method employed in this experiment to use a faecal bag to collect the total output (more or less) for 24 hours was considered to provide a more accurate method of faecal sampling than a series of 'grab samples' as in Experiment 7. A problem associated with faecal collections in such instances to estimate total faecal output is the procurement of faecal samples that are truly representative of the whole excreta. It cannot be certain that diurnal changes in composition occur. Obviously the major advantage is that it removes the time-consuming necessity of undertaking total faecal collections as with sheep in cages. (see Time-Cost Study). However, the existence of a diurnal excretion pattern of chromic oxide in the faeces has been recognised by a number of workers (Kane et al, 1952; Smith & Reid, 1955; Bloom et al, 1959; Wilkinson & Prescott, 1970). This may be attributable to a variety of factors including daily dosing pattern and physical nature of the diet. It is therefore considered that a single grab sample is not representative of the total excreta and thus a number of grab samples would be taken at the same time of day to minimise possible error due to diurnal excretion patterns (Experiment 7).

It was considered that a total collection (more or less) for 24 hours reduced the potential error even further and obviously reduces the overall time required to obtain availability data.

EXPERIMENT 9

A time-cost study of the routine procedures involved in nutritional balance trials

Introduction

The aim of this experiment is to produce an in-depth detail of the routine procedures involved in different nutritional balance trials. Experiment 4, describe earlier is discussed as an example of a standard balance trial; Experiments 7 and 8 are used to illustrate two different techniques to obtain faecal samples whilst reducing experimental time. The time-costs for each trial are detailed together with the relative accuracies of each method.

Experimental

In order to compare the time cost for different methods of balance studies, detailed records were kept of the time involved in each procedure for Experiments 4, 7 and 8. Each of these experiments were concerned with the bioavailability of different magnesium supplements.

Experiment 4 involved 24 sheep, maintained in metabolism cages, for 3 periods of 14 days. The techniques employed are described under 'Balance Trial Techniques'. A simultaneous trial (Experiment 7) was carried out with a further 24 sheep maintained in individual pens (2 m x 1 m) on slats in an identical 6 x 3 incomplete Latin Square Design. They received the same diets as described in Experiment 4. After 7 days run-in, grab samples of faeces were taken 3 times a day (07.30 h, 12.00 h and 16.00 h) on days 8 and 9, amalgamated, dried and ground. In Experiment 8 an additional 20 wether sheep were maintained on slats as for Experiment 7. After a 7 day run-in period a faecal bag was attached to the rear of the sheep by means of a harness. Thus a good, but not necessarily complete proportion of the faecal output on day 8 was collected and dried.

In Experiments 7 and 8 it was assumed that 100% of the feed intake was excreted in a regular manner. A 98.5% recovery of chromic oxide had been achieved in Experiment 2. The faecal chromium concentration was assessed and thus an estimate of the faecal DM output/day was made. When combined with the magnesium concentration in the faeces it was

possible to calculate the total faecal magnesium output/day. Obviously, unlike Experiment 4 it was not possible to estimate urinary magnesium output for the sheep maintained on the slats.

Detailed records were kept of the time involved in each procedure from the time of the initial weighing of food and supplements to having the faeces ground and ready for analyses. An additional record was made for the time involved for urine collection in Experiment 4.

Results

Tables 20, 21 and 22 give detailed records of the time taken for individual procedures in the three separate experiments.

Discussion

Many procedures were common to all three experiments described, e.g. the time taken to weigh out feed and magnesium supplements, the daily routine of putting the feed in feed buckets, replenishing water bowls etc. The only way to reduce time for weighing out feed would be to reduce animal numbers, and obviously the time taken for feeding and watering will be affected by the convenience of pen lay-out.

In Experiment 4 with the sheep in metabolism cages (Table 20) the total time/sheep was 30.9 minutes for the 7 day run-in period and 51.3 for the 7 day collection period. The latter includes 14.8 minutes for faeces and urine sub-sampling, drying and preparation for analysis giving a total of 82.2 minutes/sheep in the experiment.

In Experiment 7 where samples of faeces were obtained by grab sampling the elimination of the daily tasks of emptying faecal bags and washing down cages reduced the time/sheep for the 7 day run-in period to 26.5 minutes (Table 21). Feeding and watering took 13.9 minutes compared with 9.0 for the sheep in cages (Experiment 4) but this was partly due to the less convenient lay-out of individual pens on the slats.

The time required per sheep for the two days faeces grab sampling and subsequent grinding of faeces samples was 30.0 minutes. Grab sampling of faeces (amounting to a total of 15.0 minutes for the 2 days) was surprisingly time-consuming when compared to the 14.1 minutes in Experiment 4 to collect both the faeces and urine for a total of 7 days. The total time per sheep for the 9 day period was 56.5 minutes.

Table 20. The time taken (mins) in the separate procedures of a 14-day nutritional balance trial with 24 sheep (Expt. 4)

A. Run-in period - 7 days.	7 day total for 1 sheep	% of total time
1. Provision of food and water		
i. Feeding, filling water buckets	6.7	22
ii Lay-out of feed and supplement for following day (pm)	2.3	7
2. Faeces Collection		
Remove, empty and replace faecal bag; wash down cages; discard faeces	8.5	28
3. Wash down metabolism house	1.5	5
4. Weighing of food intake for 7 days		
a) grass nuts	6.3	20
b) supplement	5.6	18
Total (7 -days)	30.9 minutes	100
B. Collection period - 7 days		
1. Provision of food and water		
i Feeding, filling water buckets	6.7	13
ii Layout of feed and supplement for following day (pm)	2.3	4
2. Faeces and Urine collection		
Remove, empty and replace faecal bag; wash down cages. Remove, empty, replace urine collection vessel; replace glass wool filter, add acid to jug.	14.1	28
3. Wash down metabolism house	1.5	3

4. Weighing of food intake for 7 days		
a) grass nuts	6.3	12
b) supplement	5.6	11
5. Faecal and Urine subsampling		
i Weigh and label sample drying trays for faeces, and pots for urine	1.3	
ii Subsample urine	1.5	
iii Subsample faeces	2.1	
iv Wash receptacles for faeces and urine	3.3	
v Weigh trays and faeces samples before and after drying	1.2	
vi Grinding, labelling dried faecal and feed samples	4.4	
vii Wash sample drying trays	1.0	
	sub-total	14.8 29
Total (7 - days)	51.3 minutes	100

Table 21. The time taken (minutes) in the separate procedures of a 9-day nutritional trial using the faecal grab sampling technique with 24 sheep (Experiment 7).

A. Run-in period - 7 days	7 day total per sheep	% of total time
1. Provision of feed and water		
i Feeding, filling water buckets	11.2	43
ii Layout of feed and supplement for following day (pm)	2.7	10
2. Brush down slats	0.9	3
3. Weighing of feed intake for 7 days		
a) grass nuts, Cr cubes	6.3	24
b) supplement	5.4	20
Total (7 days)	26.5 minutes	100
B. Collection period - 2 days	2 day total per sheep	% of total time
1. Provision of food and water		
i Feeding, filling water buckets	3.2	10
ii Layout of feed and supplement for following day (pm)	0.8	3
2. Faeces Collection		
i Grab sample - each sheep 3 x day	15.0	
ii Weigh and label sample drying trays for faeces	0.8	
iii Weigh tray and faeces sample before and after drying	1.2	
iv Grinding and labelling dried faecal and feed samples	4.4	
v Wash sample drying trays	1.0	

	sub total	22.4	75
3. Brush down slats		0.3	1
4. Weighing of food intake for 2 days			
a) grass nuts, Cr cubes		1.8	6
b) supplement		1.5	5
Total (2 days)		30.0 minutes	100

Table 22. The time taken (minutes) in the separate procedures of an 8 day nutritional trial using 20 sheep on slats fitted with a string harness and faecal bag for a 24 hour faecal collection. (Experiment 8).

A. Run-in period - 7 days	7 day total	% of total time
1. Provision of food and water		
i Feeding and filling water buckets	11.2	42
ii Layout of feed and supplement for following day	2.7	10
2. Brush down slats	1.0	4
3. Weighing of feed intake for 7 days		
a) grass nuts, Cr cubes	6.3	24
b) supplement	5.4	20
Total (7 days)	26.7 minutes	100
B. Collection period - 1 day		
1. Provision of food and water		
i Feeding and filling water buckets	1.6	6
ii Layout of feed and supplement for following day	0.4	2
2. Brush down slats	0.2	0.5
3. Faeces collection		
i Fitting harness and bag to sheep	9.0	
ii Removing bag and harness from sheep	5.0	
iii Clear up bags, string etc.	0.5	
iv Weigh and label drying trays for faeces	0.9	
v Weigh tray and faecal samples before and after drying	1.2	

vi Grind and label dried faecal and feed samples	4.4	
vii Wash sample drying trays	1.0	
Sub total	24.1	85
4. Weighing of food intake for 1 day		
a) grass nuts, Cr cubes	0.9	3.5
b) supplement	0.8	3
Total (1 day)	25.8 minutes	100

Table 23. Comparative times (mins/sheep) to perform the various principle experimental tasks using three different experimental procedures.

Experimental Period	7 day run-in 7 day collection		7 day run-in 2 day collection		7 day run-in 2 day collection	
	mins	%	mins	%	mins	%
Weighing of feed and supplement	23.8	33.3	15.0	26.6	13.4	25.5
Putting out feed/water Brush down	21.0	29.4	19.1	33.8	17.1	32.6
Faeces collection	15.5	21.7	15.0	26.5	14.5	27.6
Faeces sampling/drying	11.1*	15.6	7.4	13.1	7.5	14.3
Total	71.4	100	56.5	100	52.5	100
Plus for urine Collection, washing etc	7.0					
Sampling etc	3.7					
Total	82.1					

* Includes determination of dry matter

Table 24. Comparative time-costs for three methods of experimentation.

Experiment	3 periods mins/sheep	Days on experiment	Mins. work per sheep per day
4. 7 days run-in, 7 days collection	246.6	42	5.8
7. 7 days run-in, 2 days grab sample	169.5	27	6.2
8. 7 day run-in, 1 day collection	157.5	24	6.5

In Experiment 8 where faeces were collected in a bag for one day (Table 22) the time taken per sheep for the 7 day run-in period was 26.7 minutes which is very similar to the 26.5 minutes taken in Experiment 7. The time involved for faeces collection was 25.8 minutes of which 14.5 minutes/sheep was associated with the attachment and removal of the harness and faeces collection bag. The total time per sheep for the 8 day period was 52.5 minutes, some 4 minutes less than for Experiment 7 which took one day longer.

The principle time components for the three experiments can be summarised in Table 23.

For all three methods the weighing of the feed and supplements and the presentation to the animals of feed and water took approximately 60% of the total time. Obviously the weighing out of the materials took longer for Experiment 4 since the time periods was 14 days compared to 8 and 9 days.

The surprising factor was that for each experiment about 15 minutes per sheep was involved in faeces collection even though Experiment 4 took 14 days, Experiment 7 took 9 days and Experiment 8 took 8 days per feeding period.

The total time of 82.1 minutes for Experiment 4 included 11.7 minutes per sheep for collection of urine which provides additional information. For a typical experiment which might involve 3 feeding periods the times involved would be as shown in Table 24 (Experiment 4 includes time allowed for urine collection and is also the only one where determination of the dry matter content of the faeces is an essential part of the total experimental procedure).

These times do not include the time necessary to erect pens on the slats, to thoroughly clean cages after use, to put harnesses and bags onto sheep (Experiment 4), to put sheep into pens and cages and to repair equipment and the prior acclimatisation of sheep in cages. For Experiments 7 and 8 there is the additional time and cost of chromium and/or copper analyses and the absence of information on urine composition. All three techniques involve a time of about 6 minutes work per sheep per day (Table 24).

Nevertheless the saving in time in Experiments 7 and 8 especially in the total days of experiments is considerable. Additionally whilst they involve much less time per day for the 7 days run-in they involve very much more on day 8 (and 9). There may however be some loss in accuracy which might be compensated for by additional replication in

Experiments 7 and 8 and these aspects are discussed with the results of Experiment 7 and 8.

Both Fishwick (1973) and Taylor (1988) have carried out time-cost studies for procedures employed during balance studies with animals in cages.

In observations carried out by Fishwick (1973) with sheep in cages the single most time consuming item involving 33% of the total time was that involved in the daily routine of removing and replacing the faecal bag, washing down cages and changing the urine collection vessel. Measurement and subsampling of the daily faeces and urine outputs took 29.4% of the time and the once weekly weighing of feed took 24.4% of the total.

Similarly Taylor (1988) in a time-cost of procedures during the continuous intravenous infusion of beta-agonists into cattle maintained in metabolism stalls found that emptying faecal collection bags took the proportionately greatest amount of time at 28.5% of the total. This increased to 39.2% when including the time taken to wash down cages and empty urine receptacles.

It is the present author's personal view that the maintenance of sheep in metabolism cages for the purpose of balance studies is both an efficient and accurate method. It allows for the collection of urine and faeces, and any spillage of feed or faeces is easily recovered. The whole procedure can be managed in a more generally efficient manner, e.g. the rare spillages of feed and faeces can be better observed.

From a welfare aspect the animals settle quickly and become very calm to handle after just a day or two. Thus attachment and removal of the faecal bag becomes a quick and simple process. It is the author's view that the animals are not adversely affected by confinement in cages as may be manifested by abnormal behavioural patterns.

Having had experience of trials with sheep in cages for both 12 and 6 week periods the author believes that it is only after about 6 weeks confinement that the animals become unsettled and start chewing the bars of the cages and restraining chains, practices that may be considered to constitute abnormal behaviour and perhaps reflects nothing worse than boredom. Such behaviour is readily copied by other sheep. It does not seem to affect experimental results.

Sheep left in individual pens on slats are not, in practice, easy to handle. The pens need to be secure and well designed and a convenient layout is not always possible. At feeding times considerable

excitement occurs. The handling of individual sheep to obtain grab samples of faeces is not always easy and straightforward and faeces are not always present.

SECTION 3

**ATTEMPTS TO CORRELATE IN VIVO ASSESSMENTS OF THE DIETARY
AVAILABILITY OF GRANULAR CALCINED MAGNESITES WITH IN VITRO
LABORATORY METHODS**

ATTEMPTS TO CORRELATE IN VIVO ASSESSMENTS OF THE DIETARY
AVAILABILITY OF GRANULAR CALCINED MAGNESITES WITH IN VITRO LABORATORY
METHODS

INTRODUCTION

The experiments carried out in Section 1 of this thesis provide extensive bioavailability data for different magnesites and other magnesium supplements obtained in virtually standardised conditions. One of the main conclusions of this work (and that of Wilson, 1981) has been that there is a wide variation in apparent availabilities between calcined magnesites from different sources. From the Time-Cost study (Experiment 9) it can be seen that a bioavailability study is a very time consuming operation and requires specialised equipment. Thus the aim of the following experiments has been to attempt to find a laboratory method to predict dietary availability which may also have commercial significance as a means by which one can identify magnesites of superior qualities in terms of bioavailability.

A number of different laboratory tests have been used to characterise magnesium oxides e.g. surface area, loss on ignition (LOI), total magnesium content, iodine number etc., and a variety of chemical methods have been suggested as predictors of availability.

Attempts have been made to relate particular in vitro 'chemical reactivity' tests to particular uses of magnesium, e.g. the determination of ammonium (NH_4^+) - exchangeable magnesium is a recognised method of estimating extractable magnesium in soils, or plant available magnesium in magnesium oxide fertilisers, determined by extraction with ammonium nitrate.

Ammerman *et al*, (1972) analysed the physical properties of different magnesium sources, one of which was a feed grade oxide (origin unknown) by dissolving the materials in 0.4% HCl, 2% citric acid and neutral ammonium citrate. (The feed grade oxide was 97.4, 98.4 and 97.6% soluble, respectively).

Jensen & Jensen (1980) advocated a dissolution test for in vitro estimations of the availability of dietary magnesium oxide to ruminant animals. The test is carried out at body temperature and at constant pH 6.5 (pH-static titration). A 'reactivity profile' is produced for each magnesium oxide giving the rate at which it dissolves and thus that which is potentially available for absorption by the animal. However no

in vivo availability data were given with which to compare the results, and thus it is merely assumed that the in vitro test predicts availability.

Wilson (1981) investigated two dissolution tests for a number of different magnesium supplements - the Citric Acid Reactivity (CAR) test and determination of NH_4^+ - exchangeable magnesium. Solubilities in ammonium nitrate solution and citric acid both reflected dietary differences up to a point. However too many anomalous comparisons, particularly with the CAR test, would not recommend either technique as a means of prediction. It was suggested that the correlations between solubility and availabilities would perhaps improve with more extensive repeated availability results.

In the following series of experiments, magnesium supplements of known bioavailability have been subjected to dissolution in ammonium acetate (NH_4^+ - exchangeable magnesium), and citric acid solution (CAR test) and the pH-static test (after Jensen).

The method of obtaining small samples of material for laboratory tests is important. Each calcined magnesite sample analysed in the experiments to be described had previously been sieved out into different particle size fractions, namely (i) material above 1 mm, (ii) between 500 μm and 1 mm, (iii) between 250-500 μm and (iv) below 250 μm , and the proportions (as a % of weight) recorded. The small samples to be analysed were then individually made up from each size fraction. It was felt that since small samples were used and particle size has been shown to be important in bioavailability studies, that this would represent a more genuine example of the whole product than simply taking a random sample of 1-2 g from a bulk sample.

EXPERIMENT 10

The determination of NH_4^+ exchangeable magnesium in various calcined magnesites

Introduction

The determination of NH_4^+ exchangeable magnesium using Molar ammonium nitrate is a recognised method for determination of extractable magnesium in soils (MAFF, 1973). Durrant & Draycott (1976) used ammonium nitrate to determine the magnesium availability of magnesium oxides as sugar beet fertilisers. Wilson (1981) used this method as an assay for animal-available magnesium using various magnesium supplements. It was decided to investigate this method but to replace ammonium nitrate with ammonium acetate, better imitating rumen conditions, using all the calcined magnesite supplements previously investigated in vivo.

Materials and Methods

2.0 g samples of each magnesium supplement were placed in 250 ml screw top glass bottles, 100 ml of Molar ammonium acetate was added to each bottle and the lids secured. The bottles 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, 4, 6, 8, 10 or 12 hours. Following this, samples of all extracts were filtered off and 1 ml removed, diluted (lanthanum chloride solution) and analysed for magnesium by atomic absorption spectroscopy.

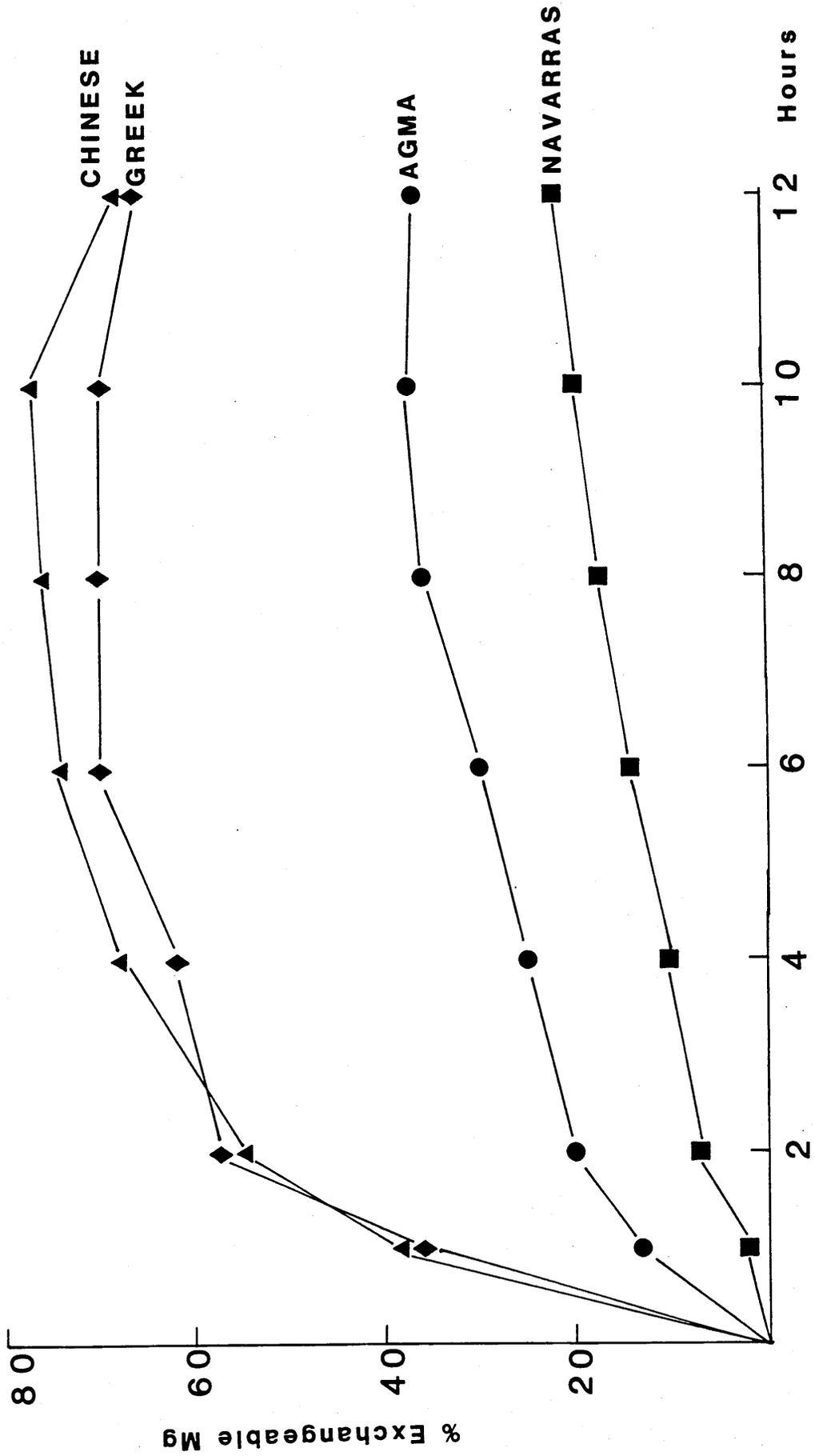
Results

Figure 5 shows the NH_4^+ exchangeable magnesium (as a % of total magnesium) as a function of extraction time for magnesium supplements used in Experiment 3. Table 25 shows the NH_4^+ exchangeable magnesium (as a % of total Mg) after 6 hours for various magnesium supplements. In addition, for comparative purposes the mean availability (as determined in the feeding trials with sheep) of each supplement is given.

Table 25. The relationship between the magnesium soluble in m. ammonium acetate after 6 hours and in vivo dietary availability.

Supplement	Expt.	% Exchangeable Mg at 6 hours	Mean S.Dev	Mean availability %
Agma	1	40.3		38.5
	2	39.8	35.5	18.5
	3	29.9	± 5.37	27.0
	4	31.8		27.0
Navarros	1	14.4		18.0
	2	11.9	13.6	22.5
	3	13.6	± 1.22	26.0
	4	14.6		23.0
Chinese	1	55.7		35.5
	2	68.5	62.0	23.5
	3	73.8	± 11.01	11.0
	4	50.0		24.0
Greek	1	67.6		33.5
	2	62.1	66.0	16.0
	3	71.2	± 4.19	15.5
	4	63.2		12.5
Turkish	3	65.7		28.5
Mg(OH) ₂	1-4	81.6		30.6
Fine grade Chinese magnesite	5	48.8		27.0

Figure 5. Expt. 10 The release of NH_4^+ - exchangeable magnesium (as a % of total magnesium) from various calcined magnesites, as a function of time.



Discussion

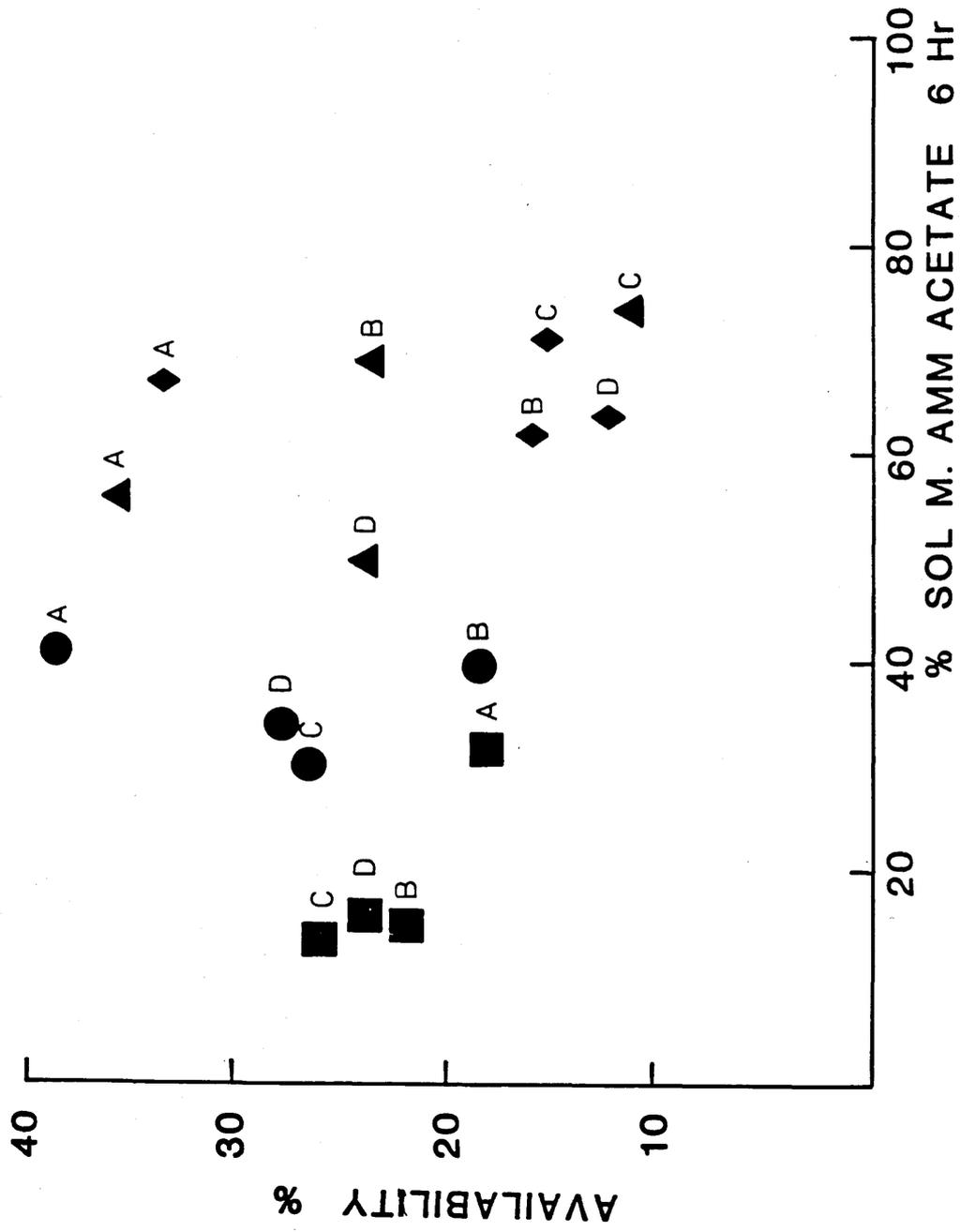
The table of results give the NH_4^+ exchangeable magnesium after 6 hours dissolution in ammonium acetate solution. It can be seen from Figure 5, showing the dissolution curves of magnesites used in Experiment 3 over a period of 12 hours, that the reaction levels off between 8 and 10 hours when there is little or no further dissolution. It is also evident from Figure 6 that in comparing the reactivity of the magnesites, expressed as percentage exchangeable magnesium, that there appear to be two different groups of magnesites, the Chinese and Greek products on the one hand and the two Spanish products on the other. The reactivity curves for the Chinese and Greek products are almost identical and appear to be more reactive in terms of NH_4^+ exchangeable magnesium than both the Agma and Navarras materials. In turn, the Agma product would appear to be more reactive than the Navarras but they follow similar reactivity patterns over the period of 12 hours.

