DEVELOPMENTAL STUDIES ON INTRARUMINAL DEVICES FOR RUMINANTS

by

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CONTENTS

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SUMMARY

This thesis deals with the development and application of a slow release intraruminal bolus for administration to cattle and sheep. In the first instance the bolus was formulated as a short term cattle trace mineral supplement which contained copper, cobalt, manganese, zinc, selenium and iodine in the form of readily available salts in proportions which fulfilled the ARC (1965) mineral requirements of cattle for 30 days. In early prototypes the salts were compressed at high pressure with 0.7 mm iron filings in 50-50 proportions by a commercial tabletting firm to produce a lozenge shaped tablet which had a density of $\geqslant 3.0\ gcm^{-3}$, sufficient to lodge in the reticulum of cattle and be retrievable from this site in fistulated cows. Further development work followed, with the acquisition of a bench press capable of compacting cylindrically shaped boluses of varying length and diameter in the laboratory. Copper oxide needles of 2-4 mm were chosen to replace iron filings in laboratory models. It was found that boluses eroded over a period of weeks partly by abrasion with another bolus and partly through dissolution of ingredients and that the pattern of erosion could be altered by changing certain features of the boluses such as coating type, surface area initially exposed, and mineral salts constituents such as zinc oxide and zinc sulphate heptahydrate. The stage was reached when a

reasonably predictable pattern of erosion took place over 4-6 weeks in fistulated cows with one particular bolus formulation. Field trials were carried out using a treatment of two boluses in fattening lambs and in a herd of beef suckler calves. While treated lambs showed enhanced blood and liver copper levels compared to control lambs, only 50% of the boluses were recovered after twenty-seven days and it was speculated that the remaining 50% had been regurgitated and possibly chewed. In the beef suckler calves' trial, improved blood copper levels were found in the treated group. An attempt was then made to produce the cylindrical mineral bolus of the same dimensions as that produced in the laboratory on a commercial factory scale. Various formulae were attempted to be produced in two sizes of cylindrical bolus, a 16 mm diameter and 25 mm diameter size. However it was found that although it was possible to produce these formulae at speed on a large scale, the length of the boluses was about half that of those produced in the laboratory, and was restricted by the physical dimensions of the Bipel press on which the boluses were produced. It was not possible to find a commercial press in Britain capable of producing a compacted bolus of the length required. The 16 mm diameter boluses containing copper oxide needles were experimentally administered to lambs and ewes, and were found to significantly increase the liver copper levels although

again there was some failure to recover boluses in the group of lambs (57% were found after twenty-four days) which was thought to be related to the density of the bolus. Further development of the bolus was continued in the laboratory, and the medicated ingredients, monensin sodium, levamisole hydrochloride, morantel tartrate and citrate and ivermectin were experimentally included in the formulation. Of these, monensin, levamisole and ivermectin boluses produced reasonably promising results, and ivermectin boluses were chosen for further development. In a controlled experimental challenge, boluses containing ivermectin were found to be>99% effective against Ostertagia ostertagi and Cooperia oncophora in housed calves. In an outdoor trial of the same bolus type over the grazing season, parasitism was apparently completely controlled in calves for a period of eight weeks which coincided with the active life of the bolus. Thereafter there was a gradual increase in faecal egg counts followed by a later increase at around four months in plasma pepsinogen levels. At necropsy the bolused group had a reduced parasite burden compared to the control group although this was not statistically significant. This trial was followed by a period of further commercial involvement during which it was attempted, with limited success, to produce a bolus containing ivermectin on a large scale. During this period many of the basic design features of the bolus were altered in an attempt to suit existing commercial machinery and processes.

SECTION I

INTRODUCTION

1. Dietary Supplementation of Trace Minerals in Ruminants

This thesis is primarily concerned with the development of an intraruminal device capable of providing the dietary trace mineral requirements of cattle and sheep over a prolonged but defined period of time.

At present there are twentytwo identified mineral elements which are deemed to be essential in all higher animal life. Calcium, phosphorus, potassium, sodium, chloride, magnesium and sulphur are known as the macronutrient elements, while of the micronutrient or trace elements iron, iodine, zinc, copper, manganese, cobalt, selenium, molybdenum and chromium are the more important. The dietary requirements of the remaining six micronutrients, tin, vanadium, fluorine, silicon, nickel and arsenic, are very low with no unequivocal evidence of deficiency having been demonstrated under normal conditions to date. Trace element deficiency disorders arising from a lack of cobalt, copper, zinc, selenium, iodine and manganese have been demonstrated in the field and in the laboratory. Therefore these are the elements which are defined as being the

desirable basic components of a trace mineral bolus.

Trace element deficiencies may occur in plants over broad geographical areas, or they may be localised on the scale of small isolated patches of plant material with lower mineral levels in an area which is otherwise adequate in mineral terms. Clinical symptoms of trace element deficiency in grazing animals generally arise when the mineral concentrations of plants in these areas reach a certain critically low level but less severe nutritional imbalance may result in subclinical effects and possibly infertility and production problems. Specific trace element deficiency disorders are often associated with particular soils or soil properties, though frequently climatic and managerial factors are also involved.

The earth's crust is composed of 95% igneous rocks, and 5% sedimentary rocks, of which about 80% are shales, 15% sandstones and 5% limestones (Mitchell, 1964). Sedimentary rocks tend to overlie the igneous rocks from which they were derived and thus are more frequent at the surface, and are therefore more important agriculturally.

(i) Factors which modify the mineral content of plants and animals via the soil

Soils derived directly from igneous rocks should have fairly well-defined total contents of trace elements. Sedimentary rock dictates the trace element content of the soil derived from it, and the subsequent history of weathering and soil management practices dictate the availability to plants of the store of trace elements contained in the soil. Few soils are deficient in the sense that they do not contain sufficient absolute quantities of trace elements, but the chemical form or association of elements may not be suitable for plant uptake. At best the total trace element content of a soil can only serve as a rough guide to its potential ability or inability to supply bioessential trace elements.