From Table 25 showing % exchangeable magnesium after 6 hours the results show a consistency between products from batch to batch. The order of reactivity in all cases gives Navarras as the least reactive followed by Agma, then Chinese, and Greek as the most reactive (mean 13.6, 35.5, 62.0 and 66.0% exchangeable magnesium at 6 hours respectively). The Turkish material appears to be of similar reactivity to that of the Chinese and Greek (66%). The fine grade Chinese products can be found in the middle of an order table of reactivity. The magnesium hydroxide, as might be expected, appears to be the most reactive with almost 85% exchangeable magnesium after 6 hours.

These values do not correlate well with comparable values of bioavailability (excluding magnesium hydroxide) in fact an inverse relationship appears to exist (Figure 6). Products that have been shown to be more reactive in terms of bioavailability are less reactive with ammonium acetate than those that have poorer availabilities and in turn are more reactive in ammonium acetate solution.

Wilson (1981) investigated the reactivity of 45 different magnesium sources in molar ammonium nitrate. These ranged from raw Spanish magnesite and dead burnt calcined magnesites through to Analar MgO and Spanish cyclone dust. A significant correlation ($P < 0.05$, $r = 0.37$) was found between mean apparent availability of supplements and their 50% extraction time in Molar ammonium nitrate solution.

Figure 6. Expt. 10 The regression between apparent availability (%) and the NH_4^+ exchangeable Mg (as a % of total Mg) after 6 hours in M. ammonium acetate. $y = 27.6 - 0.095 x$, $r = 0.25$, NS
(Key : as Figure 3)



Interestingly, included within this large selection of supplements three comparable supplements to those used in this particular experiment can be identified, namely Spanish (Agma), Chinese and Greek 'whole' products purchased in 1980. The NH_4^+ - exchange results would suggest an order of decreasing availability as Greek, Chinese and Spanish, but the reverse order had been observed in in vivo studies by Wilson (1981) which is in total agreement to that found within this present experiment. Thus it does not follow that what may appear to be a 'chemically reactive' magnesium oxide, as determined by solubility in ammonium acetate, is necessarily 'active' in terms of absorption in the ruminant digestive tract.

EXPERIMENT 11

The determination of Citric Acid Reactivity of various calcined magnesites

Introduction

Dissolution in citric acid is a well known method for the determination of extractable phosphorus from fertiliser materials. Ammerman et al, (1972) achieved 98% solubility of feedgrade magnesium oxide (unknown origin) in 2% citric acid. Wilson (1981) investigated a range of magnesium supplements, mostly oxides, using the citric acid reactivity test. In this present experiment all the calcined magnesites investigated earlier, plus the standard magnesium hydroxide have been determined for their Citric Acid Reactivity (CAR).

Materials and Methods

The method used for the citric acid reactivity tests was taken from that used by Steetley Minerals Ltd., and this is described as follows.

Citric acid solution ($\text{H}_3\text{C}_6\text{H}_5\text{O}_7\text{-H}_2\text{O}$) 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. 100 ml 0.4 N citric acid solution was measured in a 250 ml tall-form beaker containing a magnetised follower. The beaker and its contents were heated to $30^\circ\text{C} \pm 0.2^\circ\text{C}$. 2.0 g of the magnesium supplement sample was added to the beaker and a stop watch 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. The 'activity' was expressed as the time taken for the solution to change colour.

The results are quoted in minutes and the materials tested are listed in Table 26.

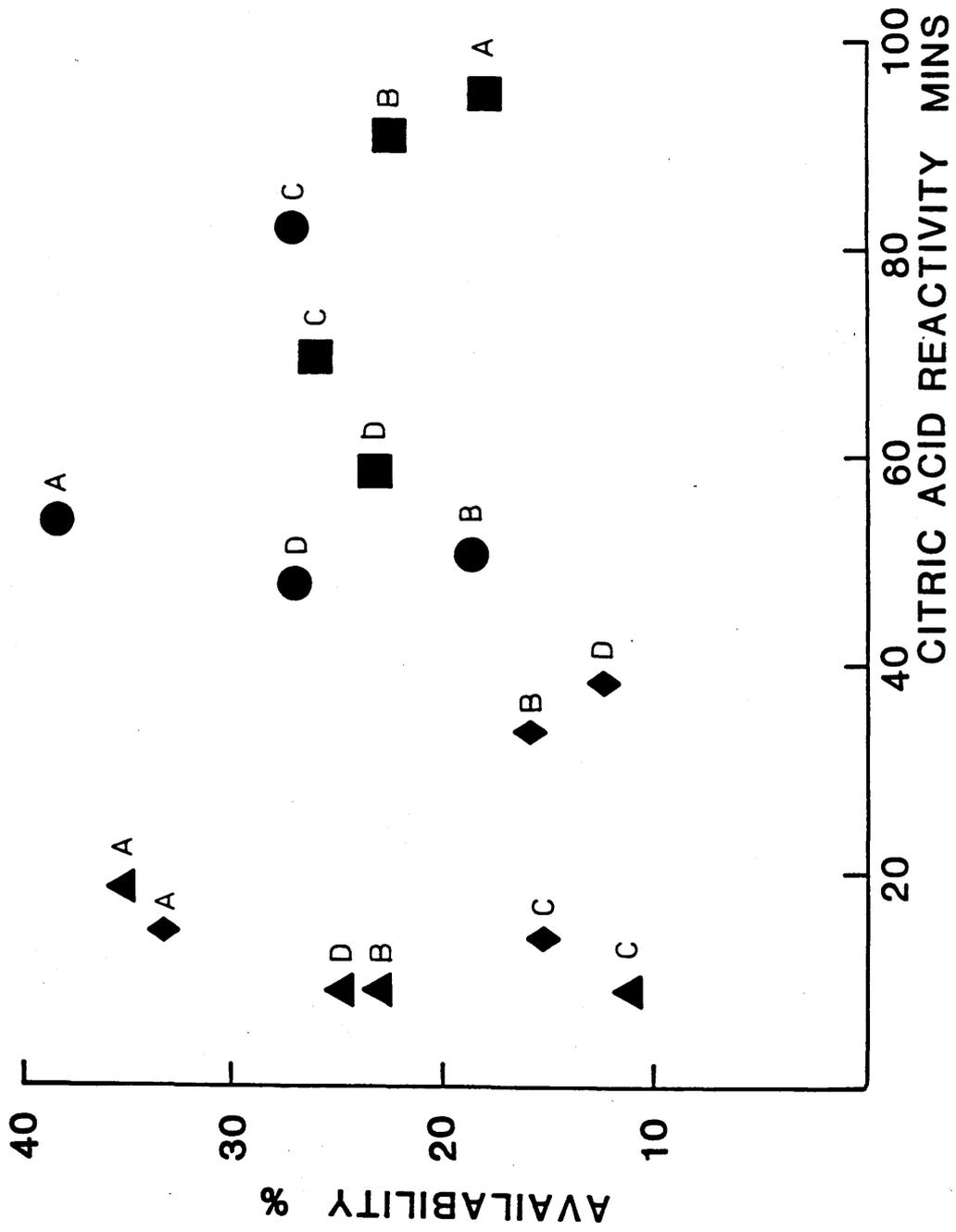
Table 26. Citric Acid Reactivity (mins) for different magnesium supplements in relation to in vivo dietary availability.

Supplement	Expt.	C.A.R. (mins)	Mean S. Dev	Apparent Availability %
Agma	1	54		38.5
	2	51	58.8	18.5
	3	82	± 15.69	27.0
	4	48		27.0
Navarras	1	95		18.0
	2	91	78.8	22.5
	3	70	± 17.1	26.0
	4	59		23.0
Chinese	1	19		35.5
	2	9	11.8	23.5
	3	9	± 4.86	11.0
	4	10		24.0
Greek	1	15		33.5
	2	34	20.3	16.0
	3	14	± 9.32	15.5
	4	18		12.5
Turkish	3	34		28.5
Mg Hydroxide	1-4	9		30.6
Chinese fine grade	5	5		27.0

Figure 7. Expt. 11 Regression between apparent availability (%) and citric acid reactivity (minutes) of various calcined magnesites.

$$y = 22.0 + 0.030 x, r = 0.11, \text{NS}$$

(Key : as Figure 3).



Results

The CAR for the different magnesium supplements ranged from 5 minutes (Fine Grade Chinese) to 95 minutes for the Navarras product (from Experiment 1). A similar pattern to that found for solubility in ammonium acetate has developed, whereby it is possible to distinguish two broad groups based on reactivity, the Greek and Chinese on the one hand and the Agma and Navarras on the other. In order of increasing reactivity the Navarras is the least reactive (mean, 79 minutes) followed by Agma (59 minutes), Greek (20 minutes) and Chinese (12 minutes). The single Turkish value falls midway between the groups (34 minutes) and the magnesium hydroxide, as might be expected (9 minutes) appeared very reactive.

Again as with the Ammonium Acetate test an inverse relationship appears to exist (Figure 7) whereby magnesium supplements that appear to be more reactive in vivo (i.e. more available) are poorly reactive in organic acids.

Wilson (1981) investigated the CAR of 45 different magnesium supplements. Of these, 21 had a CAR of less than 30 minutes and demonstrated a significant ($P < 0.01$) negative correlation between availability and CAR. However when the remaining 24 supplements with a CAR over 30 were also included, this relationship no longer existed. However, it would be fair to say that Wilson (1981) only included 3 or 4 whole products and the regression was produced using specific parameters of (a) particle size and (b) temperature; thus it would increase one's chance of achieving a significant correlation.

EXPERIMENT 12**The determination of pH-static reactivity of various calcined
magnesites****Introduction**

The pH-static in vitro determination of reactivity was developed by A. Tovburg-Jensen, Denmark and has been used for bioavailability evaluations of feed phosphates and other feed minerals. Jensen & Jensen (1980) advocated the test for use with magnesium oxides. The test is conducted at a constant pH (pH-static titration) corresponding to the acidity in the digestive tract and at body temperature. The HCl consumption is recorded as a function of time and the insoluble residue analysed. The titration curve or 'reactivity profile' gives the rate at which a particular magnesium oxide is dissolved and potentially available for absorption by the animal.

The pH-static reactivity tests were carried out by Boliden-Kemi, Helsingborg, Sweden. Due to the cost and the time required to carry out the tests only those calcined magnesites and the magnesium hydroxide used in Experiments 3 and 4 were investigated, plus a Turkish magnesite used in Experiment 2.

Materials and Methods

The mineral samples were screened and the fractions saved. A 1.000g sample was weighed out using the fraction 0.18 - 0.25 mm.

A water bath was heated to 37°C. The pH-static, with a pH glass electrode connected was adjusted against the buffer at 20°C. The temperature indicator of the pH stat was set to 37°C.

600 ml of demineralised water was added to a 1 litre round flask with three necks and suspended in the water bath. When the temperature of the water within the flask had reached 37°C the pH was adjusted to 6.5. The pH set points on the pH stat was set to 6.5 and the delay of shut out at infinity.

1.00 g of the sample to be tested was added to the pH-adjusted water and the system started.

The sample remained suspended within the water by means of a peristaltic pump. The consumption of HCl was recorded as a function of

Table 27. The amounts of magnesium dissolved (%) from original sample after 24 hours pH static titration.

	Expt.	% Mg dissolved	Apparent Availability %
Agma	3	52.1	27.0
	4	58.7	27.0
Navarras	3	36.0	26.0
	4	50.2	23.0
Chinese	3	60.1	11.0
	4	72.3	24.0
Greek	3	73.4	15.5
	4	44.4	12.5
Turkish	2	28.6	28.5
Mg(OH)	3 & 4	99.0	30.6

Figure 8a. Expt. 12 The reactivity profiles (after Jensen) of various calcined magnesites (from Expt. 3) at pH 6.5 over a period of 24 h).

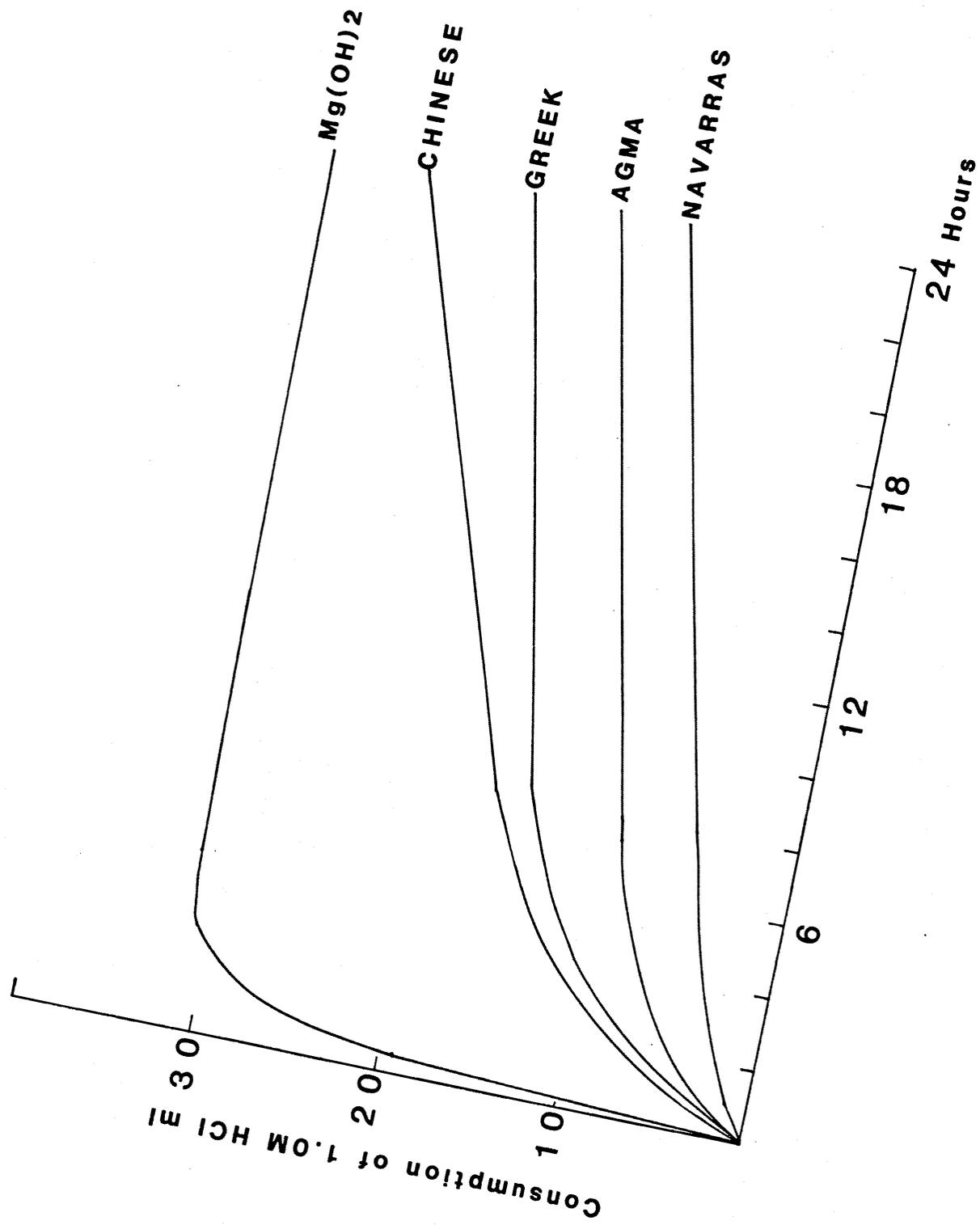
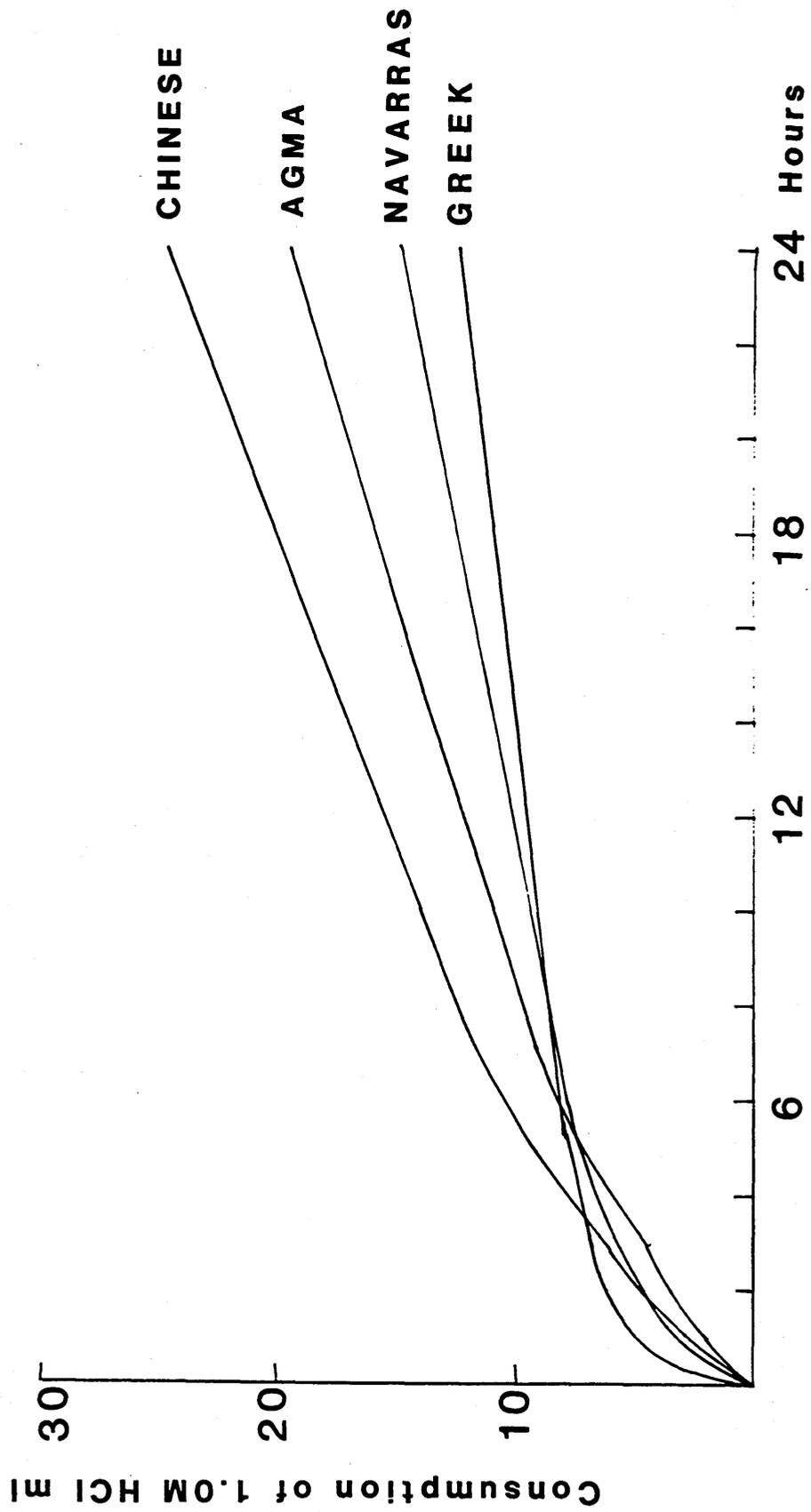


Figure 8b. Expt. 12 The reactivity profiles (after Jensen) of various calcined magnesites (from Expt. 4) at pH 6.5 over a period of 24 h).



time up to 24 hours. The undissolved residue after 24 hours was collected on a 0.2 μ m millipore filter and dried at 100°C, weighed and analysed for magnesium.

A curve was plotted to show HCl consumption as a function of time. The percentage of undissolved magnesium was calculated relative to the original sample.

Results

The 'reactivity profiles' are shown in Figures 8a and 8b. The percentages of magnesium dissolved from the original samples after 24 hours pH 6.5 static titration are given in Table 27 together with the apparent availability figures determined in the comparable balance trials.

Discussion

Figures 8a and 8b show that in all cases the magnesites are most reactive within the first hour of pH-static titration (i.e. the gradient on the reactivity curve is greatest). After approximately 8 hours the reaction appears slower with a reduced consumption of 1.0 M HCl, but apart from the magnesium hydroxide, are not complete after 24 hours. The magnesium hydroxide was the most reactive both in rate of reaction described by the 'reactivity curve' and in terms of % magnesium dissolved (99%). The hydroxide was almost totally dissolved after 4 hours titration.

The order of reactivity described in Figure 8a for rate of reaction of (i) magnesites used in Experiment 3 was Chinese, Greek, Agma and Navarros, although in terms of % magnesium dissolved the order was Greek (73%), Chinese (60%), Agma (52%) and Navarras (36%).

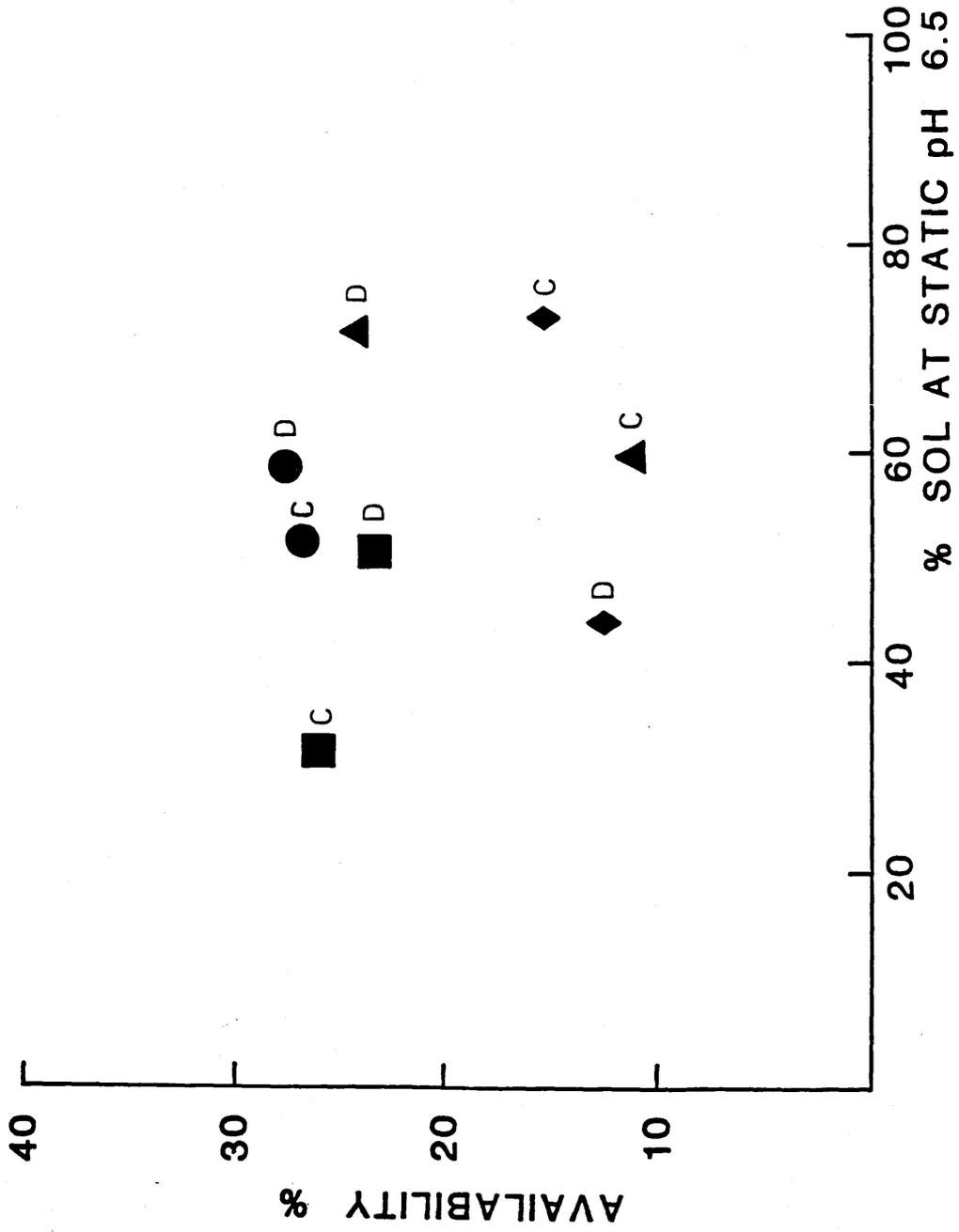
The order of reactivity (Figure 8b) for rate of reaction and % magnesium dissolved of magnesites used in Experiment 4 was Chinese (72%), Agma (59%), Navarras (50%) and Greek (44%). The Turkish material (Experiment 3) was the least reactive both in terms of rate of reactivity and % magnesium dissolved (29%).

No correlation appears to exist between these results and the comparable availability values obtained in Experiments 3 and 4, Figure 9. However there appears to be a similar trend in order of reactivity to that found with the dissolution tests in ammonium acetate and the

Figure 9. Expt. 12 Regression of apparent availability and % solubility of magnesium (pH static 6.5) of various calcined magnesites.

$$y = 25.9 - 0.0920 x, r = 0.179, \text{NS}$$

(Key : as Figure 3)



citric acid solution. A reverse relationship appears whereby granular materials that are 'chemically reactive' are poorly available to the ruminant.

SECTION 4

ATTEMPTS TO CORRELATE APPARENT DIETARY AVAILABILITY RESULTS
WITH SOLUBILITY TESTS FOR CALCINED MAGNESITES IN VIVO

**ATTEMPTS TO CORRELATE APPARENT DIETARY AVAILABILITY RESULTS WITH
SOLUBILITY TESTS FOR CALCINED MAGNESITES IN VIVO**

INTRODUCTION

The rumen is now widely believed to be a major site for magnesium absorption in the digestive tract of the ruminant and it follows that the amount absorbed will be directly affected by the amount of magnesium dissolved in the rumen liquor. The ARC (1980) states that 'animals at risk should be given a daily magnesium supplement in a form readily soluble in rumen liquor'. Jesse *et al* (1981) investigated the solubility of different particle size fractions of magnesium oxide by incubating them in rumen fluid and also reported that differences in the dietary availability of ground versus unground magnesium oxides were reflected in differences in solubility in the rumen. Similarly Rahnema & Fontenot (1983) investigated the solubilities of MgO and dolomitic limestone in rumen fluid and Lough & Beede (1988) incubated a magnesium oxide and a magnesium chelate supplemented diet in rumen liquor to compare solubilities.

Wilson (1981) used the nylon bag technique to compare rumen solubilities of different inorganic dietary magnesium supplements and related these to their apparent availabilities finding a highly significant positive correlation ($r = 0.57$, $P < 0.005$) for various calcined magnesites. This correlation however was contrived using different particle size fractions and magnesites calcined at different temperatures after 2 days in the rumen (some calcined magnesites were found to be insoluble even after 14 days). It was found that the coarser fractions (500 - 1000 μm) were markedly slower to dissolve than the finer grades (e.g. $< 75 \mu\text{m}$) and magnesites calcined at the optimum temperatures for availability (i.e. 800 - 1,100°C) showed high solubility compared with raw magnesite and that calcined at 500°C which appeared insoluble.

As early as 1938, Quin, Van der Wath and Myburgh discussed the feasibility of using cylindrical bags made of very thin natural silk for measuring the digestion of feeds in the rumen of cannulated sheep. Since then many workers have used the technique for various purposes. The technique measures the disappearance of feed constituents from bags containing the test diet after incubation in the rumen for varying periods.

Different materials have been employed to make the bag including what were described as artificial fibre, dacron, terylene, polyester and nylon bags. Schoeman *et al*, (1972) used bags made from dacron material obtained from a parachute (Mehrez & Ørskov, 1977).

Belasco *et al*, (1958) and Van Keuren & Heinemann (1962) emphasized the importance of the pore size of the material in regulating the passage of solid particles. The 'natural conditions' inside the bag will be influenced by the pore size of the nylon. Thus a minimum pore size which does not interfere with the flow of rumen fluid in and out of the bags is desirable; a surface tension effect would develop with excessively small pore sizes.

The sample size in relation to the size of the bag is important (Van Keuren & Heinemann, 1962; Mehrez & Orskov, 1977) with dry matter disappearance decreasing with larger samples. This is probably related to a reduced natural flow of digesta and agitation of the test material when it occupies a large volume of the bag.

The dietary regime of the fistulated animal can have significant effects on the results, and the position of the bag in the rumen is important. Animals at grass compared with those maintained indoors will have less solid rumen contents with a greater flow of rumen liquor. The bags themselves within the rumen will have greater movement.

Erwin & Elliston (1959) and Rodriguez (1968) found that the position of bags in the rumen had no effect on digestibility of different feeds, however the results of Miles (1951) indicated that there were wide variations in digestibility at different levels in the rumen. Balch & Johnson (1950) showed that dry matter loss from the bags in the ventral sac was almost twice that compared with bags in the dorsal sac of the rumen. They, together with Miles (1951) found it necessary to attach weights in order to anchor the bags in the ventral sac of the rumen where digestion was more rapid.

Rodriguez (1968) found that variability in dry matter disappearance from bags was less when attached to 50 cm compared with 30 cm length string. He suggested that the longer string had allowed greater movement of the bags within the rumen of the steer and thus minimised the effects of variation in the environment.

The length of time the bag remains in the rumen is important as it will influence the digestibility value observed. It may not be an accurate reflection of the length of time that the test material would remain in the rumen had it been ingested. This will also be affected by

the diet of the fistulated animal.

The use of the nylon bag technique has the advantage of giving a very rapid estimate of the digestion of nutrients in the rumen. It has the advantages of simplicity of operation, reduction of test feed replication differences and the ability to test a number of materials at one time in one rumen environment so reducing between systems effects e.g. the cow. The main disadvantage of the technique is that it is unsuitable for testing very finely powdered materials as the pore size is commonly about 24 μm .

The following experiments describe the investigation into the solubility of various calcined magnesites (used in Experiments 1-4) in rumen liquor using the nylon bag technique with fistulated cows, maintained both indoors and at grass.

The Nylon Bag Technique

Materials and Methods

The nylon bags were produced from precision made nylon cloth (Henry Simon Ltd., Special Products Division, Stockport, Cheshire) with a pore size of 24 μm . Each bag was approximately 15 x 12 cm attached to which was a length (60 cm long) of nylon cord. The free end of the cord was attached to a short length of rubber tubing. The bags containing a carefully weighed mineral sample were thus suspended in the rumen to a depth of 30-50 cm held by the rubber tubing which remained outside the fistula. After 48 h the bag was removed from the rumen and 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 mopped dry of excess water and then dried for 48 h in a glass drying oven at about 80°C. The partly digested dried sample was carefully removed from the bag, weighed and analysed for magnesium. The total magnesium remaining in the residue could then be calculated. By comparing the total magnesium present in the weighed initial sample with that of the residue from the bag it was possible to calculate the percentage loss of magnesium from the calcined magnesite.

The experimental animals were non-productive cows (Friesian and Ayrshire) which had had a rumen fistula in position for a number of years. They were used either at pasture or in a byre where they received hay and concentrates. Each cow had up to 8 bags in the rumen

at any one time and samples of rumen liquor were taken periodically for pH analysis.

EXPERIMENT 13

An investigation into the solubility of various calcined magnesites in the rumen of cows maintained indoors and at grass

Introduction

This experiment consists of a series of trials that were carried out over a period of 2 years. The calcined magnesites used in Experiments 1-4 described earlier, were placed in the rumen of fistulated cows maintained (a) indoors and (b) at grass, to determine individual solubilities and to determine if apparent differences in solubility exist between products and as a result of feeding regime (i.e indoors or at grass).

Materials and Methods

The method used was as described earlier in the 'Nylon bag technique'. The different consignments of calcined magnesites (i.e. those used in Experiments 1-4) were investigated separately over a period of two years. However the animals used and the diets received were identical and thus it was considered justified to tabulate the results together.

Whilst maintained indoors (during the autumn/winter), the cows received limited concentrates with hay and whilst at grass (summer) all values were taken using the same field with grass at a comparable growth stage.

For each individual consignment, eight dacron bags containing the four major calcined magnesites were placed in the rumen of each of four fistulated cows, i.e. 2 bags of each product per cow, and this was repeated giving a total of 16 values per treatment for cows maintained (a) indoors and (b) at grass.

A 10 g sample of each product was placed in each bag which remained in the rumen for 48 h. On insertion and removal of the bags, a sample of rumen fluid was taken and tested for pH.

The calcined magnesites investigated are detailed in the Results in Tables 28 and 29.

Table 28. Experiment 13. The mean loss of magnesium from calcined magnesites placed in the rumen (for 48 h) of cows maintained indoors.

		1	2	3	4	Mean
Mean pH		6.89	6.88	6.91	6.77	
No. values/treatment		16	16	14	14	
Agma	A	17.49	8.44	12.79	8.84	11.89
Navarras	N	11.76	18.58	4.78	9.60	11.18
Chinese	C	14.02	10.77	-3.57	4.70	6.48
Greek	G	5.39	5.54	8.71	5.41	6.26
SEM		1.449	2.028	1.183	1.863	1.016

Significance

P < 0.001	A,C>G	N>A,G	A,N,G>C	A,N>C,G
P < 0.01	A>N; N>G	N>C	A>N	
P < 0.05			A>G	
			G>N	NS

Table 29. Experiment 13. The mean % loss of magnesium from calcined magnesites placed in the rumen (for 48 h) of cows maintained at grass.

Calcined magnesites from experiment:

		1	2	3	4	Mean
Mean pH		5.78	5.75	5.83	6.01	
No. values/treatment		16	16	14	15	
Agma	A	23.70	32.50	24.97	17.76	24.73
Navarras	N	23.52	19.90	19.86	8.28	17.89
Chinese	C	18.17	17.50	14.88	11.52	15.52
Greek	G	4.25	7.60	9.55	18.71	10.03
SEM		2.430	2.817	2.652	2.314	1.403

Significance

P < 0.001	A,N,C>G	A>C,G	A>G	A>N,C,G;N>G
P < 0.01		A>N;N>G	A>C;N>G	C>G
P < 0.05		C>G		G>C

Results

Changes in the actual weight of the calcined magnesites in the bags were relatively meaningless, 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. In fact some calcined magnesites (especially the Chinese and Greek products) increased in weight after 48 h in the rumen despite thorough washing of the bags to remove adhering rumen contents. Thus all solubility results are described in terms of the percentage loss of magnesium from the original sample.

The results obtained for the cows maintained indoors are given in Table 28 and those at grass in Table 29. The mean pH analyses are also given.

(a) Solubility results with the cows on indoor diets.

The mean pH of the rumen liquor varied between 6.77 and 6.91 for the four different consignments.

In all cases, magnesium was lost from the original sample except for the Chinese product from Experiment 3, which apparently gained 3.57% magnesium. In terms of the mean percentage loss in magnesium of all four consignments, the Agma and Navarras were very similar (11.9 and 11.2% respectively) and the Chinese and Greek were also similar (6.5 and 6.3% respectively). The Agma and Navarras produced significantly greater losses in magnesium than both the Chinese and Greek ($P < 0.001$).

The largest individual percentage loss figure was obtained with Navarras (from Experiment 2) with a mean magnesium loss of 18.6% ($n=16$) followed by Agma (from Experiment 1) with a mean magnesium loss of 17.5% ($n=16$).

Within the first consignment (from Experiment 1) the Agma, Navarras and Chinese products all produced significantly greater losses in magnesium than the Greek product; the Agma was also more soluble than the Navarras ($P < 0.01$).

Conversely within the second consignment (from Experiment 2) the Navarras produced the greatest loss in magnesium being significantly greater than the Agma and Greek ($P < 0.001$) and the Chinese ($P < 0.01$).

Within the third consignment (from Experiment 3) the Chinese product apparently gained in magnesium and thus the Agma, Navarras and

Greek all produced significantly greater losses ($P < 0.001$). The Agma produced the greatest magnesium loss being significantly greater than the Navarras ($P < 0.001$) and the Greek ($P < 0.05$). The Greek meanwhile produced a significantly greater solubility than the Navarras ($P < 0.05$).

The mean solubilities of all four products within the fourth consignment (from Experiment 4) were much less than for the other three, with the Navarras and Agma producing the greatest magnesium losses but as small as 9.6 and 8.8% respectively. There were no significant differences between the different products.

In order to determine a possible relationship between availability and rumen solubility, the dietary availability for the 16 calcined magnesites investigated here were correlated with the percentage loss of magnesium from each calcined magnesite, after 48 h in nylon bags (24 μ m) in the rumen (Table 28 and Figure 10). The availability figure used for each calcined magnesite was the apparent availability result obtained earlier in this work (the particular experiments involved are given in Table 28). A significant positive correlation, $r = 0.54$ ($P < 0.05$) was shown to exist between dietary availability and rumen solubility in 48 h using cows maintained on indoor diets.

(b) Solubility results obtained with cows at grass.

The mean pH varied between 5.75 and 6.01. In all cases magnesium was lost from the original sample of calcined magnesite. The Greek product (from Experiment 1) was the least soluble (mean 4.25%, $n = 16$) and the Agma (from Experiment 2) produced the greatest percentage loss in magnesium (32.5%, $n = 16$).

The mean percentage loss of magnesium calculated from the four different consignments of each calcined magnesite produced solubilities ranging from 10.0% for the Greek product to 24.7% for the Agma. The Navarras and Chinese products produced similar losses in magnesium (17.9 and 15.5% respectively). The Agma (mean of the 4 consignments) produced a significantly greater loss in magnesium than the Navarras, Chinese and Greek products, and the Navarras gave a significantly greater solubility than the Greek ($P < 0.001$). The Chinese in turn produced a significantly greater magnesium loss than the Greek ($P < 0.01$).

Within the first consignment (from Experiment 1), Agma, Navarras and Chinese all produced significantly greater losses in magnesium than

Figure 10. Expt. 13 Correlation between apparent availability (%) and solubility (%) in the rumen of cows maintained on hay and concentrates, after 2 days.

$$y = 16.2 + 0.792 x, r = 0.54 *$$

(Key : as Figure 3)

HAY AND CONCENTRATES

DIETARY
AVAILABILITY

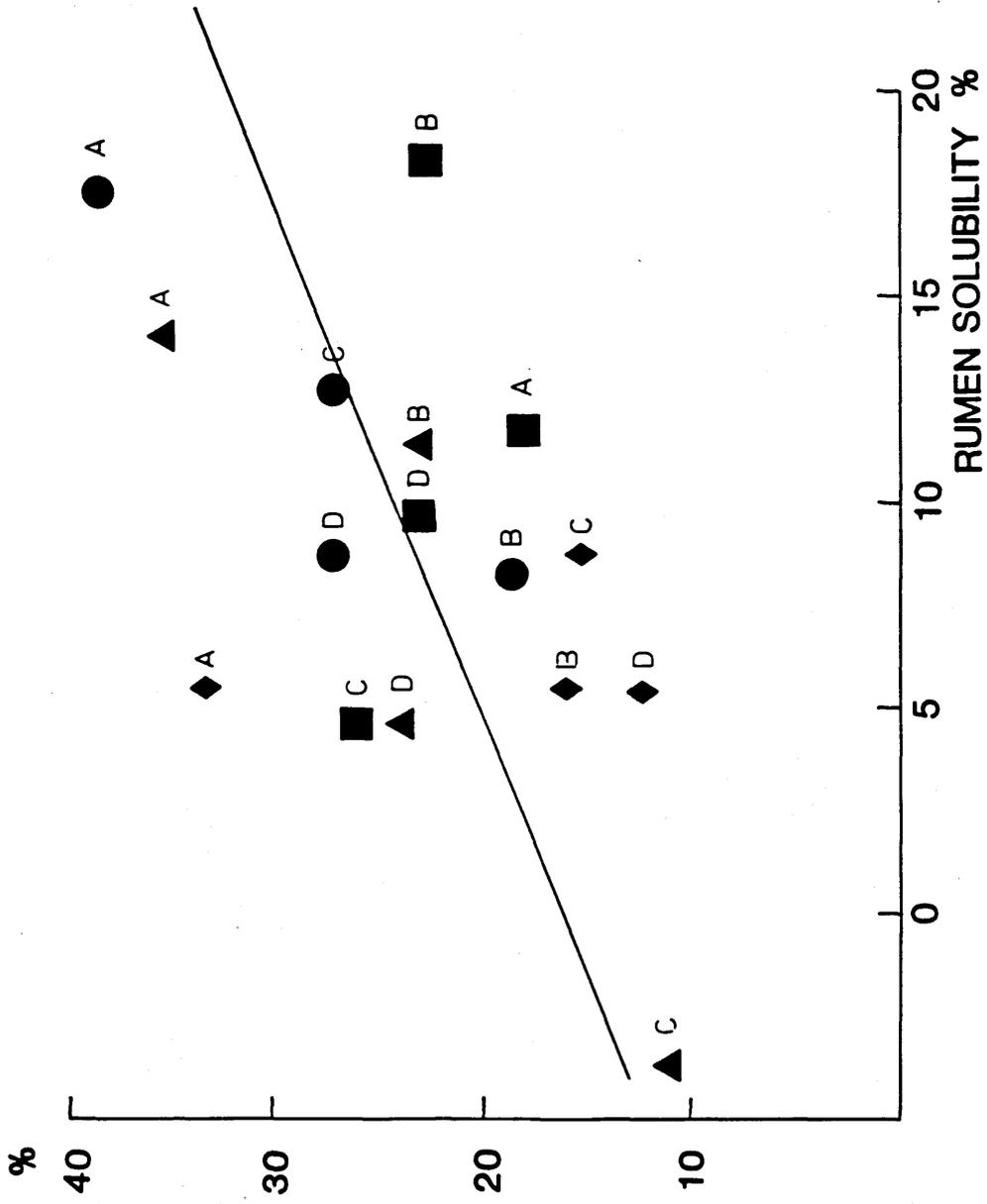


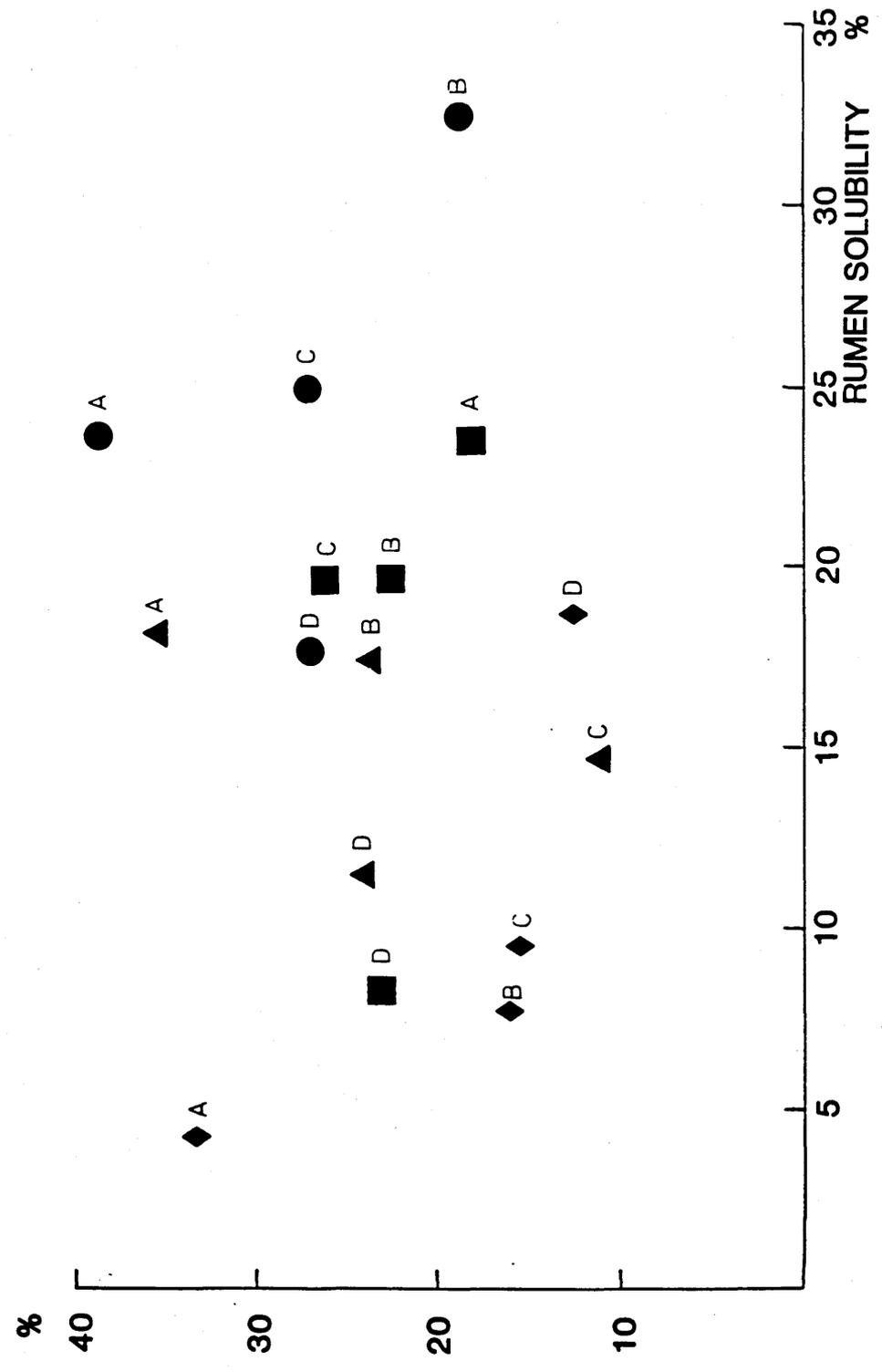
Figure 11. Expt. 13 Regression between apparent availability (%) and solubility (%) in the rumen of cows maintained at grass, after 2 days.

$$y = 22.3 + 0.0579 x, r = 0.06, \text{NS}$$

(Key : as Figure 3)

COWS AT GRASS

DIETARY
AVAILABILITY



the Greek product ($P < 0.001$).

Within the second consignment (from Experiment 2) the Agma produced a significantly greater loss in magnesium than the Chinese and Greek ($P < 0.001$) and the Navarras product ($P < 0.01$). (The Agma product gave a percentage magnesium loss of 32.50% compared to the Greek 7.60%). The Navarras ($P < 0.01$) and the Chinese ($P < 0.05$) also were more soluble than the Greek product.

Within the third consignment (from Experiment 3) the Agma product again was the most soluble being significantly greater than the Greek ($P < 0.001$) and Chinese ($P < 0.01$).

The mean solubility figures obtained for all four calcined magnesites from Experiment 4 were lower than those obtained for the previous three consignments. Surprisingly the Greek product was the most soluble (18.71% loss of magnesium) and together with the Agma product was significantly more soluble than the Navarras ($P < 0.01$), and alone was significantly more soluble than the Chinese ($P < 0.05$).

An attempt was made to correlate the rumen solubility results with the appropriate apparent availability results obtained earlier (Table 28 and Figure 11). However no correlation appeared to exist between dietary availability and rumen solubility when the cows were maintained at grass.

General Discussion

It was considered that a period of 48 h retention in the rumen best represented the natural conditions had the calcined magnesite been ingested in a normal manner.

For the various calcined magnesites investigated within each trial, both for cows maintained indoors ^{and} at grass, apparent differences in solubility in the rumen appeared to exist and these generally reflected the relative differences previously reported in their dietary availability. This agrees with findings by Wilson (1981) (the only recorded work involving solubility of calcined magnesites using this technique) who obtained a highly significant positive correlation ($r = 0.572$, $P < 0.005$) between dietary availability and rumen solubility in 2 days (apparently for both cows maintained indoors and at grass). Interestingly, Wilson found that many calcined magnesites were not fully dissolved even after 14 days.

A significant correlation was also found in this work between

dietary availability and rumen solubility ($r = 0.54$, $P < 0.05$) when cows were given indoor diets only

Solubility of the calcined magnesites tended to be less when the cows received indoor diets compared to when the cows were at grass, again in agreement with the work done by Wilson (1981). The rumen liquor tended to have a higher pH when the cows received indoor diets and it is possible that a lower rumen pH favoured dissolution of the calcined magnesites. Also, it is possible that 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.

Throughout the trials the Greek products tended to be consistently of lower solubility (except for consignment 4, at grass) and the Agma products of superior solubility.

An attempt was made to correlate the solubilities obtained at grass with those obtained on indoor diets but no significant relationship was found to exist ($r = 0.39$).

Although it had been demonstrated that by using the nylon bag technique with cows on indoor diets this may provide a relatively quick in vivo test to compare the comparative merits of different calcined magnesites, it could only be considered as a potential guide and not as a very reliable or precise method.

SECTION 5

ATTEMPTS TO INDUCE HYPOMAGNEAEMIA IN SUCKLER COWS AND EWES USING
VARIOUS DIETS LOW IN MAGNESIUM, AND ITS ALLEVIATION BY SUPPLEMENTING
WITH VARIOUS CALCINED MAGNESTIES

**ATTEMPTS TO INDUCE HYPOMAGNESAEMIA IN SUCKLER COWS AND EWES USING
VARIOUS DIETS LOW IN MAGNESIUM, AND ITS ALLEVIATION BY SUPPLEMENTING
WITH VARIOUS CALCINED MAGNESTITES**

INTRODUCTION

Magnesium deficiency has been produced experimentally in animals by many workers for a number of reasons, e.g. to study magnesium metabolism, absorption, pathogenesis of hypomagnesaemia and its treatment.

Hypomagnesaemia has been induced by a variety of different methods. Exposed environmental conditions, starvation or underfeeding have all been shown to reduce blood magnesium concentrations (Christian & Williams, 1960; Halse, 1960; Terashima *et al*, 1982). Chemical treatments have also worked successfully e.g. adrenaline infusion in ewes (Rayssiguier, 1977). Suttle & Field (1969) in feeding experiments with both dry and lactating ewes reduced plasma magnesium concentrations with dietary additions of potassium salts. When added to diets of low magnesium concentration, potassium salts induced cases of tetany.

The feeding of low magnesium diets has invariably produced some degree of hypomagnesaemia in ruminants especially when lactating. Normal indoor diets for cattle and sheep usually have comparatively high levels of magnesium, i.e. over 1.5-2.0 g magnesium/kg DM, and animals given such diets rarely develop hypomagnesaemia. Allcroft & Burns (1968) reported that hypomagnesaemia did not occur on pastures containing over 2 g magnesium/kg DM, and this value is now considered to be the desired herbage magnesium concentration for control of hypomagnesaemia.

The ARC (1980) publication gives recommended dietary allowances of magnesium for maintenance, growth, pregnancy and lactation for cattle and sheep. (All values discussed here refer to the recommended dietary allowance, using a coefficient of absorption of 17%), e.g. a pregnant 600 kg Friesian cow between 20 and 40 weeks of pregnancy has a recommended allowance of between 10.8-15.5 g Mg/d but when she is yielding 10 kg milk, as with suckler cows, this is raised to 17.9 g Mg/d and up to 32.6 g Mg at a daily milk yield of 30 kg.

Recommended allowances for pregnant ewes range from 0.71-1.33 g Mg/d for a 40 kg ewe (9-21 weeks of pregnancy) and 1.32-1.90 g Mg/d for

a 75 g ewe. The allowance for lactating ewes will be dependent upon milk yield but the recommended allowance of a 40 kg ewe producing 1 litre and 2 litres milk/day is 1.71 and 2.71 g Mg/d increasing for a 75 kg ewe to 2.32 and 3.32 g Mg/d respectively. Thus a 'low magnesium' diet will be very dependent on the production requirements of particular animals.

Much work involved in the feeding of diets low in magnesium has centred on the use of semi-synthetic diets which can be markedly magnesium deficient. However, palatability of artificial diets is extremely important and many workers have experienced feed refusals by the animals. A high proportion of purified starch-based diets results in digestive disturbances which may lead to loss of appetite, scouring and reduced production.

A number of workers have used quite unusual and abnormal ingredients for low magnesium diets with some success in terms of intake by the animal. Rook & Campling (1959) gave paper pulp, shredded and treated with molasses or invert sugar to dairy cows although some cows found it unacceptable. Tomas & Potter (1976) used tissue paper with starch, casein and molasses as a low magnesium diet for ewes (0.2-0.3 g/kg DM). Suttle & Field (1969) with non-pregnant and suckling ewes used diets as low as 0.05 and 0.075% magnesium respectively, consisting of oat hulls together with starch, sugar and blood meal.