When glaciation has taken place in the geological past, it has resulted in the transport of soils from the parent base material to be deposited some distance away on unrelated base rocks. This occurrence has resulted in the mixing up of soils formed from many different rocks, so that, for example, in Northern Scotland there are many totally different soils with widely varying trace element characteristics within small areas. Around the village of Inverurie there are forty different soils in 400 square miles area (Glentworth and Muir, 1963). Thus it is possible

that there may be several widely differing soil types within the boundaries of a single field in Scotland.

Physical weathering processes and chemical and biological forces cause soils to change progressively. Depletion of elements such as copper and cobalt may occur when crops are harvested and not returned to the soil. Conversely, other elements which are not translocated above ground extensively may tend to accumulate in roots and litter since it is likely that some plants release non-essential or potentially toxic elements by leaf or root detachment. However Mitchell (1963) compared the total amount of trace elements in the top 20 cm of soil with the quantity removed from the soil by an average crop during the growing season. It became apparent that the concentration of trace elements in the soil is sufficient for several hundreds of thousands of years. If trace elements were not held chiefly in forms unavailable to plants, within a growing season, severe toxicity might occur due to oversupply.

Very little of the total trace element content of the soil exists in free, ionic or chelated forms, in true or colloidal solution. Considerable amounts are adsorbed on the negatively charged surface of clay minerals and soil organic matter where they can be removed by processes of ion-exchange with hydrogen

and other cationic species. This labile or exchangeable fraction of the total trace element content is largely available to plants during the growing season. Trace elements occluded in precipitated oxides of, for example, aluminium, iron, manganese or carbonates, or bound within insoluble organic species, or the lattices of secondary minerals, are largely unavailable to plants during a growing season, but can replenish the soil solution or exchangeable pool within a reasonably short time. Trace elements locked within primary minerals and many secondary minerals can only be utilised as a very long-term basis. The quantity of trace elements that can become available to plants during a growing season is therefore largely restricted to that which can be transported from the labile compartment to the soil solution.

A more uniform distribution of elements in the soil has come about in relatively modern times through the intervention of man and his ploughing techniques. Traditionally he has enriched the organic matter content of the soil thus enhancing both the element status and element retention of the surface soils. More intensive arable farming with repeated high cropping densities, involving much larger removal of organic matter and increasing withdrawal of grazing animals into indoor intensive units are likely to

modify the situation.

The trace element content of plants varies depending on at which stage of the growing season sampling takes place, and from which part of the plant the sample is taken. In general the trace element content of the whole plant tends to decrease with increasing maturity (Fleming, 1965; Thomas et al, 1972). In the oat Avena sativa, the total copper content of the plant decreases progressively during the growing season, except for a slight flush as senescence sets in. Generally the copper is mobilised progressively into the seed grain, although the concentration within the grain is diluted by the storage there of organic matter. Similar patterns can be established for other trace elements, e.g. zinc and manganese, and in other cereals. Copper is an element which is generally present in similar concentrations in similar plants whether grown in deficient or copper-sufficient soils. It is usually found in grazing swards that the copper content of grass scarcely varies with the copper status of the soil, and that copper is fairly evenly distributed throughout the plant. Clover shows a marked ability to concentrate copper from marginal or sufficient soils, but serves as a good indicator plant in conditions of deficiency where many grasses show higher uptake rates than clover. In contrast to copper, zinc (Davey and Mitchell, 1968) tends to be concentrated markedly in

the young leaves of grasses such as cocksfoot and in meristematic plant tissues.

The soil management factors that chiefly influence the supply of trace elements to plants and animals are those affecting drainage, acidity, organic matter content, and, to a lesser extent, the application of chemical fertilizers. Drainage of soil is the single most important factor in relation to trace element supply from a particular type of soil in temperate areas such as the U.K. Table 1 illustrates the effects of free and impeded drainage on the trace element content of various pasture species grown on a soil derived from an argillaceous schist where it was possible to find adjacent areas of the two conditions on otherwise apparently identical soils (Mitchell et al, 1957b). Trace elements such as copper, zinc and molybdenum are much less affected than cobalts, nickel and manganese, perhaps because the former tend to be held by organic matter in the soil.

TABLE 1. Effect of soil drainage conditions on uptake of trace elements by red clover and ryegrass plants (μg/g)

Element	Cobalt		Nickel		Molybdenum		Copper	
drainage	free	poor	free	poor	free	poor	free	poor
soil extract	1.3	2.9	1.3	3.4	0.06	0.19	2.6	6.6
red clover	0.16	1.4	2.0	5.9	1.0	3.1	7.9	10.3
rye grass	0.18	1.5	1.0	3.4	0.7	1.2	4.0	3.4

(Soil extract contents in micrograms/gram of 2.5% acetic-acid soluble Co and Ni of 0.05 m EDTA - extractable Mo and Cu. Plant contents in μg/g DM plant material).