Maize products have frequently been used as they are generally low in magnesium e.g. maize gluten meal, 0.5, maize cobs 0.7 g/kg DM. However it has been reported that the inclusion of readily fermentable carbohydrates in a diet improves the availability of dietary magnesium (Rook, 1961; Giduck & Fontenot, 1987). Gerken & Fontenot (1967) and Kelley & Fontenot (1969) used diets based on shelled maize, maize cobs and maize gluten meal for steers and lactating cows (0.9-1.2 g Mg/kg DM). Ammerman et al, (1972) and Chicco et al, (1972) both used maize starch with glucose monohydrate and cellulose in diets for wethers (0.5 g Mg/kg DM). Strachan & Rook (1975) used maize starch together with washed straw, cellulose powder and casein in diets for wethers (0.04-0.06 g Mg/kg DM).

The above mentioned diets, although semi-purified or completely artificial aimed to be balanced nutritionally in all respects, except for magnesium content. Wilson (1981) carried out a number of trials with different natural feedingstuffs in an attempt to induce hypomagnesaemia. A loose diet of flaked maize, maize bran and barley

siftings (0.59 g Mg/kg DM) was most successful in terms of acceptability, ability to maintain production levels and ability to reduce blood magnesium concentrations.

The main objectives of the series of trials to be described was to investigate a number of diets based on natural feedstuffs but low in dietary magnesium. These were predominantly based on maize by-products, or barley draff, a by-product from a whisky distillery. These diets were given to pregnant and lactating ewes and suckler cows in trials carried out at various times during a period of 2 years. The primary aim was to induce hypomagnesaemia, indicative by low blood magnesium concentrations, and then supplement with magnesium in varying amounts, as various calcined magnesites. The aim was to distinguish whether differences existed between products in their ability to raise blood magnesium concentrations and to determine whether the results could be correlated with apparent availability results obtained from balance trial data.

EXPERIMENT 14**An attempt to induce hypomagnesaemia in lactating suckler cows given a low magnesium diet****Materials and Methods**

Seventeen cows (Friesian x Hereford) with single suckled Charolais X calves (approximately 2 weeks old) were housed on straw in a covered yard.

The cows received 4.5 kg of a mixed concentrate containing 3 parts flaked maize and 1 part dreg plus 2% dicalcium phosphate (FM basis). The concentrate diet was given in approximately equal portions at 07.30 and 10.00 h by means of individual headlocking feeders. The cows also received approximately 6 kg DM straw per day from a ring feeder. The diet provided approximately 721 g DCP, 97 MJ ME and 6.4 g magnesium (see Table 30).

The requirements for a suckler cow (450 kg) giving an estimated 7.5-10.0 kg milk/day are 86-99 MJ ME and 650-775 g DCP. It was considered that this diet provided adequate energy but was somewhat marginal for protein and was sufficiently deficient in magnesium to reduce blood plasma magnesium levels. (The magnesium requirement (ARC, 1980) for 450 kg cow giving 7.5-10.0 kg milk is 14.0-16.0 g magnesium, assuming 17% availability). The cows were bled from the jugular vein every three days at approximately 13.00 h.

Results

The diet was well consumed and feed refusals occurred with only one cow, and this was due to ill health.

The mean plasma magnesium concentration of the 17 cows on the first day of receiving the low magnesium diet was 0.87 m mol/l. After 6 days on the low magnesium diet the mean plasma magnesium concentration was 0.79 m mol/l and after 38 days, was 0.92 m mol/l.

During this trial period one cow died after a week from chronic diarrhoea which was believed to be unconnected with the diet. One further cow was removed with a calf suffering from pneumonia.

It was decided that it was unlikely that the blood values would fall due to the diet and stage of lactation and the cows were

Table 30. Experiment 14. Composition of dietary ingredients (g/kg).

	Dreg	Flaked maize	Barley straw
Crude Protein	500	110	38
DCP	340 +	106	9
ME (MJ)	12.5	15.0	6.5
Crude Fibre	120	24	452
Ether Extract	86	33	12
Ash	19	10	48
Magnesium	0.60	0.77	0.63

+ assumed

consequently given two magnesium alloy bullets and transferred to grass (30th April 1987) and the trial abandoned. Ironically five days after turnout the mean blood magnesium had fallen to 0.43 m mol/l with one individual as low as 0.22 m mol/l.

EXPERIMENT 15

An attempt to induce hypomagnesaemia in lactating ewes given a low magnesium diet

Materials and Methods

Twenty two lactating ewes with twins of approximately 2 weeks of age were maintained in individual pens (6'x 6') on straw, indoors. The lambs had access to creep feed.

The ewes received 0.7 kg barley husk siftings, 0.7 kg flaked maize, 0.5 kg dreg (identical material to that given to the suckler cows in Experiment 14) and 1% dicalcium phosphate per head per day, given in two approximately equal portions at 07.30 and 16.00 h. This provided approximately 251 g DCP, 20.6 MJ ME and 1.56 g dietary magnesium (see Table 31). Fresh water was available ad lib.

The ewes were bled from the jugular vein every three days at approximately 13.00 h.

Results

The diet was well consumed and the lambs gained 0.16 kg/day (n=44). A ewe with twins each with a liveweight gain of 0.16 kg/day would be producing approximately 1.6 kg milk/day. The magnesium requirement of a 75 kg ewe producing 2 kg milk/day is 3.32 g (ARC, 1980).

The mean plasma magnesium concentration of the ewes after 7 days on the diet was 0.97 m mol/l. After 14 days on the magnesium depleted diet the plasma magnesium concentration was 0.95 m mol/l and after 21 days, 0.87 m mol/l. The trial was eventually abandoned after 4 weeks when the mean blood magnesium concentration was 0.81 m mol/l.

It became obvious that it was unlikely that the blood magnesium levels would fall as a result of feeding the low magnesium diet. As the lambs became older (at the end of the trial they were at least 6 weeks old) they consumed increasing amounts of creep feed and thus reduced their dependency on milk and hence the magnesium drain on the ewe through the milk.

Table 31. Experiment 15. Composition of dietary ingredients (g/kg).

	Dreg	Flaked maize	Barley husk siftings
Crude Protein	500	110	60
DCP	340 +	106	10
ME (MJ)	12.5	15.0	5.5 ++
Crude Fibre	120	24	263
Ether Extract	86	33	11
Ash	19	10	136
Magnesium	0.6	0.77	1.03

+ assumed

++ previous findings Vet. An. Husb. Dept., Glasgow University.

EXPERIMENT 16**An attempt to induce hypomagnesaemia in suckler cows and its alleviation with various calcined magnesite supplements****Introduction**

In view of the relatively unsuccessful attempts to induce hypomagnesaemia in suckler cows given straw and a predominantly maize-based diet, the objectives of this trial were to produce a diet that would be both palatable, maintain realistic production levels and be effective in producing low blood magnesium concentrations. The barley draff that was used in this trial was a by-product obtained from a whisky distillery and contained only 0.73 g Mg/kg DM.

Materials and Methods

Fifteen Friesian x Hereford cows with single suckled Charolais X calves (mean age, 3 weeks) of mean body condition about 2.0-2.5, were housed on straw in a covered court. The cows were given 14 kg FM (3.75 kg DM) of a low magnesium draff (a by-product from a selected whisky distillery) plus a 2% limestone/salt supplement in approximately equal portions morning (07.30 h) and afternoon (13.00 h) by means of individual head-locking feeders. They also received approximately 6 kg DM straw per day from a ring feeder. The composition of the feeds and the assumed ME and DCP contents are given in Table 32. In total these provided 83.3 MJ ME, 620 g DCP and 6.14 g magnesium. The requirements for a 450 kg suckler cow giving an estimated 7.5-10.0 kg milk/day are 86-99 MJ ME and 650-775 g DCP. A liveweight loss of 0.5 kg/day by the cow would be equivalent to a further 14 MJ ME. It was considered that this diet provided about adequate energy but was somewhat marginal for protein.

The ARC (1980) magnesium requirement for 450 kg cows giving 7.5-10 kg milk is 8.2-9.2 g Mg (29% availability) or 14.0-16.0 g Mg (17% availability). It was thus considered that the basal diet could be regarded as providing less than an adequate amount of magnesium.

The cows received the low magnesium diet for a preliminary period of three weeks during which they became accustomed to the new diet. At the end of that period they were divided into 3 sets of five cows with

Table 32. Experiment 16. Dietary composition of feedstuffs (g/kg DM).

	Druff +	Barley Straw +
Dry matter (g/kg FM)	268	834
Crude Protein	204	37
Crude Fibre	206	452
Ether Extract	79	12
Ash	26	48
Assumed ME (MJ)	11.8	6.5 ++
Assumed DCP	151	9 ++
Magnesium	0.73	0.68

+ mean of 8 samples taken throughout experiment

++ MAFF (1986)

Table 33. Experiment 16. Mean blood magnesium concentrations (m mol/l) of suckler cows supplemented with various calcined magnesites on the fifth day of each dietary treatment.

Supplement	Mean, three 5x5 Latin Square (15 cows/treatment)			One 5x5 Latin Square (5 cows/treatment)			
	Mg g/d	10.0	Increase	07.30	13.00 h	Increase	
Agma	A	0.81	0.85	0.04	0.75	0.78	0.03
Navarros	N	0.79	0.84	0.05	0.79	0.80	0.01
Chinese	C	0.80	0.86	0.06	0.72	0.78	0.06
Greek	G	0.82	0.87	0.05	0.78	0.81	0.03
Nil	O	0.71	0.74	0.03	0.59	0.63	0.04
SEM		0.032	0.030		0.053	0.054	
Significance		A,C,G>O*	C,G>O** A,N>O*		A,N,G>O*	N,G>O*	

mean blood magnesium concentrations of 0.59, 0.73 and 0.80 m mol/l. By this time they were an average of 6 weeks calved.

Thereafter they formed three 5 x 5 Latin squares for a trial with five 5 day feeding periods. The five experimental treatments were either no supplementary magnesium or 10 g Mg/day as Spanish Agma, Navarras, Chinese and Greek calcined magnesites. These were the same materials as were evaluated by sheep in cages for apparent availability in Experiment 3. The pre-weighed magnesium supplements were added to the surface of the morning feed of draff. Blood samples were obtained from the cows on the last day of each period. The blood sample was obtained from either the tail or jugular vein as appropriate at 07.30 h (before feeding) and again at 12.00 h, and analysed for plasma magnesium.

A further comparable group of 5 suckler cows with calves at foot (mean 5 weeks after calving) were housed and fed identically, and randomly allocated to one of the five experimental treatments described above in a 5 x 5 Latin square. However these cows were supplemented with the equivalent of 5 g magnesium only. Their mean blood magnesium prior to supplementation was 0.83 m mol/l.

Results

The diets and supplements were readily consumed by all the cows. The morning feed of draff to which the supplements was added was generally cleared in about 8 minutes. Due to the short term nature of the trial the cows and calves were not weighed. The cows appeared to lose a little body condition but the calves grew at a satisfactory rate for the system of husbandry i.e. at an estimated 0.8-0.9 kg/day. On this basis it was assumed that the mean daily milk yield was about 8-9 kg.

The mean results are presented in Table 33. The combined mean values for the three 5 x 5 Latin squares where 10.0 g supplementary Mg was given are shown since each set of 5 cows responded in a similar manner. The overall mean blood magnesium concentrations for the three sets of five cows given 1.0 g Mg/day with initial values of 0.59, 0.73 and 0.80 were 0.81, 0.86 and 0.91 m mol/l respectively.

Both 5.0 and 10.0 g additional magnesium provided by all the supplements increased blood magnesium concentrations. Table 33 shows which of these were significant. In no case however were there

significant differences between the four supplements.

Where 10.0 g additional Mg was given blood Mg concentrations were generally 0.04-0.06 m mol/l higher at 13.00 h i.e. 5.5 h after supplementation compared with a mean increase of 0.03 m mol/l for the unsupplemented values. Where the supplement was 5.0 g Mg/day the increase was generally 0.01-0.03 m mol/l (except for 0.06 for the Chinese product) which was in the same order as the increase of 0.04 m mol/l for the unsupplemented cows.

Discussion

Each of the four products given to provide both 5.0 and 10.0 g additional magnesium/day increased blood magnesium concentrations. However no clear conclusions can be made to indicate that any one product was more efficient than the others.

The overall mean concentration for the four supplements was 0.76 m mol/l for the 5 cows given 5.0 g additional Mg/day and 0.81 m mol/l for those 15 cows given 10.0 g Mg/day. The mean value for the unsupplemented 5 cows given only 5.0 g additional Mg/day was only 0.63 m mol/l compared to 0.74 m mol/l for the other 15 cows.

The mean values given in Table 3~~2~~ are a comparison between 5 days of depletion (for the unsupplemented group) and the mean of a variable period of supplementation ranging from 5 days (for the one cow in each square given no previous supplement) to 5, 10, 15 or 20 days for the other 4 cows which would have received one or other supplement in succession before blood sampling. Evidence is presented elsewhere in this thesis (Experiment 18) to show the 3-5 days supplementation is adequate to achieve maximum blood magnesium elevations for ewes in lactation.

It is doubtful if 5 days is adequate for concentrations to fall to their minimum after withdrawal of supplementation. However the overall mean blood magnesium concentration of the 15 cows given 10.0 g additional Mg/day was 0.72 m mol/l at the start of the experiment and the overall mean value of the unsupplemented cows was 0.72. For the 5 cows given 5.0 g additional Mg/day the comparable values were 0.83 and 0.59 m mol respectively. This greater reduction is perhaps indicative that blood magnesium concentrations are lower as lactation proceeds, at least within about the first two months.

A noteworthy finding of this experiment was that supplementation

with all products generally produced increments in blood magnesium concentration which were as large at 07.30 h as they were at 12.00 h i.e. the general effect of once/day supplementation lasts for the whole 24 h and is not of a transient nature of only a few hours as has been shown for sheep elsewhere in this thesis (Experiment 18) and by Wilson (1981) for lactating sheep on indoor diets and by Ritchie & Hemingway (1963) with lactating ewes at grass.

EXPERIMENT 17**An attempt to induce hypomagnesaemia in ewes during pregnancy and lactation given a low magnesium maize-based diet, and the effects of supplementation with various dietary calcined magnesites****Introduction**

In Experiment 15 ewes were transferred to a low magnesium diet approximately 14 days after lambing. Although there were some reductions in blood magnesium concentrations they were neither marked or consistent. It was considered that improved results might be achieved if the low magnesium diet was given for an extended period during pregnancy in the hope that a measure of magnesium depletion might occur.

The objectives of this experiment were accordingly that ewes should be given a low magnesium diet during both pregnancy and in early lactation before receiving supplementary magnesium as various calcined magnesites or as magnesium hydroxide, to determine the effects on blood magnesium concentrations.

Materials and Methods

Twenty four Greyface ewes variously in their second to fourth pregnancies and in lamb to Suffolk tups, were housed in a pole barn with straw bedding at a mean period of approximately 18 days before lambing. Pregnancy scanning had taken place about seven weeks before the start of the experiment and all were assessed as carrying twins or triplet lambs. A low magnesium diet was group-fed in two approximately equal feeds each day at about 07.30 and 16.00 h.

The ewes and their lambs were individually penned at lambing for 2-3 days on straw and thereafter ewes with twin lambs were individually penned (2x2 metres) on wooden slatted floors. The remaining ewes with single lambs (the pregnancy scanning in the event being not particularly accurate) were group-fed in a straw-bedded building. Separate creep areas were established for both sets of lambs.

Various maize fractions and by-products were analysed and several palatability trials of various mixtures were undertaken. As a result the following ingredients were used to form the diet given in both

Table 34. Experiment 17. Composition of dietary ingredients (g/kg DM).

	Flaked Maize	Maize Prairie Meal	Barley Husk Siftings
Crude Protein +	110	600	60
DCP	106	516	10
ME (MJ)	15.0 +++	12.8 *	5.5 ++
Crude Fibre	24	10	263
Ether Extract	33	33	11
Ash	10	11	136
Magnesium +	0.77	0.27	1.03
Calcium +	0.08	0.07	2.01
Phosphorus +	1.97	5.14	1.07

- + Mean of 10 samples taken during course of experiment
 ++ Previous findings Vet. An. Husb. Dept. Glasgow Univ.
 +++ MAFF (1984)
 * BP Nutrition Ltd. Northwich, Cheshire.

Table 35. Mean liveweights of ewes and lambs.

	4 weeks prior to lambing	7 weeks after lambing			
Ewes with single lambs	73	69			
Ewes with twin lambs	72	61			
	Birth Weight	2 weeks	4 weeks	7 weeks	
Single lamb	5.4	9.8	14.6	21.5	
Twin lamb	4.9	9.0	12.3	19.5	

pregnancy and lactation (Table 34). These were mixed in the proportions (FM basis) 2 parts barley husk siftings, 2 parts flaked maize and 1 part maize prairie meal. Calcium carbonate (10%) and sodium chloride (1%) were added to the mixture.

The diet provided an estimated 146 g DCP, 10.8 MJ ME and 0.77 g magnesium per kg DM.

The diet was given to 4 wether sheep in metabolism cages over a period of 14 days (7 day run-in, 7 days collection) to determine digestibility and the availability of the magnesium in the diet. The mean result gave 144 g DCP, 11.4 MJ ME and a magnesium availability of 26.9%.

During pregnancy the ewes were given 1.75 kg and in lactation 2.50 kg (FM) of the low magnesium diet per day. This supplied 222 g DCP, 16.4 MJ ME and 1.23 g magnesium during pregnancy and 317 g DCP, 23.4 MJ ME and 1.75 g magnesium during lactation. Creep feed (composed of 85% barley, 15% soya plus minerals and vitamins) was made available to the lambs from 2 weeks of age.

Results

(a) Preliminary pregnancy and early lactation feeding period

The diet given in pregnancy and for the first weeks of lactation was cubed. Due to severe cubing problems the diet given subsequently was in a loose form. The diet was well consumed and the ewes maintained their good body condition (body score approx. 3) and no problems were encountered at lambing.

The results shown in Table 35 were considered to be very satisfactory according to the system of husbandry.

Blood samples were obtained for magnesium estimation at regular intervals (Table 36). Figure 12 gives the mean values presented not on the calendar date when obtained but in relation to the actual date of lambing. The points given for example at 1 week before lambing represent values for the individual sheep obtained between 1 week \pm 5 days before lambing.

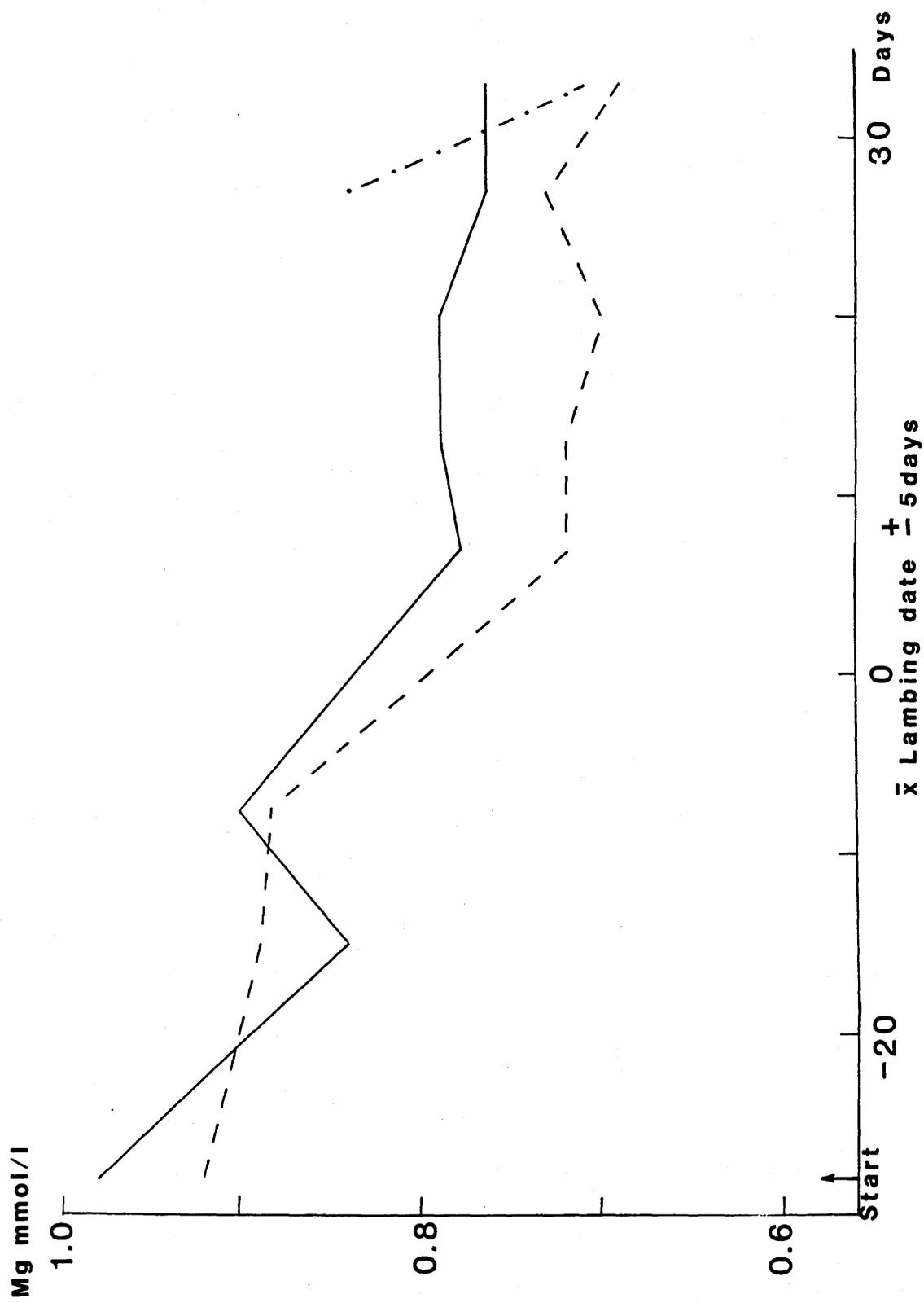
Mean plasma magnesium concentrations for ewes with single lambs fall progressively from 0.98 m mol/l four weeks before lambing to 0.84 m mol/l at lambing and to 0.77 m mol/l by four weeks after lambing. For ewes with twin lambs the mean values were 0.92, 0.80 and 0.69 m mol/l

Table 36. Experiment 17. The mean plasma magnesium concentration (m mol/l) of ewes given a low magnesium diet and the responses to supplementation.

Days before (-) or after (+) lambing	Long Term Depletion		Short term depletion			
	Singles	Twins	Singles	Twins		
n	12	12		6		
- 30	0.98	0.92		-		
- 14	0.84	0.89		-		
- 7	0.90	0.88		-		
Lambing						
+ 7	0.78	0.72		-		
+ 14	0.79	0.72		-		
+ 19	0.79	0.70		-		
+ 27	0.77	0.73		0.84		
+ 33	0.77	0.69		0.71		
After supplementation						
	+Mg	OMG	+Mg	OMg	+Mg	OMg
n	10	2	10	2	5	1
1st 5 days	0.92	0.85	0.87	0.67	0.90	0.96
2nd 5 days	0.94	0.87	0.91	0.82	0.94	0.94
3rd 5 days	0.95	0.86	0.92	0.98	1.10	0.94

Figure 12. Expt. 17 The mean blood magnesium concentration (m mol/l) of ewes given a magnesium deficient diet over a period of 33 days.

(———— ewes with single lambs; — — — ewes with twin lambs ('long term'); (•—•— ewes with twin lambs ('short term'))



respectively. The rate of fall before lambing appeared to be the same as that after lambing. The overall reductions were not as large as might have been expected from the diet composition and there was no individual value below 0.44 m mol/l.

(b) Magnesium supplementation of post-lambing ewes, and experimental design.

All the individual ewes described above, (12 ewes with twins, 12 with singles) were used in the second stage of the experiment. As the numbers of ewes with twins was less than had been anticipated a further 6 ewes of comparable breeding and age, with twins were introduced at this stage. These had not previously received the low magnesium diet but had been given hay and an unmolassed sugar beet pulp and soya mix with minerals and vitamins.

The mean age of their lambs and their mean liveweight at 4 weeks were the same as for the magnesium depleted ewes. The mean blood magnesium concentration of the ewes was 0.84 m mol/l.

The magnesium products evaluated were the four granular calcined magnesites and the magnesium hydroxide previously investigated with caged sheep in Experiment 3. Each product was given in an amount to supply 0.5 g magnesium/day at approximately 4 weeks after lambing. For individually fed ewes with twins in pens on the slatted floor this was added to the morning feed. For the group-fed ewes with single lambs it was given by oral drench in about 75 ml water at the time of the morning feed.

The experimental design was as five 6 x 5 incomplete Latin squares. Two squares were composed of ewes with single lambs and two with twin lambs, all of which had previously been given the low magnesium diet. The remaining square was made up of ewes with twin lambs which had not previously received the low magnesium diet during pregnancy.

Each feeding period was five days. The results of Experiment 18 (to be described) will indicate that three or four days supplementation are adequate to achieve a maximum and stable blood magnesium concentration. On day 5, blood samples were obtained at 07.30 h just before the morning feed and again at 13.00 h. All blood magnesium concentrations which are described and compared in the Results to follow, refer to those taken at 13.00 h. It was considered that these

results best described the effects of supplementation on blood concentrations. Previous findings by Ritchie & Hemingway (1963) and Wilson (1981) with lactating ewes, have shown peak blood magnesium concentrations to occur approximately 4 hours following supplementation.

Results of supplementation

The diet was well consumed and the ewes maintained good body condition. Lamb growth rates were good (ADLWG of twin lambs, 'long term' and 'short term' and single lambs were 309, 325 and 349 g respectively) indicating good milk production by the ewes and reflects the ability of the low magnesium diet to maintain realistic production levels.

(i) Ewes with single lambs.

The mean plasma magnesium concentrations are given in Table 37. Supplementation with 0.5 g magnesium/day increased blood magnesium over the unsupplemented in all cases. Supplementation with magnesium hydroxide produced the highest blood magnesium concentrations (0.97 m mol/l) and the Chinese and Greek the lowest (0.89 and 0.91 m mol/l respectively). None of the increases in blood magnesium concentration were significant.

(ii) Ewes with twin lambs.

The mean plasma magnesium concentrations are given in Table 37. This gives the mean values for both the 'short term' and 'long term' ewes combined, since prior to supplementation their mean plasma magnesium concentrations were similar (0.71 and 0.69 m mol/l respectively).

The lowest mean plasma magnesium value produced was for those ewes receiving Agma (0.84 m mol/l) and not for the unsupplemented ewes (0.89 m mol/l). Supplementation with the hydroxide (0.96 m mol/l) or Chinese (0.97 m mol/l) or Greek material (0.94 m mol/l) appeared to give blood magnesium concentrations which were significantly greater than those when given Agma ($P < 0.05$) but not the nil treatment.