Mitchell, R.L. and Burridge, J.C. Phil. Trans. R. Soc. Lond. B 288, 15-24 (1979)

The acidity of the soil is the next most important management factor in relation to the supply of trace elements. Plant growth benefits from the raising of pH by the addition of lime. The input of organic matter and the leaching of calcium tends to increase the acidity of soils and to make them less suitable for the production of many arable crops. However, generally an increase in pH of 1 in the region of 5-6 will halve the plant availability of most cationic trace elements with the exception of copper which is

scarcely affected, but it will considerably increase the availability of molybdenum and selenium both of which are taken up as anionic species. Thus Mitchell and Burridge (1979) found that the cobalt content of the dry matter of red clover grown on a soil derived from granitic gneiss decreased from 0.26 to 0.16 μg/g as the pH rose from 5.4 to 6.4 while the corresponding changes for manganese were 56 to 25 μg/g; nickel 2.02 to 1.04 μg/g; zinc 61 to 51 μg/g. Copper remained almost constant from 12.5 to 13.0 μg/g and molybdenum increased approximately sixfold from 0.31 to 1.78 μg/g. A similar pattern had earlier been observed by Reith (1970) in clovers and ryegrass over a wider pH range. Such changes in pH can be brought about typically by the addition of 1-3 tonnes of calcium carbonate, oxide or hydroxide per hectare, and the effects will tend to be maintained to a decreasing extent for at least four years. Elements such as cobalt, manganese and nickel which are absorbed by plants and bound in inorganic cationic forms by the soil, are more affected than those bound by soil organic matter or taken by as anionic species. The insusceptibility of copper uptake to such changes in pH is fortunate in view of the pH-enhanced uptake of molybdenum, which can induce copper deficiency in ruminants. Amendment of the soil by copper application adequate to correct deficiencies for cereals can only marginally raise the copper content of herbage.

Modern agricultural practice relies heavily on the use of the major plant nutrients such as nitrogen, phosphorus and potassium for improved crop yields. It is recognised that high levels of potassium and/or ammonium may ultimately induce hypomagnesaemia in lactating cattle. Hemingway (1962) found that phosphate and potassium fertilisation had no material effect on the micronutrient uptake, but ammonium sulphate fertilisation increased the copper, manganese and iron content of grasses and reduced the molybdenum. Reith and Mitchell (1964) reported a small reduction in the cobalt content of most herbage species when nitrogen fertilisers were applied and an erratic effect on copper content. The copper content of cereals was shown to fall (Mitchell, 1972) with regular additions of nitrogen probably because growth outstripped the available copper supply. He found that nitrogen tended to increase the molybdenum content of clover and depress that of grasses. The manganese content of grasses was also depressed by nitrogen fertilisation but there was little effect on zinc. Phosphorus fertilisers appeared to cause a very slight increase in the manganese and molybdenum content of some herbage but the effect was irregular. Generally however the effect of phosphate was to reduce the copper, zinc and iron contents of herbage very slightly.

The relationship between the trace element

content of plants and the amounts absorbed and utilised by the grazing animal is again complex and depends on a variety of factors including the proportion of grass in the animal's diet, the form and availability of ingested trace elements and interactions with other dietary constituents. Several interactions between elements in the diet are recognised, including that between copper, molybdenum and sulphur, and a further wide range of antagonistic interactions that can influence trace element absorption adversely (Mills, 1979).

Cobalt

The soils of Scotland are very varied and relatively young, having developed from glacial tills laid down some 12,000 years ago. The complex geology has resulted in soil parent materials with very varied trace element contents, usually related directly to those of the parent rock. The soil parent materials are of the most diverse type as a consequence of the complex topography and the temperate oceanic climate giving rise to varied rock-weathering processes and the ready accumulation of peat deposits. The complete glaciation of the country some 12,000 years ago removed most of the soils that then existed, and left glacial till from which the present soils are being formed.

Prior to the discovery that cobalt deficiency was responsible for pine - a well documented condition of sheep and young cattle (Hogg, 1807; McGowan and Smith, 1922; Godden and Grimmet, 1928), Grieg et al (1933) noted the associations between calcareous soil type and the incidence of pine in sheep on the island of Tiree. When cobalt was found to be the true cause of pine in Australia and New Zealand in 1935-36, it became evident that a number of different parent materials, particularly sandstones, granite and other arenaceous rocks, might not contain enough available cobalt to carry pasture herbage with sufficient cobalt (around 0.08 mg/kg DM) to support healthy ruminants. In addition to the arenaceous hill lands of Hogg's Border Country, cobalt pining in sheep has been identified and treated on soils from almost all parts of Scotland, notably those on sandstones around the Moray highlands, and on the shell sands of the Western Isles. The geographical and topographical complexity has resulted in the occurrence of cobalt deficiency throughout the country.

Copper

As in most other countries, the copper problems that have arisen in Scotland are seldom straightforward. In 1937 in Australia the association between low copper soils and neonatal ataxia was proven which led to attempts in Scotland to define a similar relationship

between soil and swayback incidence. While in some occurrences the copper contents of herbage were found to be quite low (4 mg/kg DM), in many instances relatively normal levels of copper (5-10 mg/kg) occurred.

In seeking to explain the anomaly, it was discovered (Dick and Bull, 1945) that a complex antagonistic interrelationship exists between molybdenum, copper and sulphur levels in the ruminant. At high intakes of molybdenum, copper tends to be excreted irrespective of its requirement by the body. This effect is potentiated by the presence of sulphur (Dick, 1953a, b). Relatively high lead and tin levels in the soil and plants have also been implicated (Mitchell, 1974) in causing swayback conditions in sheep on certain Scottish soils.

Copper deficiency in cattle has been verified on low copper soils derived from sandstones or other aranaceous rocks, e.g. in Aberdeenshire. There are also numerous occurrences of induced deficiency in cattle resulting from high molybdenum levels in the herbage. These often occur in quite small areas in which the soil parent material contains some agillaceous rocks and the pedological drainage is generally impeded.

Manganese

Light sandy soils tend to be deficient in several trace elements such as cobalt, copper and sometimes even selenium. Cereals growing on this type of soil tend also to be deficient in manganese. The main reason for this is that manganese, like cobalt, is less available to plants when the soil pH approaches neutrality as it does on sandy soils. Manganese deficiency is therefore prevalent on the shell sand soils of the machairs. It is interesting to note that the black oats which were widely grown in the Western Isles at one time were less susceptible to this deficiency than the common varieties.

Normal young plants contain around 100 mg manganese/kg DM and a level of less than 20 mg manganese is indicative of manganese deficiency. At maturity, the normal manganese content is less than 30 mg manganese/kg. In very acid conditions, manganese excess can occur giving rise to levels of over 300 mg manganese/kg DM.

Manganese levels in Scottish soils are generally between 1 and 50 mg/kg while a content of less than 2.5 mg is considered as likely to give rise to manganese deficiency conditions.

Iodine

A clear relationship exists between low soil levels of iodine and the occurrence of simple goitre in man and animals. In areas of iodine sufficiency in the soil, complex goitre is still a possible occurrence because of the action of certain dietary constituents which interfere with thyroid hormone synthesis.

Simple goitre is one of the most widespread of all mineral deficiency diseases. It occurs over large areas on every continent and in all classes of livestock, wherever goitre in man is endemic, and wherever animal diets are composed wholly or largely of feed, derived from the goitre region. Low soil iodine contents tend to be associated with recent glaciation, distance from the sea, and low annual rainfall. In goitre regions that have not suffered from recent glaciation, the existence of low iodine soils can generally be attributed to small or negligible accumulation of iodine of marine origin, due to their long distance from the sea, or low rainfall or both. Goitrous areas in Scotland are located mainly around the west coast (Iodine Education Bureau). Extensive areas of western coastlines extending towards central regions of England also show low levels of iodine in soil. There is evidence that ingestion helps to prevent the development of goitre (Stratham and Bray, 1975) since most soils are very much richer in iodine

than the plants which grow on them.

Selenium

Selenium is known to be essential to animals but the presence of a slight excess can lead to severe disorders in many species. Selenium toxicity has not been recorded in Scotland although it does occur on poorly drained organic soils in Ireland. There is however an incidence of selenium deficiency which can be related to arenaceous soils such as the trace element deficient sands along the shores of the Moray Firth where enzootic muscular dystrophy occurs in cattle.

An extensive survey on the effect of selenium on hill sheep in Scotland (Blaxter, 1963) has indicated that a small but significant increase in liveweight could be obtained on many soils derived from arenaceous parent materials. It is suggested from these findings that one-tenth of the land area of Scotland is mildly selenium deficient.

Zinc

There is no documentation of zinc soil deficiency in Scotland although it has been reported in British Guiana (Legg and Sears, 1960) and Greece (Spais and Papasteriadis, 1974). The sparse grazings on which the deficiency occured mostly contained 20-30 ppm zinc but values as low as 6 ppm were observed in some

samples from sandy soils. Experimental diets which contain <20 ppm zinc are often found to be adequate, therefore it is possible that some antagonistic effect with as yet unidentified factors may be involved.

2. The Biochemical Role of Individual Trace Minerals and the Effects Ensuing from Deficiency

In order to determine whether a trace mineral is indeed essential to an animal, six basic points of definition were established by Cotzias (1967). An essential trace element should meet the following criteria:

i. It is present in all healthy tissues of all living things.

ii. Its concentration from one animal to the next is fairly constant.

iii. Its withdrawal from the body induces reproducibility the same physiological and structural abnormalities regardless of the species studied.

iv. Its addition either reverses or prevents these abnormalities.

v. The abnormalities induced by deficiency are always accompanied by specific biochemical changes.

vi. /

vi. These biochemical changes can be prevented or cured when the deficiency is prevented or cured.

Living tissues also contain 20 to 30 other trace elements occurring in variable concentrations, which do not meet these criteria, such as aluminium, antimony, cadmium, mercury, germanium, rubidium, silver, lead, gold, bismuth, titanium, zirconium and others. However, these are believed to be non-essential and their presence is thought to be entirely incidental arising from the animal's contact with the environment.

Minerals play three main roles in ruminant biochemistry. Firstly some are structural components of body organs and tissues. Calcium, phosphorus, magnesium, fluorine and silicon are found in bones and teeth, and phosphorus, sulphur and calcium are present in muscle protein. Secondly some minerals are constituents of body fluids and tissues as electrolytes concerned with maintaining osmotic pressure and/or acid base balance, or influencing membrane permeability and tissue irritability. Sodium, potassium, chlorine and magnesium are present in the intracellular, extracellular and cerebrospinal fluids and in gastric secretions.

However, as a third role, many trace minerals are

predominantly present as catalysts in enzyme and hormone systems as integral and specific components of the structure of metalloenzymes or less specific activators within these systems. In metalloenzymes, a fixed number of metal atoms are bound to the protein fragment of the enzyme and any loss of the metal results in the loss of enzyme activity. The following table summarises the deficiency conditions which may arise as a result of interference in enzyme activity due to a physiological deficiency of a particular trace element.

The research work involved in establishing that trace minerals were dietary essentials of ruminants was spread over a period of almost a century. Iodine was discovered as an essential trace mineral during the 19th century while selenium was not identified as such until the 1950s. In general, the association between specific diseases and trace elements was made when the administration of a particular trace mineral cured or prevented a particular disease. Later, biochemists were able to produce detailed explanations as to why the lack of a particular trace mineral caused certain disease conditions to be manifest.