(iii) Combined ewes with twin and single lambs.

The mean blood magnesium concentrations are given in Table 37, and

Table 37. Experiment 17. The mean plasma magnesium concentration (m mol/l) at 13.00 h of lactating ewes given a low magnesium diet on the fifth day of supplementation.

		Ewes with single lambs	Ewes with twin lambs	All ewes
n		6	6	3
Supplement				
Hydroxide	H	0.97	0.97	0.97
Agma	A	0.95	0.84	0.90
Navarros	N	0.95	0.93	0.94
Chinese	C	0.89	0.97	0.93
Greek	G	0.91	0.94	0.93
Nil		0.89	0.89	0.89
SEM		0.035	0.034	0.026
Significance		NS	H,C,G>A*	H> Nil*

Figure 13. Expt. 17 The mean blood magnesium concentrations (m mol/l) of ewes on the fifth day of receiving a magnesium supplement. (An empty circle denotes comparable ewes receiving no supplement). (———— ewes with single lambs; — — — ewes with twin lambs ('long term'); •— •— ewes with twin lambs ('short term'))

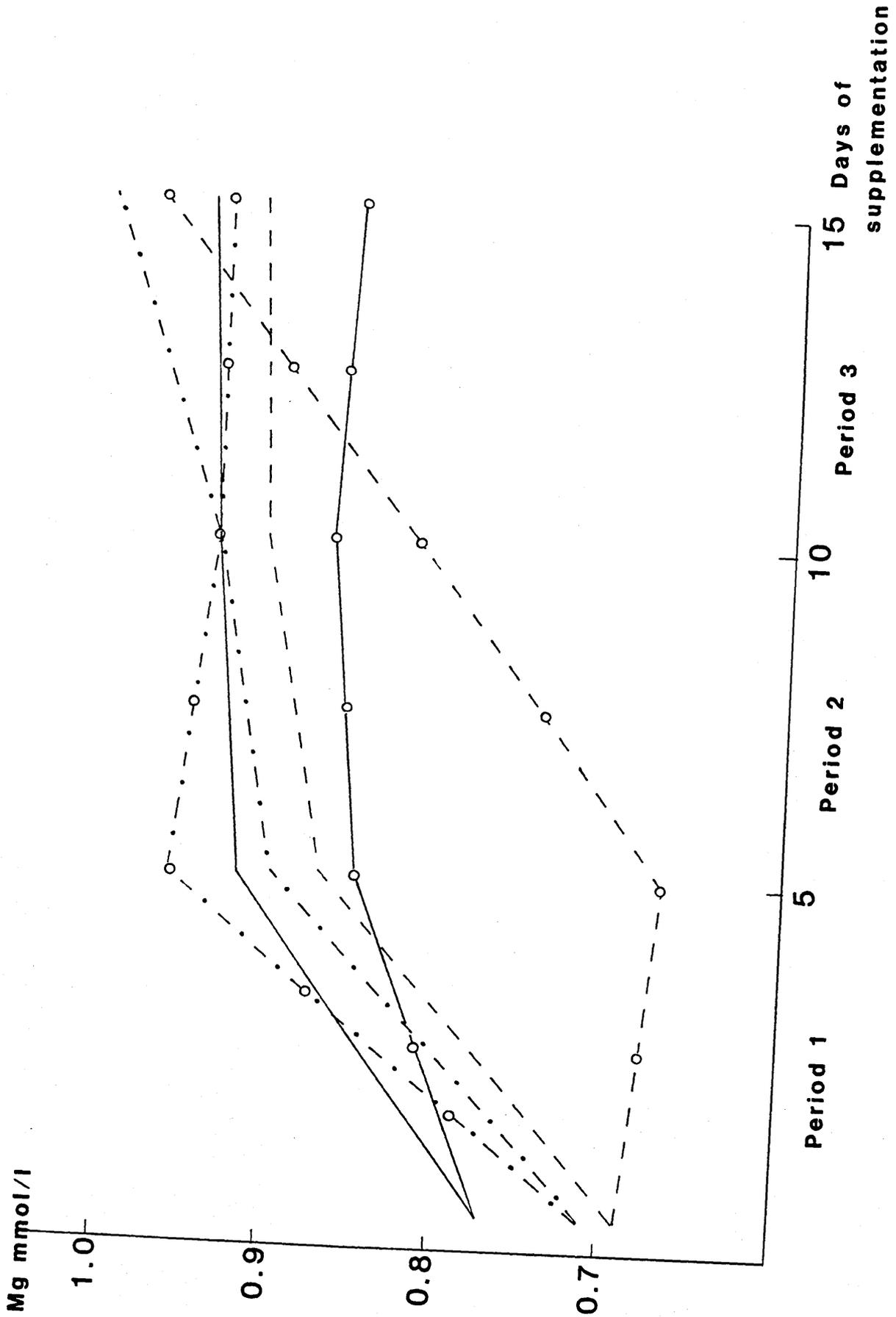


Figure 13 describes the effect of supplementation for the three different groups of ewes over the total 15 days of supplementation.

Ewes supplemented with Agma calcined magnesite produced similar blood magnesium concentrations (0.90 m mol/l) to those of the unsupplemented ewes (0.89 m mol/l).

Ewes supplemented with magnesium hydroxide produced the highest blood magnesium concentrations (0.97 m mol/l) which were significantly greater than the unsupplemented ewes ($P < 0.05$). The Navarras, Chinese and Greek products all produced similar blood magnesium concentrations (0.94, 0.94 and 0.93 m mol/l respectively). No other significant differences occurred between treatments.

As the trial progressed the mean blood magnesium concentrations of all the ewes involved in the experiment consistently increased. The mean blood magnesium concentrations in Period 3 were significantly higher ($P < 0.05$) than at the beginning of the trial (0.89 and 0.96 m mol/l respectively).

Obviously, as the mean blood concentrations were rising the actual 'responses' to supplementation were less meaningful. The rising blood concentrations of magnesium were probably due to a combination of increasing creep feed intake by the lambs and also reduced milk yields. The experiment was therefore discontinued after the third period.

Too much emphasis should not be placed on any apparent significant differences between treatments. Any differences were small and all values (including those for the nil treatments) would be considered as fully normal and at or above the threshold value.

EXPERIMENT 18

An attempt to induce hypomagnesaemia in ewes during lactation when given a low magnesium draff-based diet, and the effects of supplementation with various dietary calcined magnesites

Introduction

All the previous experiments discussed involving feeding low magnesium diets to both suckler cows and pregnant and lactating ewes have involved the use of maize products as a basal ingredient to the diet. The success achieved by using such diets had been mixed. This may have been partly due to the effectiveness of the diet but also to the length of time the animals received the diet relative to the stage of pregnancy/lactation. There is also the possibility that whilst flaked maize may be very low in magnesium, it may well be of high availability and/or give an atypical fermentation pattern in the rumen.

The objective of this trial was to attempt to induce hypomagnesaemia in lactating ewes given a draff-based diet (the draff from the same source had previously been given to suckler cows in Experiment 16) and then to supplement with magnesium in the form of different calcined magnesites.

Materials and Methods

Eighteen Greyface ewes (mean liveweight 66 kg) with either Suffolk or Texel cross twin lambs were housed in a covered yard with straw bedding at a mean period of approximately 2 weeks post-lambing. The ewes were initially group-fed and after receiving the low magnesium diet for 7 days the ewes and their lambs were individually penned in 2 x 2 m pens on a wooden slatted floor. A separate creep area was established for the lambs.

Initially each ewe received approximately 8 kg FM (2.22 kg DM) draff, a by-product from the same whisky distillery used in Experiment 16 with suckler cows, plus 1% limestone/salt supplement. However due to the lower than expected consumption the quantity of draff given was reduced and at the end of 7 days each ewe received approximately 4 kg FM (1.11 kg DM) draff and 1.4 kg (FM) (1.22 kg DM) of the low magnesium diet given in Experiment 17 with pregnant and lactating ewes (2 parts

Table 38. Experiment 18. Analyses of Dietary Compositions (g/kg DM).

	Draff	Flaked Maize	Barley Siftings	Maize Prairie Meal
Dry Matter (g/kg FM)	277	865	871	867
Crude Protein	204	110	59	649
DCP +	151	106	10	516
Crude Fibre	206	24	263	10
Ether Extract	79	33	11	33
Ash	26	10	136	11
ME MJ	11.8	15.0	5.5 ++	12.8 +++
Mg	0.73	0.77	1.03	0.27

+ Assumed using a digestibility coefficient 0.74 (MAFF, 1986)

++ Prev. findings. Vet. Animal Husb. Dept. Glasgow Univ,

+++ BP Nutrition Ltd. Northwich, Cheshire

o Mean of 8 samples taken throughout experiment

barley siftings, 2 parts flaked maize and 1 part maize prairie meal) plus a limestone/salt supplement. The whole diet supplied approximately 347 g DCP, 26 MJ ME and 1.75 g magnesium per head per day (Table 38). The low magnesium diet and draff were weighed out on an individual daily basis, thoroughly mixed and given to the ewes in two approximately equal feeds at 07.30 and 16.00 h.

The ewes were bled after receiving the diet for a preliminary 7 days and the blood analysed for magnesium. The blood magnesium concentrations fell from a mean of 1.04 (S. Dev. \pm 0.24) to 0.47 (S. Dev. \pm 0.21) after 7 days which provided evidence that the diet was deficient in magnesium.

The experimental design was as six 3 x 3 Latin squares, and each ewe was blocked into a group of 3 according to their initial blood magnesium concentrations. Each ewe within a block was randomly allocated to one of 3 diet treatments. By the end of the trial each ewe would have received each diet treatment. Each feeding period was 5 days. These short periods were again used so that the experiment could be completed during early lactation.

The magnesium products evaluated were Agma and Chinese magnesites (used in Experiment 3) and a nil treatment. Each product was given in an amount to supply 0.5 g magnesium/day and was added to the morning feed.

During the first period the ewes were bled each day at 07.30 h (before feeding) and again at 12.00 h. Inspection of the results for the first period of 5 days showed that supplementation consistently increased blood magnesium concentrations so that each supplemented sheep reached a peak value by day 4 or day 5 (Figure 14). As a result of this it was decided to obtain blood samples on days 1, 4 and 5 only for the following two feeding periods.

Results

The diet was well consumed over the whole experimental period of 15 days. However the ewes lost body condition from about 2.5 to 2 with a mean weight loss of 9.4 kg over the 23 day trial. This is perhaps rather more than would be expected for ewes with twin lambs, but at the same time it was not exceptional.

The mean plasma magnesium concentrations are given in Table 39 and Figure 14.

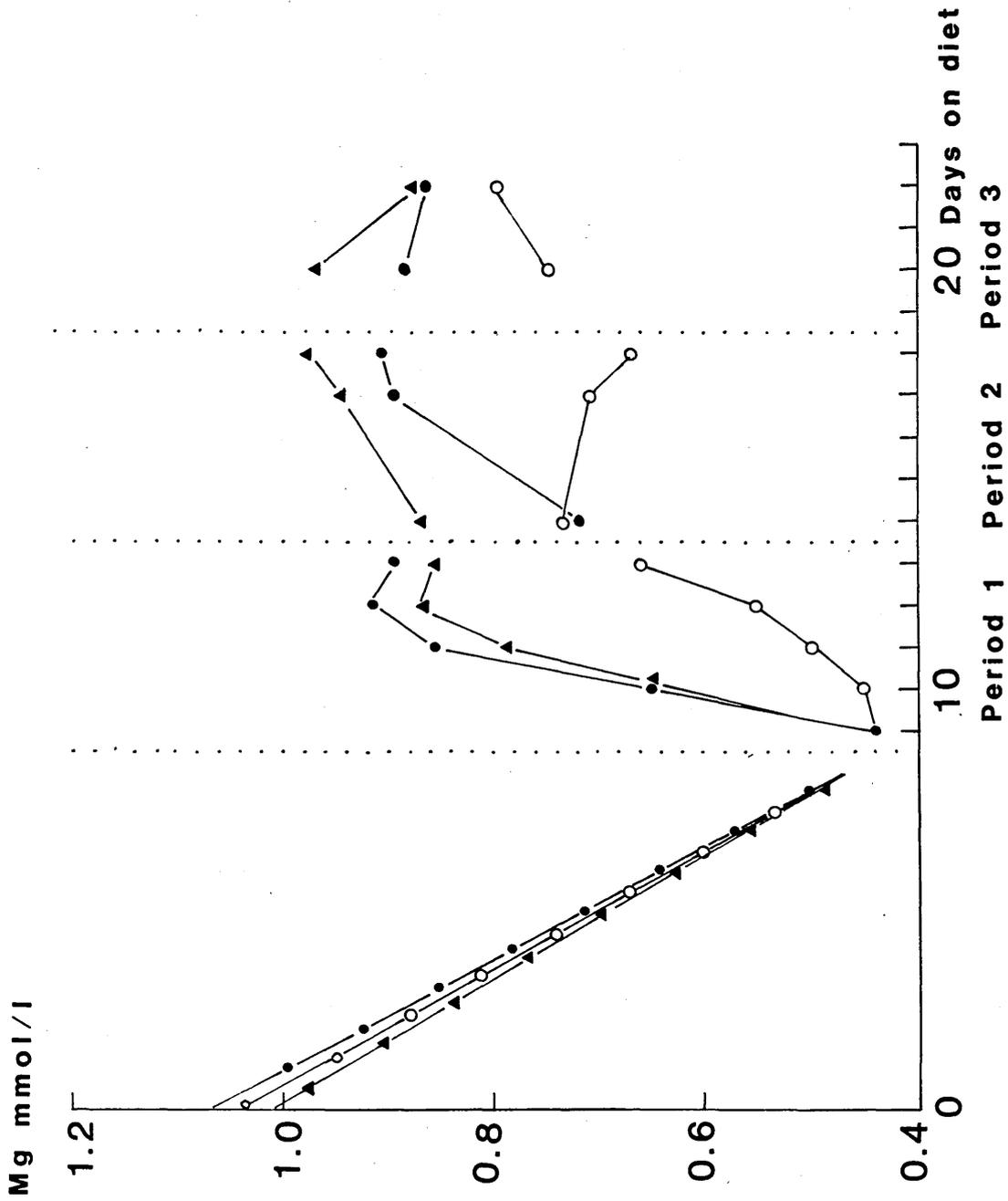
Table 39. Experiment 18. The mean effects of two calcined magnesites on the blood magnesium concentration (m mol/l) of lactating ewes with twin lambs given a low magnesium diet (6 ewes/treatment for each period, 18 in total).

Supplement	Period	1	2	3	Mean
Agma	A	0.92	0.90	0.87	0.90
Chinese	C	0.88	0.95	0.88	0.90
Nil	O	0.55	0.71	0.80	0.69
SEM		0.031			
Significance		A,C>O	P< 0.001		

The initial mean concentration was 1.05 m mol/l. This was reduced over 7 days to be 0.47 m mol/l at the start of the experiment.

Figure 14. Expt. 18 The mean blood magnesium concentrations (m mol/l) of lactating ewes given a low magnesium deficient diet and the effects of supplementation with calcined magnesites

(o—o nil supplement; ●—● Agma; ▲—▲ Chinese)



During period 1 (days 1-5) both supplements progressively increased mean blood magnesium concentrations from about 0.47 (Day 0) to about 0.90 m mol/l (Day 5), an increase of about 0.4 m mol/l. At the same time the mean value for the unsupplemented ewes increased steadily to about 0.65 m mol/l. By day 5 the difference between supplemented and unsupplemented ewes was thus about 0.25 m mol/l.

In Period 2 the six ewes given the unsupplemented diet would have received one or other of the supplements for the previous 5 days. Their mean blood magnesium concentrations fell progressively in the 5 days of Period 2 from about 0.84 to about 0.67 m mol/l as measured by the blood samples obtained at 12.00 h. Most of the fall occurred within the first day. Equally, four of the six ewes given either of the supplements would also have had supplementary magnesium in Period 1. There was thus a rather less mean effect of supplementation, but by day 5 of Period 2 the mean concentrations for ewes given either Agma or Chinese calcined magnesites were both about 0.95 m mol/l and were again about 0.3 m mol/l greater than for the unsupplemented ewes.

By the end of Period 3 the mean blood magnesium concentration of the unsupplemented ewes had increased to about 0.8 m mol/l and the response to supplementation had almost entirely disappeared. Nevertheless both supplements had equal and highly significant overall mean effects (Table 39).

Discussion

The large fall in the overall mean blood magnesium concentration of the ewes over the preliminary feeding period of 7 days when the low magnesium diet was introduced, demonstrated quite clearly that at that stage of lactation the ewes were developing marked hypomagnesaemia. The rate of fall in blood magnesium concentration (0.07 m mol/l per day) was greater than that previously demonstrated elsewhere with other low magnesium diets, e.g. Ritchie *et al* (1962) with non-pregnant, non lactating Cheviot ewes achieved a fall in blood magnesium concentration of 0.03 m mol/l/day during the first 8 days of receiving a low magnesium diet consisting of 650-700 g barley straw, 100 g sucrose and 100 g casein per day.

The rise in mean blood magnesium concentration of the 6 ewes given no magnesium supplementation during Period 1, i.e. with no change in diet, is perhaps most likely to reflect a reduction in milk yield of

the ewes as the increasing age of the lambs (now 4 weeks old) led to a greater consumption of creep feed. The steady increase in mean blood magnesium concentration of the unsupplemented ewes (Figure 14) throughout the whole 3 periods of the experiment perhaps provides further evidence for this supposition. Figure 14 also gives the impression that 0.5 g supplementary magnesium given in one feed at 07.30 h was unlikely in the circumstances of this experiment to give a mean blood magnesium concentration at 12.00 h higher than about 0.9 mmol/l.

EXPERIMENT 19**The induction of hypomagnesaemia in pregnant and lactating suckler cows given a low magnesium draff-based diet, and its alleviation with various calcined magnesites****Introduction**

Experiment 16 describes an attempt to induce low blood magnesium concentrations in lactating suckler cows given a diet based on a low magnesium draff (0.74 g Mg/kg DM). The objective of this experiment was to reduce blood concentrations during pregnancy and the cows were given a barley-draff obtained from a specific whisky distillery which contains a low concentration of magnesium (0.64 g Mg/kg DM).

Materials and Methods

Twenty two suckler cows (Friesian x Hereford) in calf to a Charolais bull were housed on straw in a covered yard approximately 12 weeks prepartum, to become accustomed to a diet low in magnesium.

The cows individually received approximately 14 kg Draff (FM) from a specific whisky distillery plus 50 g salt/limestone mixture per head per day divided into two feeds given at 07.30 h and 16.00 h. They also received approximately 7 kg barley straw per head group-fed from a ring feeder. This provided 604 g DCP, 82 MJ ME and 6.1 g magnesium (Table 40).

After 3 weeks one of the cows died from hypomagnesaemic tetany and the cows were moved to a covered yard with headlocking feeders and were divided into 3 groups of 5 cows with an overall blood magnesium of 0.40 m mol/l. Three other cows with extremely low blood magnesium levels (mean 0.15 m mol/l) were supplemented with magnesium as calcined magnesite separately to the experimental group. A further three cows with higher blood magnesium levels (mean 0.76 m mol/l) continued on the low magnesium in order to obtain further low blood magnesium values, again separately to the main group.

At this point the cows received 0.5 kg flaked maize per head daily which was increased to 1.0 kg four weeks later due to the poor body condition of the cows. This, with the straw provided 680 g DCP, 95 MJ ME and 6.7 g magnesium. A further reason for the addition of the flaked

Table 40. Experiment 19. Composition of feedstuffs (g/kg DM)
(mean of 12 samples).

	Draff	Flaked Maize	Barley Straw
ME MJ	11.8	15	6.5
Crude Protein	204	92	38
DCP	151	88	9
Crude Fibre	217	32	452
Ether Extract	95	39	12
Ash	27	13	48
Magnesium	0.64	0.71	0.63

Table 41. Experiment 19. Mean blood magnesium concentrations of suckler cows supplemented with different calcined magnesites supplying different amounts of magnesium (5 cows/treatment); all values refer to bloods obtained on day 5 of supplementation).

Supplement		Initial blood Mg concentration prior to supplementation				
			2 g	4 g	5 g	6 g
Nil	O	0.40	0.40	0.41	0.45	0.51
Agma	A	0.43	0.43	0.50	0.57	0.63
Chinese	C	0.40	0.39	0.46	0.64	0.68
SEM	-	-	0.055	0.139	0.046	0.062
Significance	-	-	NS	NS	C>O*	NS

Supplement				
Nil	O	0.24	0.22	0.13
Navarros	N	0.24	0.26	0.34
Greek	G	0.24	0.24	0.26
SEM			0.048	0.073
Significance			NS	NS

maize was to give the cows more rumen-soluble carbohydrate.

The fifteen cows were divided into 3 groups of five. One group received supplemental magnesium as Agma, another as Chinese and the third received no supplement. The materials were identical to those used in Experiment 3 where the apparent availability of the Agma was 27.0 % and the Chinese 11.0 %. The cows received 2 g supplemental magnesium for one week. They were bled on days 5 and 7 of supplementation at approximately 12.00 h and the blood plasma analysed for magnesium. The supplementary magnesium was increased to 4 g, 5 g and 6 g for the following consecutive weeks.

After 4 weeks of supplementation all the cows received no supplement in order to reduce blood magnesium levels again. After 2 weeks the mean blood magnesium was 0.24 m mol/l and the cows were regrouped into 3 groups of five and received supplemental magnesium as Navarras and Greek calcined magnesite, (again identical materials to those used in Experiment 3) with one group receiving no supplement. The cows received increasing amounts of supplemental magnesium each week as described earlier and were bled on days 5 and 7 of supplementation.

Results

At the beginning of the experiment the consumption of draff was good. Broadbent (1967) fed suckler cows 18 kg of fresh draff with straw and more recently M. Lewis of the ESCA persuaded fistulated steers (500 kg) to consume 9 kg/day (R. Pass, Pentlands, Personal Communication, 1989). Thus 14 kg fresh draff seemed a suitable amount to give to suckler cows. However after approximately 9 weeks into the experimental period feed refusals became relatively frequent particularly for specific cows. The trial was eventually abandoned after 10 weeks due to more frequent feed refusals. Accordingly the treatments involving the addition of 5 g and 6 g magnesium as Navarras and ~~Greek~~ calcined magnesite were not given.

The flaked maize was fed due to increasing concern over the body condition of the pregnant cows. A number of cows had a body condition score of about 2.

The mean calving date was 16 March, approximately 9 weeks after the start of the experimental period.

One cow died from clinical hypomagnesaemia and three of the original group of twenty two had critically low blood magnesium levels

(mean 0.15 m mol/l) although none of these three cows actually exhibited outward signs of hypomagnesaemia. The three cows were supplemented with 10 g magnesium as Agma calcined magnesite and two of the cows responded with increased blood magnesium levels. However the third cow started to show signs of hypomagnesaemia e.g. excitability and tremors and produced a blood magnesium concentration of 0.09 m mol/l. Magnesium sulphate was administered subcutaneously and the cow was also drenched with 40 g of Agma for the following three days and received a diet of sugar beet pulp and hay. Within 3 days the cow had totally recovered.

The ARC (1980) recommended dietary allowances of magnesium for a pregnant cow between 28 and 40 weeks of pregnancy is 11.7 - 15.5 g magnesium per day. The diet supplied approximately 6.8 g magnesium and thus can be considered to be magnesium deficient. The diet was very successful in reducing blood magnesium concentrations.

Three weeks after the introduction of the diet the mean blood magnesium concentration of the fifteen cows, which were subsequently to be supplemented was 0.41 m mol/l. Table 41 and Figure 15 describes the effect of supplementation on the blood magnesium concentrations.

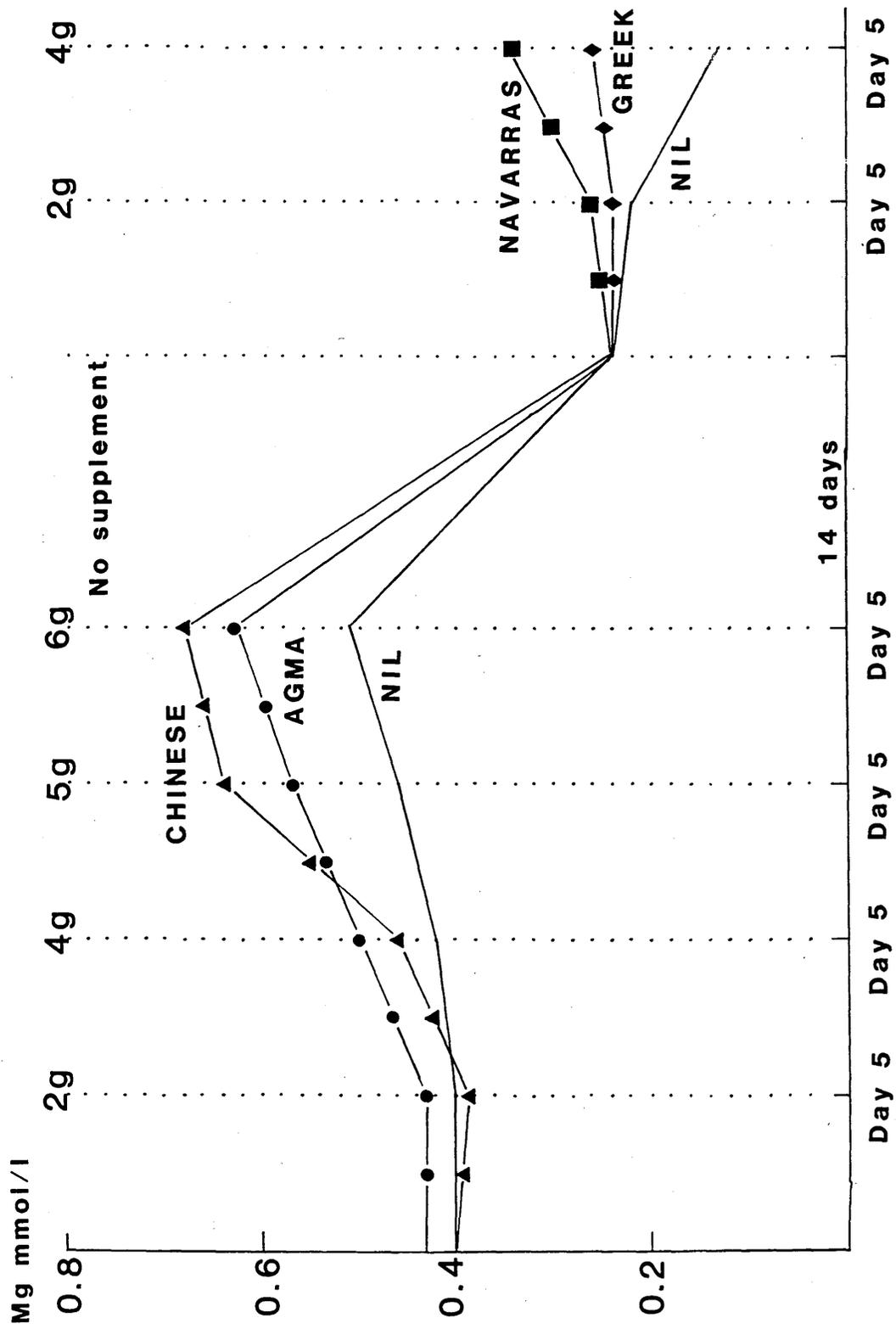
All blood concentration values refer to those obtained on day 5 of supplementation. The values obtained on day 7 were very similar to those obtained on day 5. This agrees with other findings e.g. Experiment 18 with sheep where a peak in blood magnesium concentrations occurred after 5 days of supplementation.