TABLE 2. Principle pathological and metabolic defects in essential trace element deficiencies

Deficiency	Pathological Consequences	Associated Metabolic Defect
Copper	Defective melanin production	Tyrosine/DOPA oxidation
	Defective keratinization, hair, wool	-SH oxidation to S-S
	Connective tissue defects	Lysyl oxidase
	Ataxia, myelin aplasia	Cytochrome oxidase
	Growth failure	?
	Anaemia	?
	Uricaemia	Urate oxidase
Cobalt	Anorexia	Methyl malonyl CoA mutase
	Impaired oxidation of propionate	
	Anaemia	Tetrahydrofolate methyl transferase
Selenium	Myopathy: cardiac/skeletal	Peroxide/hydroperoxide destruction
	Liver necrosis	Glutathione peroxide
	Defective neutrophil function	OH O_2 generation
Zinc	Anorexia, growth failure	?
	Parakeratosis	Polynucleotide synthesis, transcription, translation
	Perinatal mortality	
	Thymic involution	
	Defective cell-mediated immunity	
Iodine	Thyroid hyperplasia	Thyroid hormone synthesis
	Reproductive failure	
	Hair, wool loss	
Manganese	Skeletal/cartilage defects	Chondroitin sulphate synthesis
	Reproductive failure	?

C.F. Mills. Trace Elements in Animal Production and Veterinary Practice. Occasional Publication No. 7. B.S.A.P. 1983. Edit. N.F. Suttle, R.G. Gunn, W.M. Allen, K.A. Linklater and G. Wiener.

Cobalt

A wasting disease of cattle and sheep on apparently luxuriant pastures in Australia and New Zealand was well documented in the early 20th century and was known as bush-sickness. In 1937 Askew and Dixon discovered that a drench of cobalt chloride providing 8 mg cobalt per week was completely successful in curing the disease. This discovery led to the identification of cobalt as an essential nutrient. Further research on the disease continued in the field and in the laboratory. A breakthrough occurred in 1948 with the discovery in both the United States and Britain (Rickes et al, 1948, and L. Smith, 1948 respectively) that, when isolated, the anti-pernicious anaemia factor vitamin B_{12} contained 4% by weight of cobalt. It became apparent that the consequences of cobalt deficiency in sheep were attributable to a lack of vitamin B_{12} and that cobalt was required only to permit synthesis of the vitamin by microorganisms in the rumen.

Severe cobalt deficiency in sheep is characterised by a loss of appetite, a severe wasting condition, a progressively developing anaemia with associated pallor of the mucous membranes, skin fragility and moderate to severe lachrymation. There is increased susceptibility to infection due to a lowering of the body's defence mechanisms which detrimentally affects

the viability of new born lambs.

Subclinical cobalt deficiency, although not so readily recognised, may be of greater economic importance. In this condition, large numbers of animals fail to achieve optimum performance. Overt clinical symptoms may never appear but animals do not thrive despite an apparent abundance of feed.

The symptoms of the clinical condition in cattle are very similar to those seen in sheep. Inappetence and listlessness are the early indicators of the condition which, if left untreated, result in ill-thrift and progressively developing anaemia. Eventually loss of live weight, which can be quite rapid, may ensue, leading to severe emaciation and death. The subclinical condition is again hard to define or diagnose and can probably be definitely confirmed only on the basis of dose-response trials. However, as with lambs, young growing cattle fail to thrive and their coats become lack lustre in appearance.

The main energy source of ruminants is from acetic and propionic acids and smaller amounts of butyric and other fatty acids produced by fermentation in the rumen. The enzyme which catalyses the breakdown of methyl malonyl CoA during propionate metabolism, is a vitamin

B_{12} dependent enzyme methyl malonyl CoA isomerase. In sheep with inadequate cobalt supplies, this enzyme has been found to be deficient (Marston et al, 1961). Consequently, if deficiency continues, the rate of clearance of propionate from the blood is depressed and the intermediary metabolic methyl malonyl CoA accumulates (Marston et al, 1972). Raised propionate levels appear to exert an inhibitary effect on acetate metabolism, and it also accumulates. The most significant evidence linking inappetence with impaired propionate levels is an inverse relationship between the voluntary feed intake of deficient sheep, and the half time for propionate clearance (Marston et al, 1972).

A second vitamin B_{12} containing enzyme 5-methyl tetrahydrofolate homocysteine methyltransferase catalyses the reformation of methionine from homocysteine. The activity of this methyltransferase is depressed in the liver of vitamin B_{12}-deficient sheep (Gawthorne and Smith, 1974) which could lead to a deficiency of available methionine. This provides a possible basis for the impaired nitrogen retention found in vitamin B_{12}-deficient sheep and could be a critical factor in wool and body growth.

Copper

In 1928 Hart et al showed that copper was essential for growth and haemoglobin formation in rats.

Shortly afterwards a number of disease conditions were identified in different parts of the world as being responsive to copper therapy, such as salt-sick cattle in Florida (Neal et al, 1931) and "lecksuckt" of sheep and cattle in the Netherlands (Sjollema, 1933). Gradually, a number of metalloenzymes containing copper were later identified in the cells and tissues, such as ascorbic acid oxidase, lysyl oxidase, cytochrome oxidase and superoxide dismutase. The concentrations and activities of many of these enzymes were then related to the specific functional and structural disorders that develop in the copper-deficient animal.

Copper deficiency in grazing stock cannot be linked in a direct way to low herbage copper levels. Molybdenum and sulphur levels are also critically important, in that they adversely affect copper absorption by the animal. Indeed, a particular level of dietary copper can, under certain natural grazing conditions, induce either copper deficiency or copper toxicity in animals, depending on the concurrent levels of molybdenum and sulphur in the herbage. The 'teart' pastures in Somerset are a well known example of copper deficiency induced by high molybdenum and sulphur levels.

Severe signs of copper deficiency are rare in animals aged two years or older and are most evident in

cattle which are 3 to 12 months old. The earliest features of the syndrome in young cattle are changes in the texture and colour of the hair coat, reduction in growth, changes in the leg bone formation, and in some instances of severe deficiency, diarrhoea.