All supplements increased the blood magnesium concentrations. However it would not be justified to compare the effects of the Navarras and Greek with the Chinese and Agma since the cows were beginning to refuse feed by day 5 of supplementation with 4 g magnesium as the Navarras and Greek products.

Although blood magnesium concentrations were higher following supplementation with different levels of magnesium, the only treatment that produced a significant increase in concentration was 5 g magnesium in the form of the Chinese product ($P < 0.05$). All other differences were not significant.

Following supplementation with 6 g of magnesium the blood concentrations were allowed to fall as a result of no supplementation. Within 14 days the mean blood magnesium of all 15 cows had fallen to 0.24 m mol/l. The cows were then supplemented with magnesium in the form of Navarras and Greek (see Figure 15). Again, magnesium

Figure 15. Expt. 19 The mean blood magnesium concentrations (m mol/l) of suckler cows following supplementation with various calcined magnesites (obtained on day 5 of supplementation) (5 cows/treatment)



supplementation increased blood magnesium concentrations but to no significant effect. However by this stage the cows were refusing feed and the unsupplemented cows had a mean blood magnesium as low as 0.13 m mol/l. One individual cow on the nil treatment had a blood magnesium value as low as 0.08 m mol/l. The decision was thus taken to abandon the experiment.

EXPERIMENT 20**An attempt to induce hypomagnesaemia in ewes during pregnancy when given a low magnesium draff-based diet, and the effects of supplementation with various calcined magnesites****Introduction**

Experiment 17 described earlier attempted to induce hypomagnesaemia in ewes during pregnancy in the hope that a greater depletion in blood magnesium might occur. Also in Experiment 18 marked hypomagnesaemia in lactating ewes was achieved with a diet consisting of draff and a 'flaked maize mix' from Experiment 17. The aim of this particular trial was to attempt to induce hypomagnesaemia using a similar diet given to ewes during pregnancy.

Materials and Methods

Twenty one Greyface pregnant ewes (in lamb to either a Suffolk or Texel ram) of mean liveweight 70 kg were housed in a covered yard with straw bedding at a mean period approximately 7 weeks pre-partum. The ewes were group-fed throughout the trial and received approximately 0.82 kg draff (identical material fed to suckler cows in Experiment 19) and 0.82 kg (DM basis) 'flaked maize mix' (2 parts barley husk siftings, 2 parts flaked maize and 1 part maize prairie meal plus 1% limestone/salt mix). This supplied approximately 241 g DCP, 18.5 MJ ME and 1.14 g magnesium per head per day during pregnancy (Table 42).

The ewes all produced twin lambs (mean birth weight 5.6 kg). After lambing the ewes received 1.10 kg DM draff and 1.10 kg DM 'flaked maize mix' but due to poor consumption the diet was changed to 0.82 kg draff and 1.10 kg 'flaked maize mix' (DM basis). This supplied approximately 280 g DCP, 21.5 MJ ME and 1.35 g magnesium per head per day. The 'flaked maize mix' and draff were weighed out on a daily basis, mixed and given to the ewes in two approximately equal feeds at 07.30 h and 16.00 h.

The ewes were bled initially every 7 days up to lambing when they produced a mean blood magnesium concentration of 0.93 m mol/l. Fourteen days later the mean blood magnesium concentration was 0.43 m mol/l. The blood concentrations were considered sufficiently low to commence

Table 42. Experiment 20. Composition of feedstuffs (g/kg DM)
(mean of 12 samples).

	Draff	Flaked Maize	Maize Prairie Meal	Barley Siftings
ME MJ	11.8	15.0	12.8	5.5
Crude Protein	204	92	600	60
DCP	151	88	516	10
Crude Fibre	217	32	10	263
Ether Extract	95	39	33	11
Ash	27	13	11	136
Magnesium	0.64	0.71	0.27	1.03

supplementation (approximately 2 weeks after lambing).

The experimental design was as three groups of seven ewes. The three treatments evaluated were calcined magnesite supplemented as Agma and Chinese (products from Experiment 3 with comparative availabilities of 27.0 and 11.0 %) and a nil treatment. Each of the seven ewes within the group received the same treatment. The ewes received the magnesium supplemented at 1.0 g, 0.5 g and 0.75 g, each for a period of 5 days in sequence as an oral drench in minimal water (approximately 75 ml) immediately before feeding at 07.30 h.

The ewes were bled on day 5 of supplementation at approximately 13.00 h.

Results

A few problems were encountered with poor consumption of feed post-lambing. However when the ratio of 'flaked maize mix' to draff was increased the diet was well consumed. Two ewes lost body condition, but on the whole the diet appeared to maintain realistic production levels and the lambs grew well.

The mean plasma magnesium concentrations are given in Table 43 and Figure 16. During Period 1 (days 1-5) both supplements increased mean blood magnesium concentrations from 0.43 (day 0) to about 0.70 (day 5). At the same time the mean value for the unsupplemented ewes increased to 0.46 m mol/l. By day 5 the difference between supplemented and unsupplemented was thus about 0.24 m mol/l.

In Period 2, despite receiving half the amount of supplemental magnesium (0.5 g) the mean value for the supplemented ewes was about 0.79 m mol/l (day 5) and that of the unsupplemented ewes 0.55 m mol/l, again a difference of about 0.24 m mol/l.

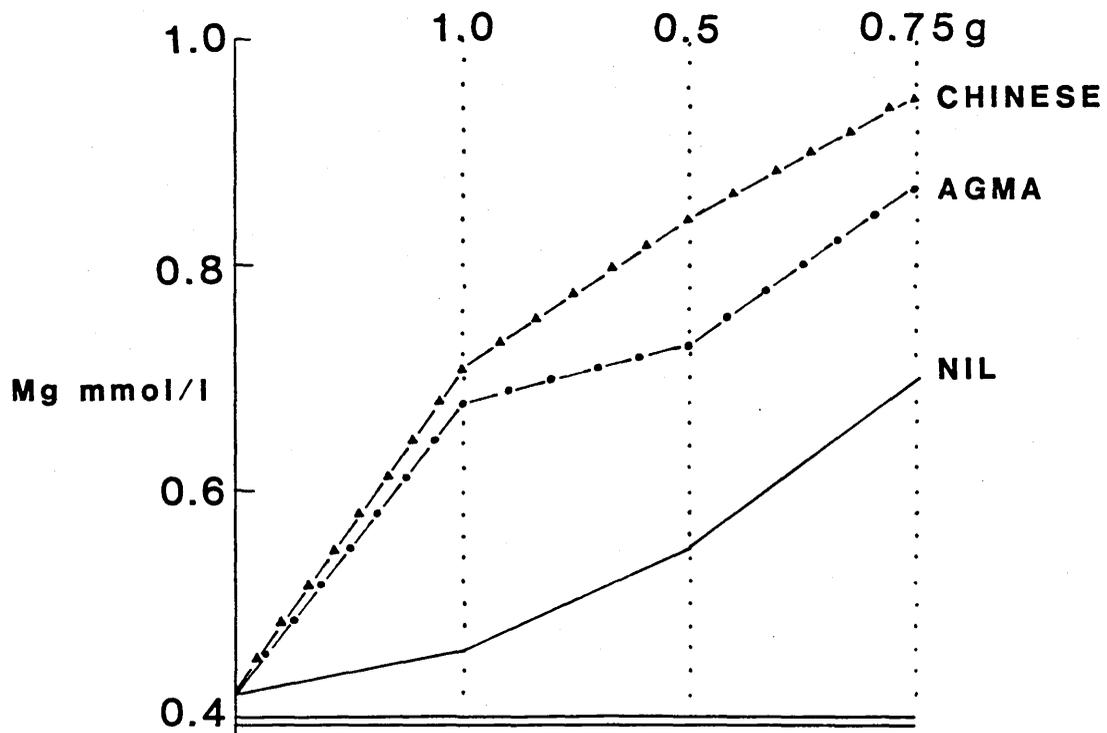
In Period 3 ewes receiving 0.75 g supplemental magnesium produced a mean blood magnesium concentration of about 0.91 m mol/l compared to a value for the unsupplemented ewes of 0.70, a difference of about 0.21 m mol/l.

At all levels of supplementation the Chinese material produced significantly higher blood magnesium levels when compared with unsupplemented ewes (0.5 and 1.0 g, $P < 0.05$; 0.75 g, $P < 0.01$). The Agma produced significantly higher blood magnesium concentrations ($P < 0.05$) when supplemented at 0.75 g. By this stage of the trial (approximately four weeks post-partum) the blood values of the supplemented and

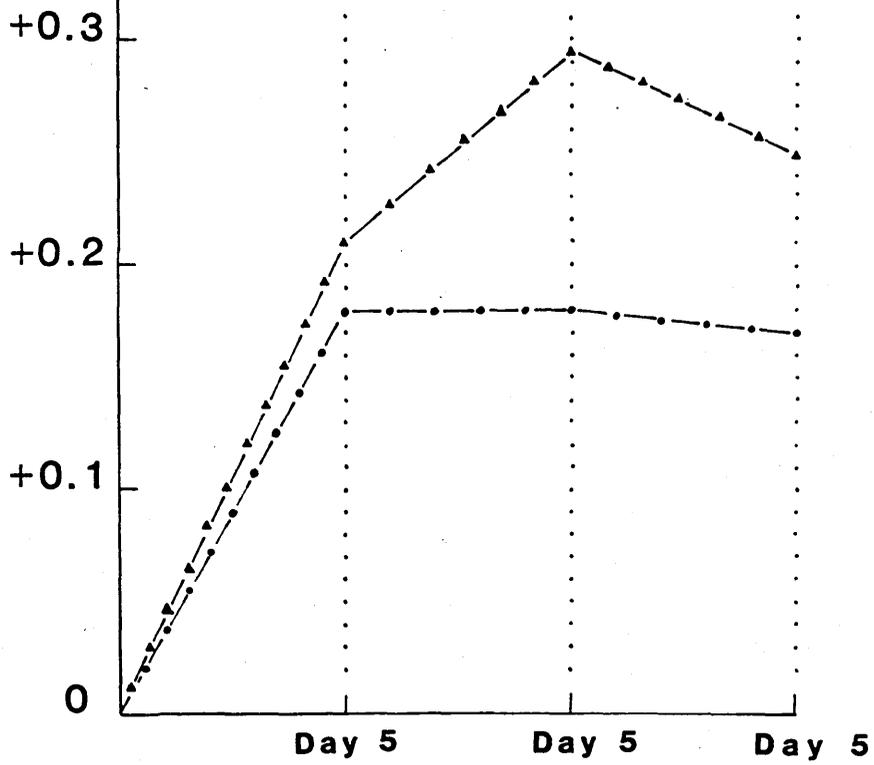
Table 43. Experiment 20. Mean blood magnesium concentrations of lactating ewes supplemented with different calcined magnesites supplying differing amounts of magnesium (7 ewes/treatment; all values refer to bloods obtained on day 5 of supplementation).

Supplement		Initial blood Mg concentration prior to supplementation	1 g	0.5 g	0.75 g
Nil	O		0.43	0.46	0.55
Agma	A	0.43	0.68	0.73	0.87
Chinese	C	0.43	0.71	0.84	0.95
SEM	-	-	0.072	0.076	0.054
Significance					
P < 0.01					C > N
P < 0.05			C > N	C > N	A > N

Figure 16. Expt. 20 The mean blood magnesium concentrations of lactating ewes following supplementation with various levels of magnesium as calcined magnesite. (7 ewes/treatment) (a) The increase in blood magnesium as a result of supplementation (m mol/l)



(a)



unsupplemented ewe had achieved what would be considered as normal concentrations, and as a result the trial was discontinued.

Discussion

Problems were encountered earlier with the feeding of draff to ewes (Experiment 18). It would appear from these findings and those recorded here that ewes will not consume much more than 1 kg DM (approximately 4 kg fresh weight) draff per head per day. In association with maize products this provides a satisfactory diet that is both low in magnesium, palatable and able to maintain satisfactory production levels.

It was interesting to note that reducing the supplemental magnesium by half (i.e. from 1.0 to 0.5 g) the blood magnesium concentrations did not fall. In fact the increase in mean blood magnesium concentration due to supplementation was identical for both the 0.5 and 1.0 g levels of supplementation. Quite small additions of magnesium may have large effects on blood magnesium. Ritchie et al (1962) found that 150 mg magnesium given as a daily drench maintained plasma magnesium levels between 0.66 - 0.74 m mol/l, compared to 0.45 - 0.49 m mol/l for the unsupplemented ewes.

GENERAL DISCUSSION

The main objective of all the experiments described in this section was to induce hypomagnesaemia in the animals and then to study the relative efficacies of different calcined magnesites in terms of plasma magnesium response and to determine whether these results could be correlated to apparent availability results.

In all the experiments magnesium was supplied in the form of calcined magnesite evaluated in Experiment 3. In Experiments 16 and 17, all supplements were used but in Experiments 18, 19 and 20 only the Agma and Chinese products were investigated against a nil treatment. These two products showed the greatest disparity in terms of availability (27.0 and 11.0 %) and it was hoped to determine whether they would raise blood magnesium levels to any greater extent.

The aim was to provide diets that were well balanced nutritionally in all aspects except for the magnesium deficiency. It was considered preferable to use entirely natural feeding stuffs as these would be generally more acceptable to the animal and should produce a more 'natural' rumen fermentation. Since all trials described involved pregnant and lactating ewes and suckler cows it was important to maintain satisfactory levels of production, i.e. milk yield. Also some diets had to support the animals for long periods - during pregnancy and lactation.

Some diets were successful in achieving hypomagnesaemia to a greater degree, and others appeared to have no effect.

Experiment 14 with lactating suckler cows and Experiment 15 with lactating ewes given a magnesium deficient diet based on flaked maize and dreg were both unsuccessful in inducing hypomagnesaemia. After 21 days on the diet the ewes had a mean blood magnesium concentration of 0.87 m mol/l and the mean blood concentration of the suckler cows after 38 days was 0.92 m mol/l. Experiment 16 with lactating suckler cows given a draff-based diet was slightly more successful in reducing blood magnesium concentrations. However these did not fall below 0.59 m mol/l and thus cannot be considered to be markedly hypomagnesaemic. Although significant differences in blood magnesium concentrations occurred when comparing supplemented and unsupplemented, the unsupplemented cows had relatively normal blood magnesium concentrations (i.e. a mean of 0.63 and 0.73 m mol/l).

In Experiment 17 ewes were given a low magnesium maize-based diet

during pregnancy and lactation in the hope that a greater measure of magnesium depletion might occur. The rate of fall in blood magnesium concentrations appeared to be similar pre- and post-partum. After 8 weeks on the diet the mean blood magnesium concentration of ewes with single lambs was 0.77 m mol/l and for ewes with twin lambs, 0.69 m mol/l. However the overall reductions were not as large as expected and there were no individual values below 0.41 m mol/l.

One of the most successful inductions of hypomagnesaemia occurred with lactating ewes on a draff and maize diet (Experiment 18). After 7 days on the diet the blood concentrations fell from a mean of 1.04 to 0.47 m mol/l. As a result of this success, in Experiment 19, pregnant and lactating suckler cows received a draff/maize/straw diet. The mean blood magnesium concentrations were 0.40 m mol/l with individual blood values as low as 0.09 m mol/l.

The final trial (Experiment 20) involved feeding a draff based diet to pregnant and lactating ewes. They received the diet approximately 7 weeks pre-partum and at lambing the mean blood magnesium concentration was 0.93 m mol/l, however 14 days later the mean magnesium concentration had fallen to 0.43 m mol/l.

Despite extremely marked hypomagnesaemia there were only two clinical cases of hypomagnesaemic tetany occurring in suckler cows (from Experiment 19) out of a total of seven trials, one of these cases was fatal. This agreed with findings of Hemingway & Ritchie (1965) that ruminants appear able to experience severe hypomagnesaemia for long periods without actual signs of tetany.

In Experiment 18 with ewes, a peak concentration of blood magnesium occurred on day 5 of supplementation. As a result of this finding all blood samples were henceforth taken at this time. Except for Experiment 16 with suckler cows, a magnesium supplement given once daily (at 07.30 h) appeared to have a transient effect on plasma magnesium concentration. This agreed with findings by Wilson (1981) where a peak concentration was observed at 4 h after the administration of the supplement to ewes indoors, and also with findings by Ritchie & Hemingway (1963) with ewes at grass. Plasma magnesium levels steadily decreased after the 12.00 h peak to approximately the original at 07.30 h. This obviously has very important implications for the practice of administering magnesium supplements for the prevention of hypomagnesaemic tetany. Many casualties occur at night and if supplementary magnesium is given once daily in a morning feed, animals

would have their lowest plasma magnesium concentrations at night when stress factors e.g. cold temperatures are also implicated. Thus frequency rather than quantity of magnesium supplementation may be the critical factor.

The pattern of blood response seen only a few hours after administration of magnesium supplements indicates rapid absorption from the rumen.

Wilson (1981) distinguished between magnesium supplements that gave better responses in blood concentrations than others. Supplements with higher apparent availability raised plasma magnesium to a greater extent and associated with this, fine calcined magnesite appeared better than coarse fractions.

In the trials discussed here supplementation in some cases significantly increased blood magnesium concentrations, but it was difficult to distinguish between supplements, although the Chinese on the whole appeared to be more effective when compared to the Agma (Experiments 18, 19, 20) but not significantly so. This obviously does not agree with Wilson (1981) since the Chinese had been shown to be the least available in balance trials (Experiment 3).

Quite small additions of magnesium (75-150 mg) have been shown to have large effects on blood magnesium (Ritchie & Hemingway, 1962) and it is important that when comparing products too much should not be given as they may then appear to be equal.

SECTION 6

SCANNING ELECTRON MICROSCOPY

SCANNING ELECTRON MICROSCOPY

Introduction

The aim of this investigation was to look at four different consignments of Agma, Navarras, Chinese and Greek calcined magnesites investigated earlier for bioavailability (Experiments 1, 2, 3 and 4), under low and high resolution to identify any possible differences between the various sources. At the same time the samples are subjected to X-ray microanalysis to determine the presence of impurity ions.

In addition the magnesites from Experiment 3 were exposed to rumen conditions for 48 hours by the Nylon Bag Technique (see Experiment 13) and were investigated to identify the effects of rumen conditions on the different magnesite samples. Further samples of the same materials were then exposed to the rumen for 7 days. The objective of this investigation was to determine the prolonged effect of exposure to rumen conditions, and identify more clearly the effects of rumen liquor etching on the crystals.

Method and Materials

The materials investigated using the Scanning Electron Microscope were the four major calcined magnesites from each of the four different batches investigated earlier in Experiments 1, 2, 3 and 4 together with the Agma, Navarras, Chinese and Greek materials used in Experiment 3 having been exposed to the rumen for both 48 hours and for 7 days.

The calcined magnesite samples were prepared for Scanning Electron Microscopy as follows:

Samples of the different materials were mounted on double sided tape on aluminium stubs. They were then coated for two minutes with gold palladium and viewed in a Phillips 501 B Scanning Electron Microscope at 15 KV from x 20 to x 2,500 magnification. Simultaneous microanalysis was carried out using an Edax micro analyser to identify the presence of impurity ions.

Results and Discussion

(i) Calcined magnesite samples.

The gross appearance of the calcined magnesite samples show the different products to differ both in texture and colour; Navarras and Agma are finer and darker with a light brown colouration and the coarse grained products from China and Greece are white.

The Scanning Electron Microscopy confirmed the differences in grain size and extended the original observation to reveal that the Greek particles are quite different to the other three products. The Agma, Navarras and Chinese samples show particles of a rectangular and faceted nature with straight sides, which although similar were not identical. The Greek sample on the other hand was characterised by the rounded nature of the particles (Figures 17, 18, 19 and 20). The presence of planes along which particles can cleave, may affect the ease with which acids can attack the crystals.

Comparative analyses between the four different batches of the four major magnesites show a uniformity in gross morphology suggesting that they are structurally similar with regard to their country of origin. This similarity in gross morphology only suggests that the magnesites were formed under identical environmental conditions. Unfortunately it appears that this technique cannot be used as a means to identify materials derived from the same magnesite mine.

(ii) Calcined magnesite samples after 48 h in the rumen.

There appeared to be no appreciable difference in the gross morphology of the magnesites after the samples had been placed in the rumen for 48 hours. The Agma, Navarras and Chinese materials still possessed a straight edged faceted appearance, resembling many building blocks. The Greek particles were similarly of a rounded nature (see Figures 21, 22, 23 and 24).

More notable however, were the appearance of cracks on the surface of the crystal blocks which could easily be identified on low magnification (x 80). These cracks were not present on the original material and show the effect of rumen acids by the particles. No individual magnesite appeared fissured to any greater extent than the others.

Figure 17 Agma x 80 (Spanish calcined magnesite)

Figure 18 Navarras x 80 (Spanish calcined magnesite)



Figure 19 Chinese calcined magnesite x 80

Figure 20 Greek calcined magnesite x 80



Figure 21 Agma after 48 h in bovine rumen x 80

Figure 22 Navarras after 48 h in bovine rumen x 80



Figure 23 Chinese after 48 h in bovine rumen x 80

Figure 24 Greek after 48 h in bovine rumen x 80



Figure 25 Agma after 48 h in bovine rumen x 2500

Figure 26 Navarras after 48 h in bovine rumen x 2500

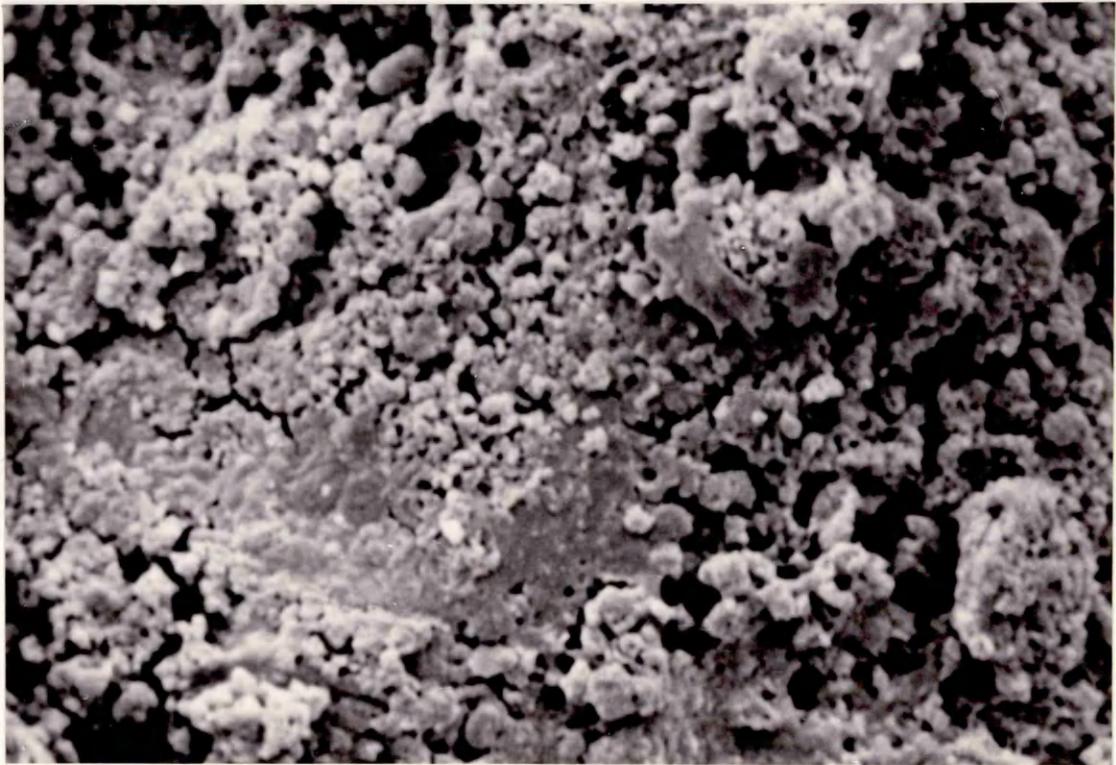
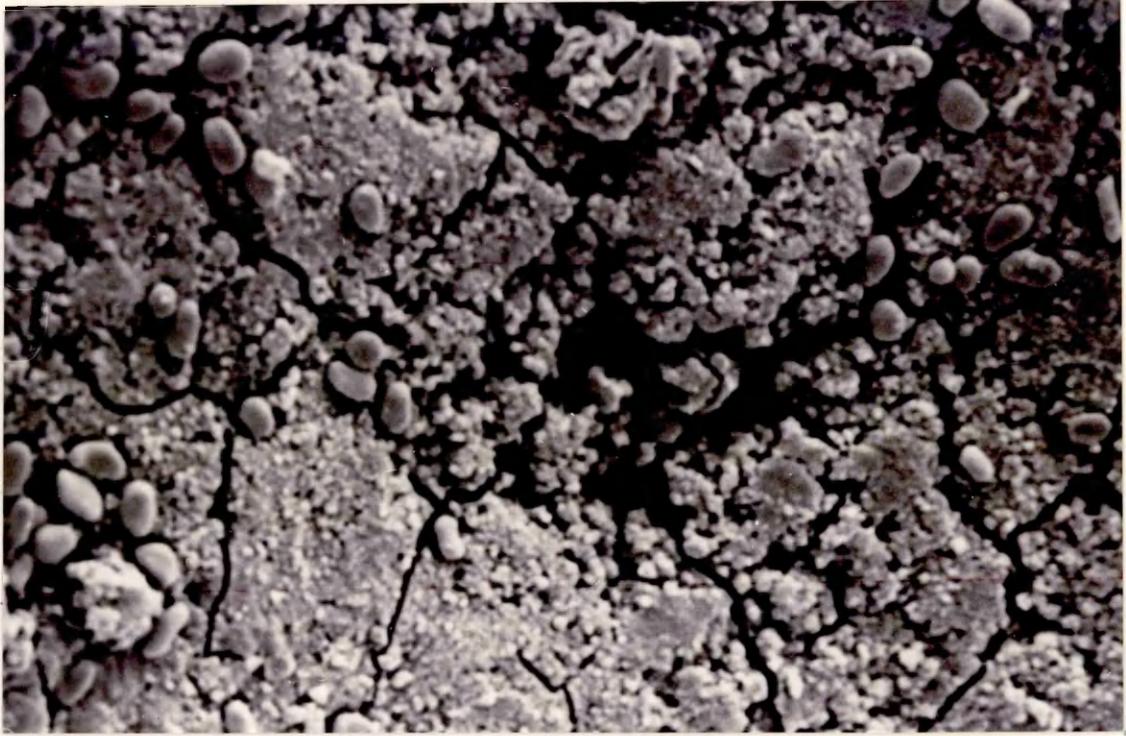