The process of pigmentation is very sensitive to copper levels and achromotrichia is often the first obvious symptom of copper deficiency due to interference in the conversion of tyrosine to melanin. Tyrosine conversion to melanin is catalysed by copper containing polyphenyl oxidases. When this effect is manifest, depigmentation usually starts on the head around the eyes (known as "spectacle-eye"). The loss of crimp from sheep's wool is another early feature of copper deficiency, and is known to be due to the disruption of sulphur bridges in hair fibres. Recent research, however, has shown that growth failure from copper deficiency is not always preceded by hair colour change. The appearance of "spectacle eye" described in earlier reports such as Jamieson and Allcroft (1949) is not always evident (Mills et al, 1976).

The change in structure and density of the metatarsal bone is a highly specific and early clinical manifestation of copper deficiency in cattle. Measurement by callipers shows that there is a significant decrease in the ratio of width at midshaft

to width at epiphysis (Mills et al, 1976). Normal bone becomes relatively narrower at midshaft, but wider at the epiphysis. Skeletal changes are less common in copper deficient sheep, but an increased susceptibility to fractures has been noted in lambs reared on improved hill pastures.

Diarrhoea is also a symptom of copper deficiency in cattle. It clears rapidly when copper is administered orally in quantities that are normally insufficient to increase the concentration of copper in blood or liver.

This, and evidence obtained with other species suggests that the gastrointestinal tract is particularly sensitive to copper depletion whether this arises from a generalised tissue deficiency, or from the presence of dietary antagonists inhibiting locally, the utilization of copper by the intestinal mucosa.

The most significant economic losses caused by copper deficiency arise from the decreased growth rate of young cattle, and their reduced efficiency of feed conversion. In contrast to cobalt deficiency, such effects usually develop gradually and are rarely accompanied by an abrupt decline in feed intake.

Young lambs confined to improved hill pasture show ill-thrift, poor wool quality and increased

susceptibility to bone fractures. Swayback in lambs is the most common sign of copper deficiency in sheep. Congenital swayback is apparent at birth but the delayed form is manifest at 6 to 8 weeks of age. It can range from complete paralysis of the newborn lamb to a less severe staggering gait which in some cases may be virtually asymptomatic. Death from acute cardiac failure or massive haemorrhage in cattle has been recognised as being due to copper deficiency in many parts of the world (Bennets et al, 1942; Bennets et al, 1948) though not in the U.K.

At preclinical stages of the deficiency, degenerative changes occur in the collagen and elastin matrix of tendons and probably in the collagen matrix of bone. There are structural changes in the heart mitochondria and connective tissue matrix of heart muscle in conjunction with cardiac enlargement (Leigh, 1975). There is growing evidence that degenerative changes also arise in the sympathetic nervous system of copper deficient cattle, and particularly in that part of it serving the gastrointestinal tract (Fell et al, 1975). Copper is involved in at least two stages of the metabolic events leading to the synthesis of haemoglobin. Nevertheless the supply of tissue copper for these processes appears to be strongly conserved during copper depletion. Thus, anaemia, is a late feature of the copper deficiency syndrome.

Copper is essential for the absorption of iron from the intestinal mucosa, the mobilisation of iron from the tissues, and its utilization in haemoglobin synthesis. These functions are carried out by ceruloplasmin, a copper containing enzyme necessary for the formation of iron III transferrin, and the transport vehicle for copper. This has been demonstrated by Evans and Abraham (1973) and the methods assessed by Frieden (1971).

Osteoblastic function is vulnerable to copper deficiency in foetal and neonatal lambs (Suttle et al, 1972). This is due to a reduction in the activity of lysyl oxidase, a copper containing enzyme responsible for cross-linking polypeptide chains in bone collagen.

Neonatal ataxia or swayback is associated with myelin aplasia and degeneration of the motor neurones of brain and spinal cord (Mills and Williams, 1962; Howell et al, 1964). The precise biochemical interaction causing these lesions is not fully known. Two possible explanations exist. Ataxic lambs have subnormal levels of the copper containing enzyme cytochrome oxidase, which is the terminal respiratory enzyme of the electron transport chain. Gallagher and Reeve (1971) have shown that depressed cytochrome oxidase activity in rats impairs phospholipid synthesis. Since myelin is composed largely of phospholipids, this

could lead to myelin aplasia and therefore the symptoms displayed in swayback.

The second explanation has arisen from more recent research. Smith et al (1976) have shown that the magnitude of depression of cytochrome oxidase activity is insufficient to lead to the respiratory constraints which would bring about the degeneration of myelin. Also, neurotransmitters such as noradrenalin as well as plasma amine oxidase are lower in concentration in the brainstem of ataxic lambs compared to normal animals (O'Dell, 1976). The depressed amine oxidase activity could interfere with myelination through a decrease in the supply of oxygen to nerve cells, because lack of this enzyme in copper deficiency tends to thicken the vascular wall.

The elastin content of the aorta was shown to be reduced in copper deficient pigs and chickens (Hill et al, 1967) because of an increase in lysine with relation to desmosine. Desmosine is the cross linkage group in elastin and it is found when lysine is oxidatively deaminated. The enzyme responsible for this function is lysyl oxidase, a copper metalloenzyme which has diminished capabilities for catalysing the transformation of lysine to desmosine when copper levels are reduced. Therefore cardiovascular disorders and ruptures arise because of reduced elasticity in the blood vessels.

Selenium

The role of selenium as a toxic agent in higher animals was first recognised in a series of studies beginning in 1929 (Franke, 1934). However, it was not until many years later in 1957 that selenium was recognised as an essential nutrient in rats and chicks by Schwarz and Foltz, and Patterson et al respectively. Within two years it was found that the muscular dystrophy that occurs naturally in lambs and calves in parts of Oregon and New Zealand is caused by selenium deficiency and can be prevented by selenium therapy. Kubota et al (1967) subsequently determined that the selenium deficient areas of the U.S. are much larger than the selenium toxic regions. The effects of selenium deficiency are often modified substantially by changes in the dietary supply of vitamin E. The two nutrients have closely related biochemical functions.

The most common clinical symptom of selenium/ vitamin E deficiency in cattle and sheep is nutritional myopathy which is know as white muscle disease or paralytic myoglobinuria.

Calves and lambs from dams severely deficient in selenium/vitamin E may be born dead or die within a few dyas of birth due to sudden heart failure with no obvious previous symptoms. Delayed mopathy can occur at up to two years of age in affected animals, usually

resulting in a peculiar stiff gait and reluctance to move. Older animals are generally less severely affected, indeed those suffering only mildly may recover spontaneously.

On post mortem examination the affected muscles are mostly those of the neck, legs, and trunk. They are pale in appearance and in more severe cases show white striations, probably due to the deposition of calcium. When the heart is involved, the myopathy appears as white plaques which may extend up to 1 mm into the myocardium.

Where nutritional myopathy is endemic in Australia and New Zealand an "ill-thrift" syndrome of sheep and cattle has been reported (Wilson, 1964; Dodson and Judson, 1973). Affected stock fail to grow and respond favourably to selenium therapy, but not to vitamin E. Other disorders such as retained placentae in cattle have been shown to be responsive to selenium/vitamin E prophylaxis (Trinder et al, 1969). Evidence from U.S.A. (Mace, 1963; Julien et al, 1976a, b) and New Zealand (Hartley, 1963) indicates that some reproductive problems in sheep and cattle are responsive to selenium therapy. All the selenium responsive diseases are accompanied by biochemical changes in the blood and tissues, particularly subnormal selenium and glutathione peroxidase values. Glutathione peroxidase

(GSH-Px) is a selenium containing enzyme and catalyses the reduction of peroxide and hydroperoxide formed from the breakdown of fatty acids and other substances, thus protecting tissues against oxidative damage.

Vitamin E also acts as an antioxidant though its relationship to selenium is not fully understood. Noguchi et al (1973) have sought to explain the situation by postulating that GSH-Px is located internally in the cell cytosole whereas vitamin E is present as an antioxidant in the cell membrane. On this basis, GSH-Px would be the first line of defence in detoxifying metabolites produced in the cell while vitamin E would prevent antioxidation of membrane lipids.

Selenium appears to be involved in the metabolism of sulphydryl compounds (Broderius et al, 1973; Sprinker et al, 1971), in the oxidative processes of the tricarboxylic cycle (Whanger, 1973; Godwin et al, 1974), and in fatty acid and glucose metabolism (Fischer and Whanger, 1977). Animals suffering from white muscle disease have increased amounts of serum aspartic aminotransferase and lactic dehydrogenase.

Iodine

Human goitre has been successfully treated with salts of iodine since the 19th century. Subsequently

the thyroid gland was found to contain a high concentration of iodine, and reduced amounts were found in goitrous thyroids (Marine et al, 1908). By 1953 the active principle of the thyroid gland had been isolated, identified as tetra iodothyronine, and named thyroxine (see Harington, 1953). Subsequently triiodothyronine which has three to four times the potency of thyroxine was shown also to be secreted by the thyroid gland, and to circulate in low concentrations in the blood. Extensive goitrous areas were discovered in every continent associated primarily with environmental deficiency of iodine. A high degree of control of the disease was achieved by raising individual iodine intakes, usually through the medium of iodised salt.

The most common manifestation of iodine deficiency in farm animals in the U.K. is late abortion, or the birth of dead lambs or calves with enlarged hyperplastic thyroids (Andrews and Sinclair, 1962). Clinical manifestations of goitre attributable to a deficiency of dietary iodine rarely appear at later stages of development, but are not uncommon when brassicae with a high content of goitrogens form a major part of the diet. Although infertility, loss of male libido and hair or wool loss have been clearly associated with a severe deficiency of dietary iodine in other parts of the world (Underwood, 1977), there is no clear

evidence that such effects arise under Scottish conditions.

Thyroid hormone primarily controls the rate of oxidation of all cells. In simple goitre the rate of energy exchange and the quantity of heat liberated by the tissues are reduced and the basal metabolic rate declines. Motility in the alimentary tract is reduced with a slower rate of passage of food through the digestive tract of cattle (Miller et al, 1974). The decline in basal metabolic rate is accompanied by a fall in concentration of protein-bound iodine of the blood serum and in the free or unbound thyroxine.

Manganese

Manganese composes 0.1% of the earth's crust and is the twelfth most abundant element. It is widely distributed in very low concentrations in the cells and tissues of the animal body and it is necessary for the normal development of bone and to maintain a proper functioning of the reproductive processes in both male and female. Manganese was first shown to be essential for growth and fertility in mice by Kemmerer et al in 1931 and in rats by Orent and McCollum in 1931.

It is an essential component of enzymes involved in the synthesis of constituents of the organic (mucopolysaccaride) matrix of bone, cartilage and

other connective tissues (Leach, 1971). Studies with manganese deficient laboratory animals suggest it also has important roles in some key enzymes of carbohydrate metabolism (Underwood, 1977) and may be involved in the synthesis or metabolism of fats and sterols (Plumlee et al, 1956; Anke et al, 1973). Pathological changes induced during such experiments include skeletal defects, pancreatic degeneration, and defects in the balancing mechanism of the inner ear that result in ataxia (Hurley et al, 1960). There appear to be marked genetic variations in susceptibility to the pathological lesions of manganese deficiency.