Figure 27 Chinese after 48 h in bovine rumen x 2500

Figure 28 Greek after 48 h in bovine rumen x 2500

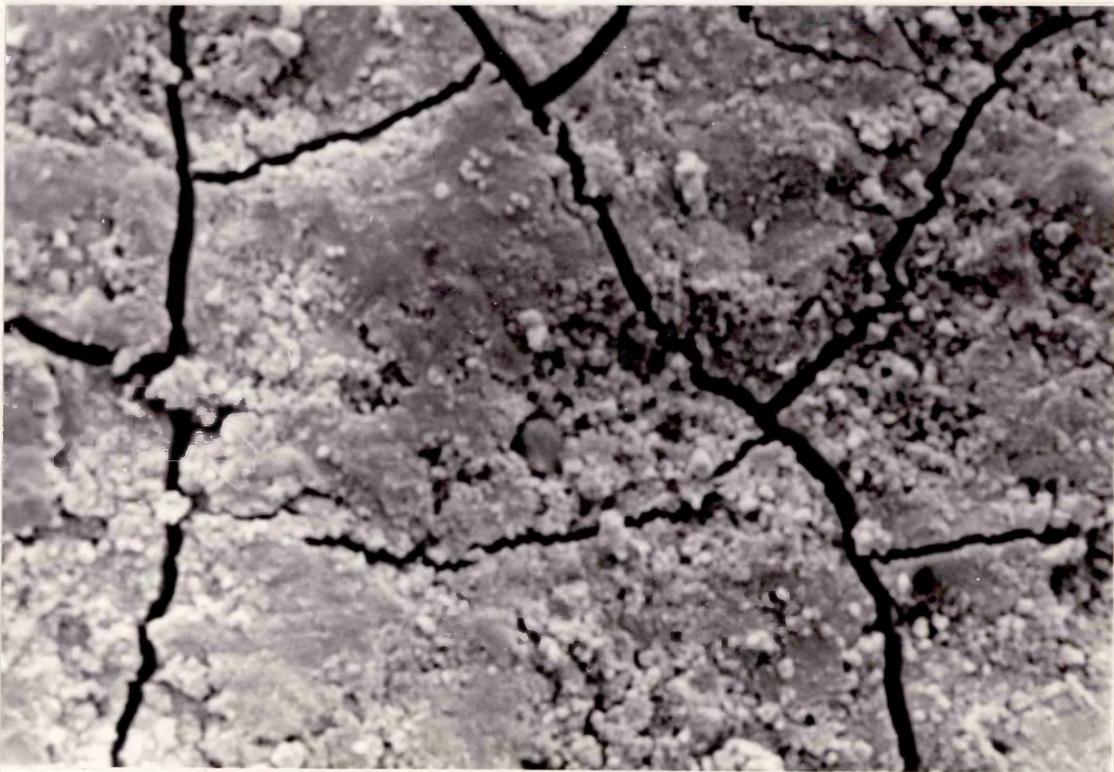


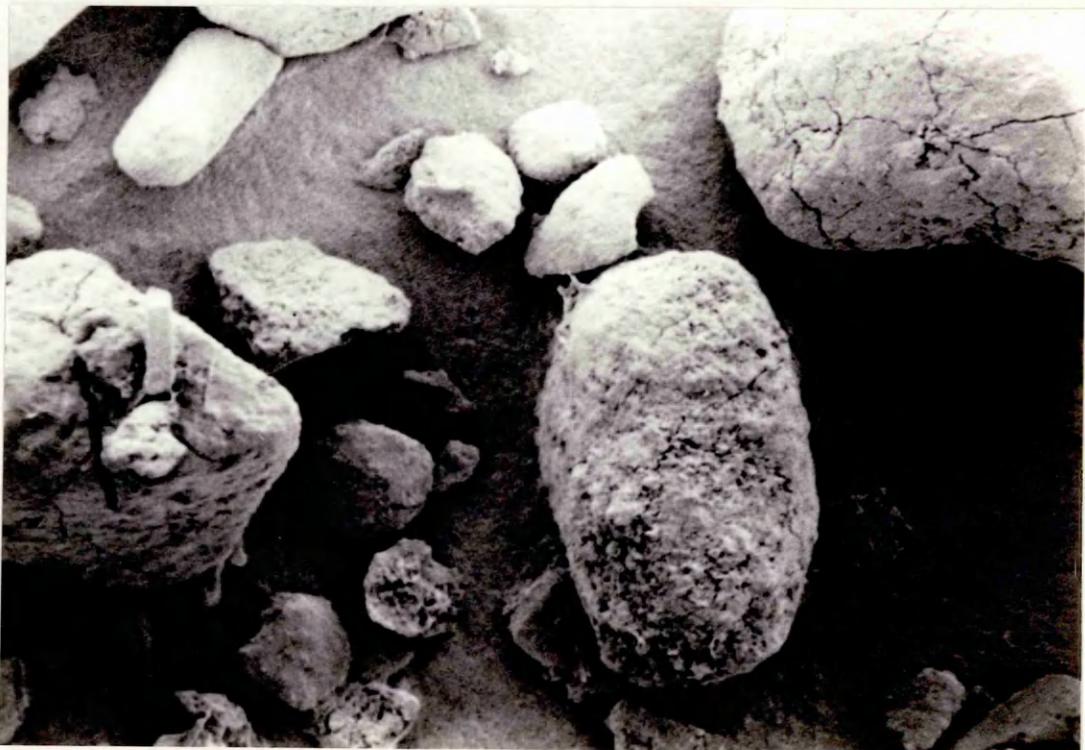
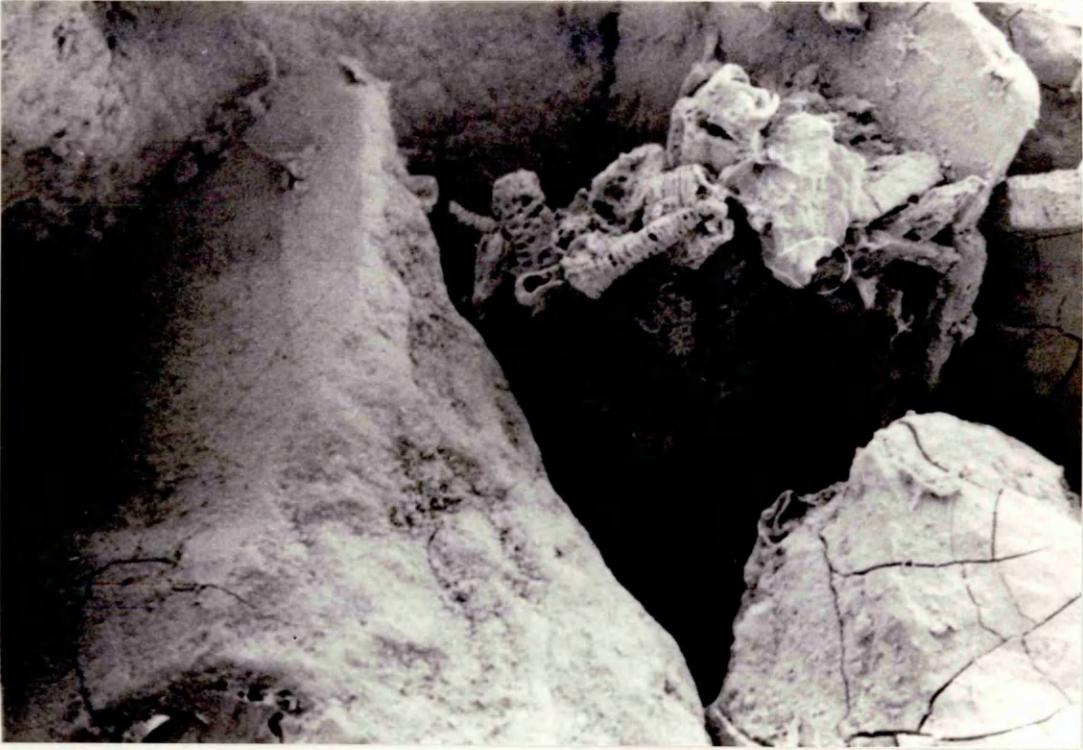
Figure 29 Agma after 7 days in bovine rumen x 160

Figure 30 Navarras after 7 days in bovine rumen x 160



Figure 31 Chinese after 7 days in bovine rumen x 160

Figure 32 Greek after 7 days in bovine rumen x 160



The cracks on some individual Greek crystal blocks appeared quite deep and at a magnification of x 2500 created a wadi-type appearance (Figure 28). The surface of the Agma blocks on the other hand took on a 'flaky appearance' and at a magnification of x 2500 both the Navarras and the latter appeared to have a 'crumbly-textured' surface (Figures 25 and 26). This may have been due to some extent to the presence of debris and also micro-organisms which are very evident on the Agma particles.

(iii) Calcined magnesite samples after 7 days in the rumen.

The most notable difference between the samples exposed to the rumen for 48 hours and 7 days was the extent of fissuring on the surface of the crystal blocks. As might have been expected, after extensive exposure to the rumen the blocks have been largely eroded through acid-etching and in some instances have been rendered more porous.

The most conspicuous feature of extensive exposure to the rumen was in the shape of the particle blocks. The Agma, Navarras and Chinese samples had become eroded to the point where some individual blocks began to resemble the rounded nature of the Greek particles. The erosion of the Agma appeared to be more extensive (Figures 29, 30, 31 and 32) resulting in a more open appearance with softened crystal edges.

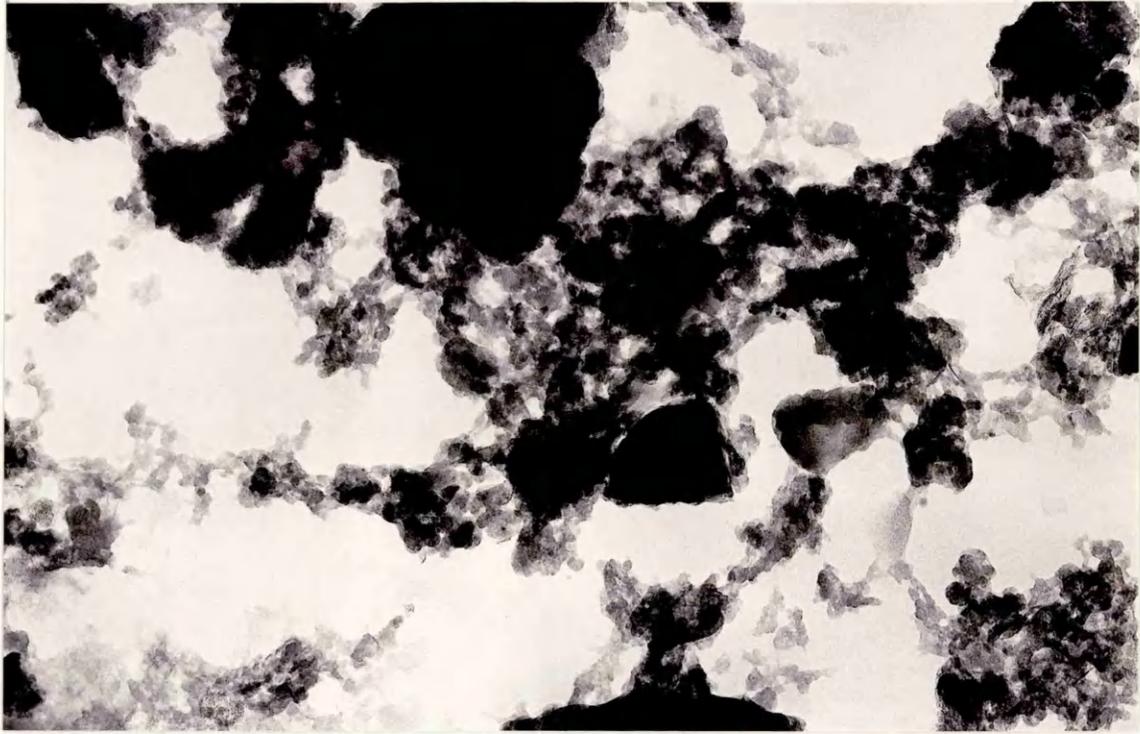
The acid-etching has thus opened up the crystal blocks, increasing the surface area internally and externally for exposure, and thus it is assumed increased the 'potential' available magnesium to the ruminant.

MICROANALYSES

X-ray microanalyses showed that an appreciable difference exists between samples in the level of impurities and these levels are quite characteristic of each type. Impurities were found in all samples and quantitative measurements were made. Phosphorus, silicon and some mercury have been found but the levels of phosphorus and silica in the Greek and Chinese samples were consistently higher than in the Spanish products. The Chinese sample had the highest level of impurity ions and this might explain its more open appearance at individual crystal level

Figure 33 Agma x 80,000.

Figure 34 Chinese x 80,000.



identified under high resolutions (Figure 34) compared to the Agma (Figure 33).

The calcined magnesite samples were subsequently analysed in the laboratory for phosphorus content. The results obtained do not suggest any significant difference in phosphorus levels between the Greek and Chinese and the Spanish products as found by microanalysis. The mean phosphorus concentration of the four different consignments of calcined magnesites were Agma, 2.65 g/kg; Navarras, 1.84 g/kg; Chinese, 2.27 g/kg and Greek 2.80 g/kg. Fairly similar concentrations were found for each consignment of the Agma and Navarras. However the Chinese sample from Experiment 1 and the Greek sample from Experiment 4 contained higher levels of phosphorus, 3.92 and 5.22 g/kg respectively.

The presence of ion impurities will affect the release of magnesium in so far as they cause distortion and distress. This could make the magnesium more available and it has been shown in Experiments 10 and 11 that the Chinese and Greek samples, both containing higher levels of impurity ions than the two Spanish products, are more reactive in dissolution tests with organic acids compared to the latter. However this hypothesis does not correlate well with the bioavailability results obtained with sheep in Experiments 1, 2, 3 and 4.

TRANSMISSION MICROSCOPY AND ELECTRON DIFFRACTION

Introduction

The main objective of this study was to look more carefully at the calcined magnesites following investigation under the Scanning Electron Microscope (SEM) and microanalyses.

Transmission analyses were carried out at high resolutions (>x50,000 magnification) to identify crystal make-up and appearance. Electron diffraction was carried out at the same time to determine the presence of other compounds, findings that were substantiated by Thermal Gravimetric Analysis.

The materials used in this study were identical to those used for the SEM, although only one sample of each source of calcined magnesite was used - the procedure was not repeated for each batch. Samples of Agma and Chinese after exposure to the rumen for 48 h were also

investigated for changes in the crystal appearance.

Method

The calcined magnesite samples were prepared as follows:

The samples were first ground in water using a pestle and mortar to produce individual crystals and particles less than 75 μm in size. The ground samples were then dried on a Formvar coated copper grid. (The Formvar coating prevented the crystals falling through the grid). When dry, the samples were placed in a Jeol Transmission Microscope 100 CX for transmission and electron diffraction.

Transmission Microscopy

In this investigation two extremes were compared with reference to surface area, namely Agma and Chinese. Both samples broke down equally easily with sonic treatment and the crystal morphology was similar at high magnification (i.e. $\times 300,000$). The samples were not initially pure magnesium oxide although they oxidised quite readily to the latter in the beam. (This finding was substantiated with electron diffraction techniques and thermal gravimetric analyses).

During preparation of the samples for transmission the Agma was more readily dispersed than the other magnesites indicating that withdrawal from individual crystals would be more easily facilitated. The Agma is a softer material and went into suspension more easily.

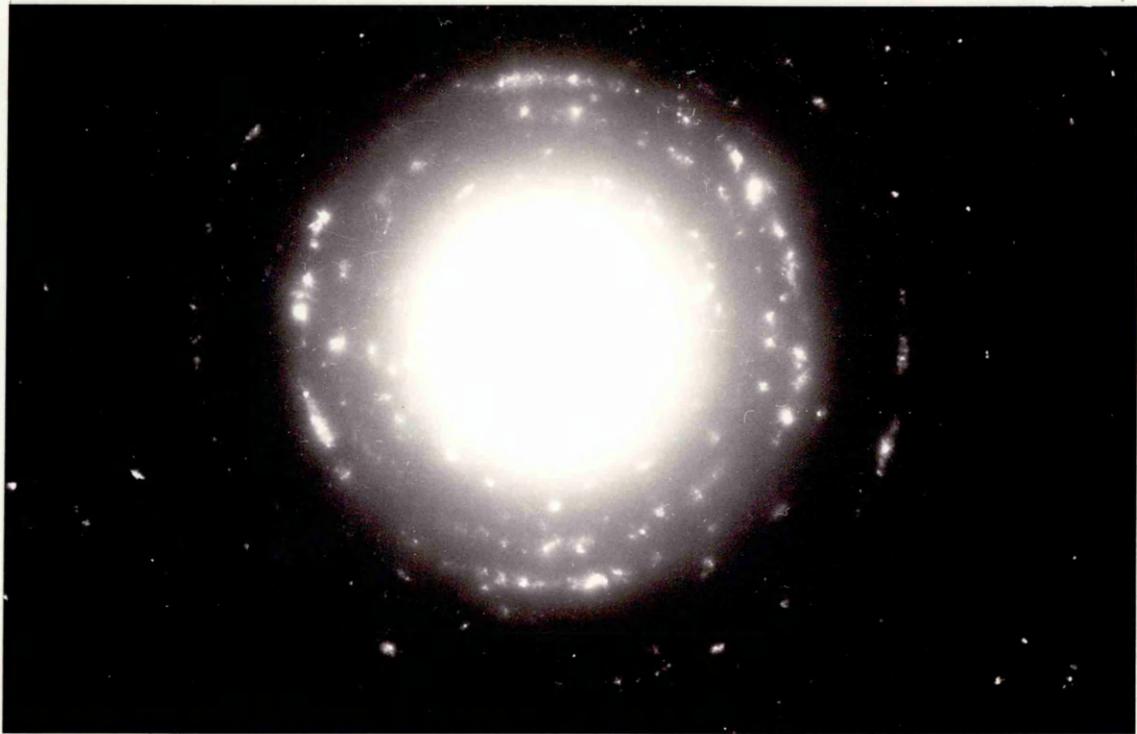
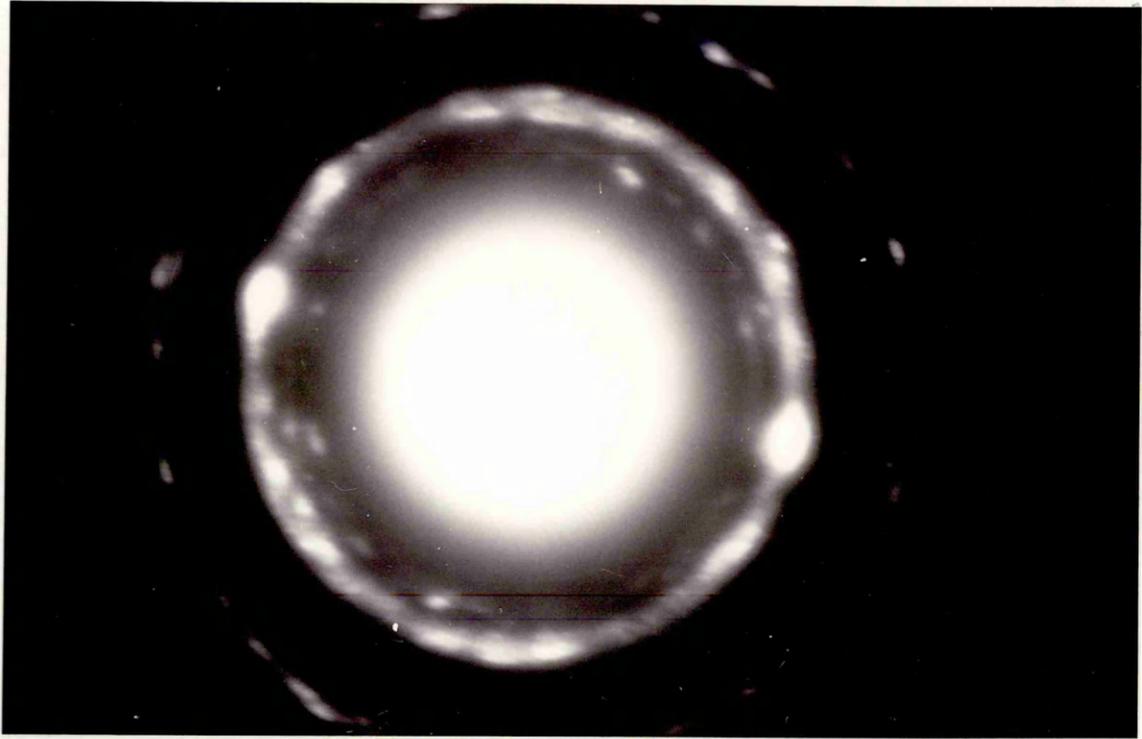
High resolution analyses of Agma and Chinese samples reveals a change in crystal appearance following exposure to the rumen for 48 h. Individual crystals have been etched giving them a more porous appearance. This is evident to a greater extent in the Chinese magnesite where the breakdown of the crystal is particularly well demonstrated (Figure 34) with evidence of much greater porosity than the Agma sample (Figure 33).

Electron Diffraction

The electron diffraction results showed that the calcined magnesite samples were not pure magnesium oxide and confirmed the presence of both hydroxide and carbonate (Figures 35 and 36). This was

Figure 35 Diffraction pattern of Agma

Figure 36 Diffraction pattern of Chinese



further verified by Transmission Thermal Gravimetric Analysis and Loss on Ignition (LOI). During the Gravimetric analysis, when heated there was a greater weight loss from both the Chinese and Greek materials compared with the Spanish. The weight loss corresponded to the presence of both hydroxide and carbonate. This inferred that the Agma was a cleaner and 'well-calcined' product. It has been further postulated that the presence of these other magnesium compounds will affect the surface area and this may be an explanation for the greater surface areas found in the Chinese and Greek products (Experiments 1-4) (S.E. Solomon, personal communication 1989). Table 44 gives the LOI of all the calcined magnesites investigated in this section. All the calcined magnesites contained less than 1% moisture content. As can be seen from Table 44 the Chinese and Greek products had higher LOI than the two Spanish products, agreeing with the Transmission and Thermal Gravimetric Analysis. This greater LOI indicates less severe or incomplete calcination.

Table 44. Loss on Ignition of calcined magnesites used in Experiments 1-4.

	Expt.	Agma	Navarras	Chinese	Greek
Agma	1	3.42	4.67	14.54	5.72
	2	3.29	1.00	9.67	9.04
	3	4.22	1.72	8.18	4.42
	4	1.93	2.59	4.78	7.27

All calcined magnesites contained less than 1% moisture content.

The presence of carbonate in the Chinese and Greek products as suggested by these results may therefore affect the availability of the magnesium within these products. A number of workers have compared the availabilities of magnesium from both magnesium carbonate and dolomite versus magnesium oxide. Meyer & Grund (1963) with cattle, Ammerman et al, (1972) with sheep and Wilson (1981) also with sheep, all found magnesium carbonate to be poorly available. Likewise, Gerken & Fontenot (1967), Moore et al, (1971) and Rahnema & Fontenot (1983) all found dolomite to be less available than magnesium oxide.

GENERAL CONCLUSIONS

The main conclusions that can be drawn from these studies can be subdivided into three categories:

(i) Shape of Crystal Blocks.

The Agma, Navarras and Chinese products are essentially similar, but not identical in their gross morphology having straight-sided faceted crystal blocks. The Greek material differs in that the crystal blocks are essentially of a rounded nature. Their gross morphology characteristics cannot be used as a means of identifying a specific site of potential extraction.

The shape of the crystal blocks are little altered after exposure to the rumen for 48 h although the presence of cracks and fissures appear on the surface of the blocks indicating uptake. After 7 days in the rumen some of the crystal blocks from the two Spanish and Chinese samples begin to resemble the rounded nature of the Greek sample due to extensive erosion, and become more open in appearance.

(ii) Impurity Ions.

All calcined magnesite samples were shown to contain impurity ions, the levels of which are quite characteristic of each sample. The Chinese and Greek samples contain proportionately higher levels of impurity ions, particularly phosphorus and silica, than the Spanish products. Impurity ions will affect the packing of the crystal and will affect the release of magnesium in so far as they cause distortion and distress.

(iii) Presence of other compounds.

Electron diffraction techniques, Thermal Gravimetric analysis and LOI indicates the presence of both hydroxide and carbonate in the Chinese and Greek samples. The Agma is a 'cleaner' and 'well-calcined' material.

The presence of these compounds may be an influencing factor in the greater surface area measurements obtained with these samples.

All these features point to differences in the performance of the

materials. The differences found in bioavailabilities between the different calcined magnesites are more likely to be the result of differences in the packing properties of individual particles rather than any inherent porosity properties of individual crystals.

SECTION 7

THE USE OF MAGNESIUM OXIDE AND SODIUM BICARBONATE TO
INFLUENCE THE YIELD AND COMPOSITION OF MILK BY COWS

THE USE OF MAGNESIUM OXIDE AND SODIUM BICARBONATE TO
INFLUENCE THE YIELD AND COMPOSITION OF MILK BY COWS

INTRODUCTION

There is extensive literature concerning the use of magnesium oxide as calcined magnesite (MgO) and sodium bicarbonate (BIC) in diets for dairy cows but with one exception, the work is American. The intention of the American work has generally been to use both BIC and/or MgO to buffer rumen pH of cows given mainly maize silage and high concentrate diets to improve voluntary feed intake, milk yields and milk fat outputs. Much of the work has been conducted with complete diets. The mean amounts of BIC and MgO given in 22 and 10 experiments respectively have been 225 and 110 g and the ranges for individual experiments have been from 120-450 g BIC and from 35-150 g MgO. Where both have been used in the same experiment the ratio of BIC:MgO has generally been about 2:1.

The experimental treatments and results of 24 references in the literature are summarised in Tables 45 and 46, listed in chronological order according to the nature of the roughage components of the diet. Only the work of Edwards & Poole (1983) has been conducted in Britain, the remainder being American. Table 45 gives the proportions of roughage and cereal (normally ground maize) in the diets. In over half the experiments more than 30% and often more than 40% of cereals have been used and this, with a low roughage intake, would be conducive to the production of milk with a low fat content.

Table 46 necessarily has many omissions as all the experiments did not include all the treatments nor measure all the parameters and some did not define the ingredient composition of the concentrates. Dry matter intakes are not included where the experiment did not involve complete diet ad libitum access.

It is not intended at this stage to review all these publications. The experiments have been conducted in a wide range of circumstances including changeover of diets shortly after parturition, at different stages and for different lengths of lactation, possibly by using some cows with a rumen fistula and frequently by employing basal diets to deliberately induce the production of milk of low fat content. Ten of the references in Table 46 indicate that with the unsupplemented diet milk with less than 3.0% fat was produced.

Table 45. Experiments involving the addition of magnesium oxide and/or sodium bicarbonate to the diet of lactating cows with the roughage and cereal contents of the diets given.

Reference	Date	Inclusion in complete diet		Approx. intake from complete diet g/day	Intake from diet g/day	Intake from fixed		Whole diet composition		
		BIC %	MgO %			BIC	MgO	Silage/hay	DM Cereals	
Maize silage-based diets.										
1. Erdman et al.	1980	1.5	0.8	300	150			40	38	
2. Killmer et al.	1980	0.7	-	150	-			60	40	
3. Killmer et al.	1981	0.8	-	160	-			50	?	
4. Chase et al.	1981	1.6	-	300	-			50	?	
5. Jesse et al.	1981	-	0.8	-	135			25	51	
6. Erdman et al.	1982	1.0	0.8	170	150			40	37	
7. Rogers et al.	1982	2.0	-	400	-			75	20	
8. English et al.	1983	0.75	0.25	100	35			50	14	
9. Snyder et al.	1983a	1.2	-	250	-			50	33	
..	1983b	1.2	-	250	-			75	6	
10. Thomas et al.	1984	1.0	0.5	170	85			25	48	
11. Rogers et al.	1985	1.2	-	250	-			40	40	
12. Teh et al.	1985	0.8	0.8	150	150			50	28	
Maize silage with grass silage-based diets.										
13. Thomas & Emery	1969	0.75	-	135		310	103	30	?	
14. Boisclair et al.	1986a	0.75	-	135				50	36	
	1986b	0.75	-	135				50	43	
Grass silage-based diets.										
15. Vandemark et al.	1981	1.0	0.4	180	90			30	?	
16. Edwards & Poole	1983	2.0	-	300	-			40	52	
17. Stokes et al.	1986	0.7	0.28	140	60			30	27	
18. Stokes & Bull	1986	0.7	-	120	-			50	29	

Table 46. (cont'd)

Reference Table 45	14a	14b	15	16	17	18	19a	19b	20	21	22	23	24
DM intake kg													
Nil	17.7	17.5		13.9	19.8	17.6					18.0		
BIC	18.3	17.9		14.9	19.8	16.8							
MgO					20.1								
BIC + MgO											17.3		
Milk yield kg													
Nil	31.3	34.5	40.1	24.1	39.1	25.7	12.7	10.7	16.8	24.2	16.0	17.8	22.9
BIC	32.5	34.1	38.3	24.9	38.3	24.6	12.6	11.3	15.9			17.8	24.3
MgO					38.0				15.3				
BIC + MgO			38.7							21.4	16.7		
Fat %													
Nil	3.31	3.19	3.43	3.98	3.58	3.99	3.40	2.10	2.68	2.50	2.78	2.96	3.25
BIC	3.47	3.63	3.41	4.00	3.66	3.81	3.70	2.90	3.12			3.42	3.60
MgO					3.73				3.16				
BIC + MgO			3.52							3.40	3.22		
Fat yield kg/d													
Nil	1.04	1.10	1.38	0.96	1.40	1.01	0.43	0.22	0.45	0.50	0.42	0.53	0.74
BIC	1.13	1.24	1.31	1.00	1.39	0.98	0.47	0.33	0.50			0.62	0.87
MgO					1.41				0.48				
BIC + MgO			1.36							0.73	0.54		
Acetate/Propionate													
Nil					1.68	2.50		3.71	1.10	1.57		1.90	
BIC					1.50	1.70		3.70	1.51			2.30	
MgO					1.87				1.38				
BIC + MgO					2.08					3.41			

A review of particular aspects relating to initial fat % and dietary cereal inclusion will be included in the discussion following the experimental part of this section. A summary can however be made of the overall mean effects of MgO and BIC of the data in Table 46.

The effects on dry matter intake have generally been small. Sometimes high inclusion rates of MgO and/or BIC have resulted in loss of palatability and BIC has generally increased and MgO generally decreased milk yields, but by small amounts. Almost invariably both MgO and BIC have increased both fat % (+ 0.3) and daily fat yield (+ 0.08 kg/day) and there are indications that the separate effects are additive. For a cow giving say 25 kg milk with 3.5% fat the increase in fat yield is about 10% and of economic importance. Both MgO and BIC improve rumen acetate/propionate ratios to a similar extent.

In these comparisons it is important to appreciate that generally twice as much BIC has been given as MgO (Table 45) and so MgO may be the more effective agent.

A primary effect of the MgO and/or BIC inclusion in diets is to increase rumen pH. Most authorities accept that pH differences are at their maximum 3-4 hours after feeding. Some examples of changes so described are listed in Table 47.

Table 47. Some observed effects of magnesium oxide and sodium bicarbonate on rumen pH.

	Nil	BIC	MgO	BIC + MgO
Thomas & Emery (1969)	6.7	6.8	6.8	6.9
Erdman <u>et al.</u> (1982)	5.6	6.0	6.0	6.2
Rogers <u>et al.</u> (1982)	5.7	6.0	-	-
Snyder <u>et al.</u> (1983)	5.4	5.9	-	-

Increasing rumen pH may improve the digestibility of dietary fibre. As illustrative examples from Table 45, Erdman et al., (1982) found both MgO and BIC increased the digestibility coefficient of acid detergent fibre from about 0.40 to about 0.48 and Snyder et al., (1983) found an increase from BIC of about 0.48 to 0.52.

The management change to a high concentrate intake at calving should be as rapid as possible and inclusion of MgO and/or BIC may be beneficial. For example, Erdman et al., (1980) found that inclusion of

1.5% BIC in a complete diet enabled peak milk production to be attained 2-3 weeks earlier and this was accompanied by a 2 kg DM increase in feed intake and increases of 1.6 kg milk and 0.14 kg milk fat/day. The rumen acetate/propionate ratio improved from 1.7 to 2.2. In the same experiment further addition of 0.8% MgO had only a small effect on feed intake and rumen acetate/propionate ratio but increased milk yield by 3.8 kg and milk fat output by 0.28 kg/day.

Very few of the references in Table 45 quote effects on milk protein. Kilmer *et al*, (1980) reported that BIC had no effect and Teh *et al*, (1985) found no improvement from either MgO or BIC. Thomas *et al*, (1984) found that MgO, magnesium hydroxide and BIC all reduced milk protein marginally. In contrast, Stokes & Bull (1986) considered that as BIC reduced rumen ammonia concentrations this could be consistent with an observed increase in the transfer of dietary protein to milk protein.

Newbould, Thomas & Chamberlain (1988) found that BIC together with sucrose added to the diet of sheep given silage increased the fixation of ammonia in microbial protein. Newbould, Chamberlain & Thomas (1988) also showed with non-lactating cows that progressive additions of BIC to a silage, molasses, barley, soya bean meal diet increased rumen pH and rumen liquid outflow rates. The rumen degradation rate of both soya bean meal and rapeseed meals (but not fishmeal) were thereby increased.

In the context of the present work the rate and extent to which MgO may dissolve in the rumen is important. Only two of the references given in Table 45 either detail the form of the MgO or investigate different physical (i.e. particle size) forms. Jesse *et al*, (1981) used 0.43-1.70 mm and 0.14-0.66 mm prilled and < 0.14 mm ground prilled MgO. Each was given to cows as 0.8% of the concentrate. All markedly increased milk fat % (2.19 to 2.78-2.95) and daily milk fat output (0.47 to 0.56-0.59 kg) but only the < 0.14 mm ground MgO improved rumen acetate/propionate ratio (1.78 v 1.44) as expected from its high solubility and much superior effect on rumen pH.

Thomas *et al*, (1984) investigated the use of 0.5% MgO in the concentrate part of the diet. This was in the form of either 0.43-1.7 mm prills, < 0.43 mm ground prills, < 0.045 mm material described as of 'greater chemical reactivity'. Powdered magnesium hydroxide was also given. All the materials other than the 0.43-1.7 mm prills reduced feed intake and milk yield because of unpalatability. Nevertheless only the 0.43-1.7 mm product did not improve rumen acetate/propionate ratios by

about 0.5. Because of the marked effects on feed intake it was hard to evaluate the effects on milk fat and protein outputs.

Nevertheless both these references indicate that fine grade, more reactive MgO is superior if diet palatability is not reduced.

The general conclusion of this largely American work with MgO and BIC is that it would be interesting to investigate their use with lactating cows given a winter diet such as is commonly used in Britain at the present time. i.e. A diet based on grass silage using brewers grains and molassed sugar beet pulp and the minimum amount of cereals. For practical purposes it seemed important that both MgO and BIC should be presented to the cow in the minimum amount of compound feed consistent with palatability. Such an experiment is now described.

EXPERIMENT 21

The separate and combined effects of calcined magnesite and sodium bicarbonate on the yield and composition of milk of cows given grass silage

Materials and Methods

Sixteen Friesian cows at peak milk yield (30 kg milk/day at a mean of 55 days post calving) had 24 h access to grass silage from a clamp and were group-fed 2 kg molassed sugar beet pulp, 2 kg wheat dark distillers grains and 10 kg fresh brewers grains (plus minerals and vitamins). These were estimated to provide for the first 14 kg milk/day.

Four groups each of four cows were formed on a basis of previous and current milk yields and composition. Four diets were given in a balanced design of four 4 x 4 Latin squares. The four feeding periods were each of four weeks. Milk yields were recorded during the last 14 days of each period to reduce the possibility of any carry over effects. Fat and protein were determined in all samples using a Milkoscan 300 Infra-red Analyser operated by the Central Laboratory of the Scottish Milk Marketing Board, Paisley.

The diets were:

- A. 50 g Agma 85 calcined magnesite (MgO) in 1 kg concentrate feed
- B. 225 g Alkacarb sodium bicarbonate in 1.67 kg concentrate feed
- C. Diet A plus Diet B
- D. No supplement

Diets A, B and C were given via two separate out-of-parlour feeders (Hunday). Diet D was given in the milking parlour in additional (and varied) amounts to make the total concentrate allocation 0.4 kg/kg milk produced after allowing for (a) the 14 kg produced from the group-allocated feeds and (b) the amounts provided by A or B or A + B.

The concentrates A, B and D necessarily had varying individual ingredient compositions. However at the overall mean milk yield of the whole experiment of 26.5 kg milk per day the various concentrate combinations all provided 0.60-0.68 kg cereals, 0.20-0.22 kg vegetable proteins, 0.02-0.03 kg animal proteins and 0.08-0.16 kg fat per 1 kg total concentrate feed.

Composition of the concentrate feeds and estimations of metabolisable energy and digestible crude protein contents.

Estimates were made of the ME and DCP contents of the three concentrate feeds by feeding each to three adult wether sheep in metabolism cages. In each case there was a 7-day run-in period followed by a 7-day collection period. Initially all the sheep were given 800 g dried grass plus 200 g rolled barley (FM basis) and assessments made of the ME = DE x 0.81 (MAFF, 1984) (i.e. digestible energy assessed by bomb calorimetry of feeds and faeces).

In the second feeding period the sheep were given 667 g fresh matter of the appropriate compound plus 333 g fresh matter of the mixture of 4 parts dried grass to 1 part rolled barley. The ME and DCP contents of the concentrate were derived by difference on the assumption that the values for the dried grass/barley mixture remained constant.

Table 48 gives the proximate analyses of the three concentrates.

Table 48. Experiment 21. Proximate analyses and estimated ME and DCP contents of the concentrates.

Product	MgO	BIC	Parlour
Dry matter g/kg	883	895	853
Composition of DM g/kg			
Crude protein	216	203	195
Crude fibre	45	25	82
Ash	96	159	86
Gross energy (MJ/kg)	17.4	16.5	18.3
ME (MJ/kg) +	12.4	10.5	11.3
DCP (g/kg)	185	160	148

+ DE x 0.832

Table 49. Experiment 21. The mean separate and combined effects of magnesium oxide (MgO) and sodium bicarbonate (BIC) on the yield and composition of milk of dairy cows.

	Yield kg	Fat g/kg	Protein g/kg	Fat g/day	Protein g/day
Nil	26.7	37.6	29.7	1.01	0.79
BIC	26.6	37.4	30.5	0.99	0.81
MgO	26.2	37.6	29.7	0.98	0.78
BIC + MgO	26.7	37.2	30.2	0.99	0.80
SEM	0.38	0.50	0.20	0.012	0.01

There were no significant differences.

The inclusion of such substantial quantities of MgO and BIC within 1.67 kg fresh matter raised considerable formulation problems and it was not possible to obtain identical ME and DCP values for each.

When 1.67 kg of the MgO product was given it provided about 2 MJ ME and 30 g DCP more than the BIC compound. The MgO compound also provided about 2 MJ ME and 60 g DCP more than the parlour concentrate. Within the context of total requirements at the overall mean milk yield of 25.5 kg of 190 MJ ME and 1750 g DCP, these small differences were not considered to be important.

Results

Much of the past American work with both MgO and/or BIC has included the materials within complete diets. One objective of the present experiment was to assess the palatability of the concentrate feeds containing MgO and BIC. With only one or two exceptions all the cows readily and completely consumed both feeds within two days of introduction to the diet.

There were no problems with the cows. The performances with respect to overall milk yield and composition were as would have been expected for these cows in relation to previous years when given the same type of basal diet. Other cows in the herd at the same time received the same basal diet together with Concentrate D throughout. Their performance was similar to the 16 experimental cows.

Table 49 gives the mean milk yields together with the fat and protein contents and the mean daily fat and protein yields. There were no significant differences, nor any indications that either MgO or BIC had any effect on milk yield or composition.

Discussion

The mean amounts of BIC and/or MgO given in the previous largely American work (Table 45) were 197 \pm 92 g and 112 \pm 44 g respectively. In this present experiment the amounts were 225 g BIC and 50 g MgO. These amounts were chosen largely so that they would be readily consumed in only 1.67 kg concentrate which would be important if feed products were to be marketed. The 50 g MgO is only about half that generally given in American research and was partly chosen to be a familiar amount given to cows going to grass in the spring and where the MgO might have a

dual effect in the prevention of hypomagnesaemia and in a possible improvement in milk fat concentration. Accordingly some caution should be expressed in relating the results obtained with MgO to American work.

In Figure 37 the separate and combined effects of MgO and BIC on daily butter fat yield are plotted against the initial fat content of the milk. The solid circle points represent maize-based diets and the solid square points represent hay-based diets. The encircled solid circle point is for the present experiment. Significant regressions were found for diets which include BIC (% increase in daily fat yield = $65.7 - 16.6 \times \text{initial fat } \%$, $n = 24$), for BIC + MgO (% increase in daily fat yield = $42.1 - 10.5 \times \text{initial fat } \%$, $n = 14$) but not for where MgO was given alone perhaps because of the fewer number of experiments ($n = 10$). Where notable increases in daily fat yield are found, the initial fat concentration in the milk of cows given unsupplemented diets has frequently been below 3.0%. Inspection of the American literature shows that frequently the basal diets were designed to produce low fat milk and often the cows would be Holstein and with a different genetic background.

In the present experiment the initial fat concentration was about 3.8% and any improvement is thus much less likely.

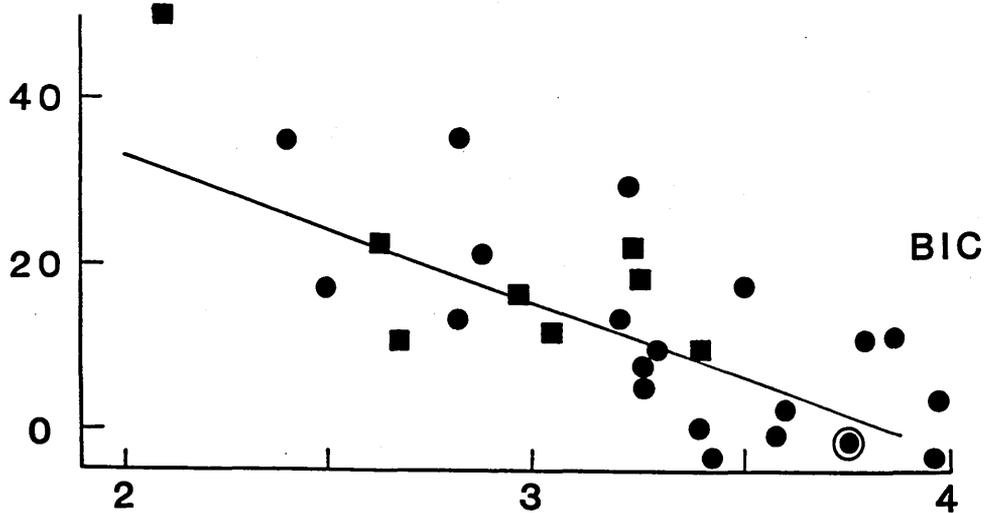
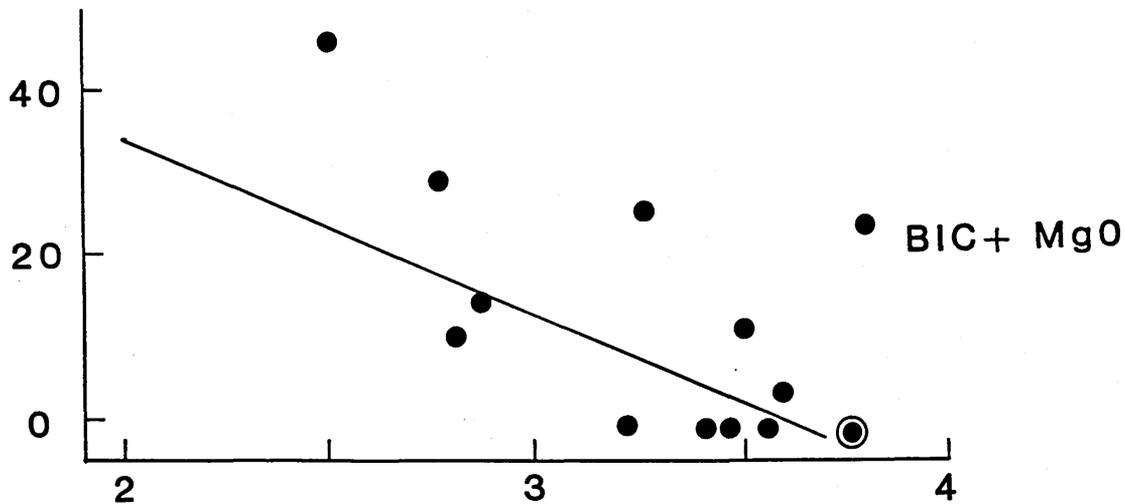
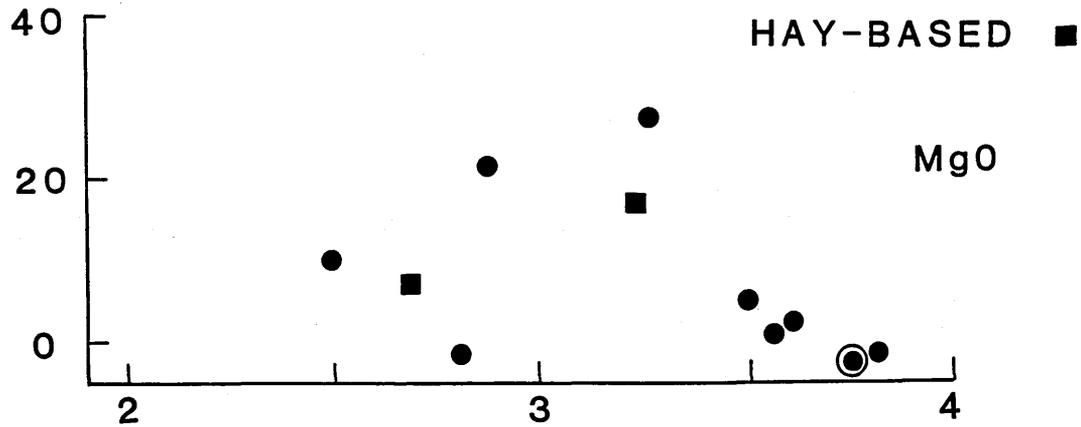
A major nutritional factor leading to reduced fat concentrations in milk is a low roughage/high cereal combination.

In Figure 38 the American work shows a marked tendency for both BIC and MgO to increase daily fat yield as the percentage of cereal in the diet progressively increases. Only for BIC plus MgO was there a significant regression. (% increase in daily fat output = $0.942 \times \%$ cereal in total diet - 16.1, $n = 6$). Only five of the 38 diets shown in Figure 38 contained less than 20% of cereals and most contained 30-35%. In marked contrast, in the present experiment (encircled solid circle) the whole diet at a mean milk yield of 26.5 kg contained only 10% of cereals. It is therefore perhaps not unexpected that neither MgO and/or BIC improved milk fat yields.

It must accordingly be concluded that it is unlikely that either MgO and/or BIC will influence the milk fat output of dairy cows with an initial fat content of about 3.7% when given grass silage and limited amounts of cereals.

Figure 37. Expt. 21 The relationship between the fat concentration in the milk of cows given unsupplemented diets and the increases in fat yield when given MgO and/or BIC

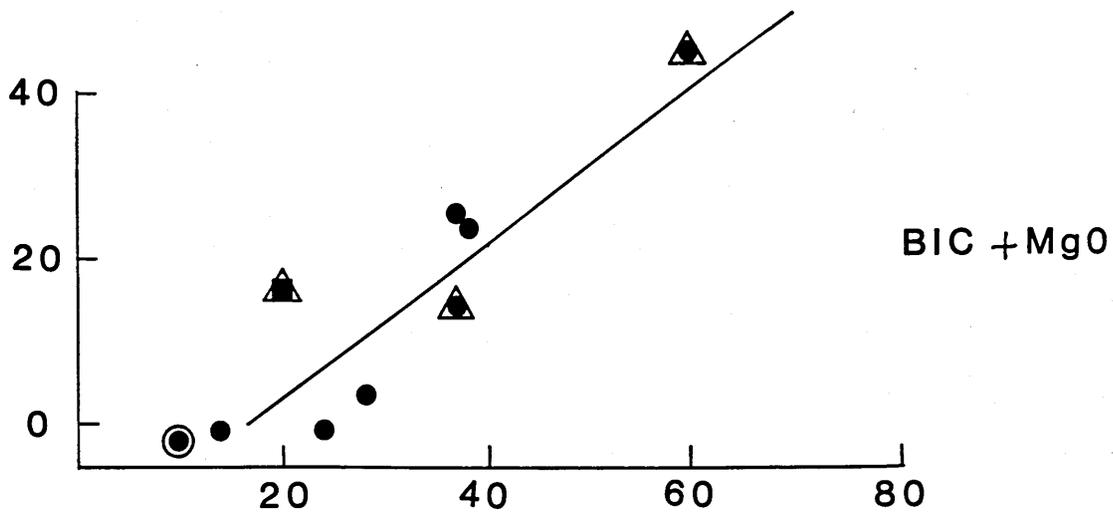
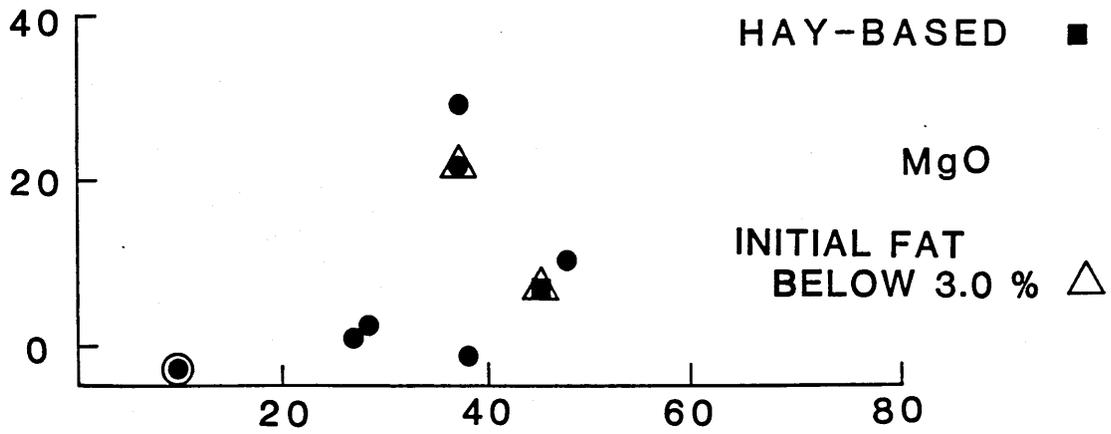
% INCREASE IN DAILY FAT YIELD



% FAT WITH UNSUPPLEMENTED DIET

Figure 38. Expt. 21 The relationship between the amount of cereal in the unsupplemented diets and the increases in daily fat yield when supplemented with MgO and/or BIC

% INCREASE IN DAILY FAT YIELD



% CEREAL IN TOTAL DIET

Since this work was completed, Staples & Rough (1989) have published a comprehensive review of the effects of supplementary sodium bicarbonate and, to a lesser extent magnesium oxide on the yield and composition of milk of cows. Most of the data quoted have been summarised in Table 45. Essentially for 41 experiments where maize silage diets included a mean of 57% concentrates leading to acidic rumination the benefits included 0.8 kg more milk/day with 0.22% more fat. They concluded that (a) with hay and/or grass silage the effects of BIC were inconsistent and (b) whilst there were benefits from giving MgO the combined effects of MgO and BIC on milk yield and composition were not additive.

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APPENDIX 1**Analytical Methods****(i) Dry matter**

The dry matter (DM) in the feedstuffs and faecal samples was determined by heating 0.5 to 1.0 kg quantities in a hot air oven at 90°C for 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, feedstuffs, calcined magnesites, faeces and urine samples was determined by atomic absorption spectrophotometry (Perkin-Elmer, 1976). Prior to analysis, samples of faeces and feed were digested in a 3:2:1 mixture of nitric, perchloric and sulphuric acids. The calcined magnesite samples were digested in hydrochloric acid. The solutions were diluted before analysis.

(iv) Calcium

The calcium content of blood and feed samples was determined by atomic absorption spectrophotometry (Perkin-Elmer, 1976). Prior to analysis, feed 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) Copper

The copper content of feedstuffs and faeces samples was determined by atomic absorption spectrophotometry (Perkin-Elmer, 1976). Prior to analysis the samples were digested in a 3:2:1 mixture of nitric, perchloric and sulphuric acids. The solutions were diluted before analysis

(vii) Phosphorus

The phosphorus content in feed samples was determined by modification of the colorimetric method of Cavell (1955). Phosphorus in blood samples was determined by the colorimetric method of Fiske & Subbarow (1925).

(viii) pH

The pH of rumen liquor samples was determined using a Pye Unicam Ltd pH meter. The combined glass and reference electrode was immersed directly into the fluid.