Experimentally induced manganese deficiency in calves, lambs and goat kids born of dams maintained on low manganese diets causes deformity of long bones, increased bone fragility, joint stiffness and swelling (Rojas et al, 1965; Howes and Dyer, 1971; Lassiter and Morton, 1968; Groppel and Anke, 1971). In some studies unco-ordinated voluntary muscle movements in deficient animals have been shown (Groppel and Anke, 1971). It has been suggested that skeletal and joint defects are relatively early manifestations of manganese deficiency; neurological lesions are only likely to develop if deficiency is severe.

Manganese deficiency was originally shown to impair reproductive performance in small laboratory animals

though its precise role was not identified. Some studies have proved the association between manganese deficiency, delayed or irregular oestrous and reduced conception rates under experimental conditions in cows but whether this can be applied to practical husbandry situations is not clear (Bentley and Phillips, 1951; Munro, 1957; Rojas et al, 1965).

Manganese functions as an activator of the glycotransferases necessary for polysaccharide and glycoprotein synthesis (Leach, 1971) which are vital structural components of cartilage, thus leading to bone deformities in deficiency situations.

The role of manganese in reproductive disorders is subject to speculation. Hidiroglou (1975) has suggested that this element has a possible role in the functioning of the corpus luteum while Doisey (1973) suggests that a lack of manganese inhibits the synthesis of cholesterol and its precursors which in turn limits the synthesis of sex hormones and possibly other steroids with consequent infertility.

Little is known of the defective lipid and carbohydrate metabolism in ruminants which has been reported in rats and guineapigs experiencing manganese deficiency (Everson and Schrader, 1968; Zhuk, 1964). Manganese is also involved in the

formation of prothrombin, a glycoprotein, through its activation of glycotransferases. The clotting response from vitamin K is reduced in manganese deficient chicks (Doisey, 1973) but the precise involvement of manganese with vitamin K in the conversion of prethrombin to prothrombin is not clear.

Zinc

Shortly after Todd et al (1934) demonstrated that zinc was necessary for growth and health in rats and mice, it was also experimentally shown to be necessary for pigs, poultry, lambs and calves. The deficiency was associated in all species with severe inappetance and growth depression, impaired reproductive performance and abnormalities of the skin and its appendages. Zinc was found to both prevent and cure parakeratosis in pigs (Tucker and Salmon, 1955), a discovery which stimulated further research into zinc's role as a nutrient for farm animals.

Zinc deficiency in all species is clinically characterised by inappetance, slowing or halting of growth, and lesions in the skin and hair, wool or feathers. All phases in the female reproductive process in rats from oestrous to parturition and lactation have been shown to be adversely affected (Hurley and Swenerton, 1966; Hurley and Mutch, 1973; Hurley and Shrader, 1975). In the male, spermatogenesis

and the development of primary and secondary sex organs may be impaired as in calves and ram lambs (Pitts et al, 1966; Millar et al, 1961)

Reduced growth in affected animals is partly due to a decrease in voluntary intake of food, and partly due to impaired protein metabolism (Miller et al, 1968; Somers and Underwood, 1969). A primary factor in growth inhibition is that thymidine kinase activity is reduced in zinc deficient animals (Prasad and Oberleas, 1974; Dreosti and Hurley, 1975). Thymidine kinase is a zinc metalloenzyme which catalyses the formation of thymidine triphosphate, a necessary metabolite for DNA synthesis and cell division.

In calves zinc deficiency is manifest in subnormal growth, and parakeratosis localised mainly on the muzzle, neck, ears, scrotum and back of the hind limbs. Bowing of hind limbs, stiffness of the joints and swelling of the hocks also occurs (Miller and Miller, 1962). Similar symptoms are shown by affected lambs. Changes in the wool and horns of sheep are particularly obvious, and the horns are ultimately replaced by soft spongy outgrowths that continually haemorrhage (Mills et al, 1967). Changes can also occur in the hoof structure. Wool growth alters markedly - it becomes thin and loose and easily shed, and does not start to regrow until additional dietary zinc is provided (Underwood

and Somers, 1969).

The functional and structural abnormalities of zinc deficiency described are associated with a wide variety of biochemical changes in the blood and tissues. The amounts of zinc in hair, wool and feathers is normally high (100-200 ppm) but as zinc deficiency develops there is usually a marked decline in their zinc concentration and a small decline in the zinc in concentration of the liver, kidney, heart, bone and muscle. The zinc contents of the male sex organs and secretions which are also normally high, similarly decline (Underwood and Somers, 1969).

Zinc occurs in blood plasma, erythrocytes, leucocytes and platelets. Almost all of the zinc in erythrocytes occurs as carbonic anhydrase, an enzyme which cannot be detected in white blood cells or plasma. The activity of carbonic anhydrase is depressed in zinc deficient calves (Miller and Miller, 1962) and plasma zinc levels are reduced (Mills et al, 1967).

Alkaline phosphatase activity is reduced in the blood serum and bones of deficient cows (Kirchgessner et al, 1975). The blood serum of lambs (Saraswat and Arora, 1972) also has reduced activity of alkaline phosphatase and of liver alcohol dehydrogenase. Vitamin A metabolism is also affected by zinc levels.

When zinc levels are inadequate, vitamin A deficiency can be manifest despite its adequate dietary provision (Saraswat and Arora, 1972) and zinc therapy can alleviate this condition.

3. Mineral Requirements of Ruminants

An accurate assessment of the absolute quantities of minerals required in the ruminant diet is necessary in order to minimise the incidence of preventable clinical and subclinical deficiency states. Two basic approaches have been adopted in the calculation of requirements. Factorial studies measure mineral necessity from a detailed study of endogenous losses of an element from the animal's body post utilisation and of quantities retained in new tissues for growth and pregnancy. A summation of losses plus retention then produces an absolute value for the requirement of a particular mineral. Absorption coefficients must be taken into account in calculating the daily amount needed in the diet. The other approach is the more traditional one from which most requirements are presently assessed. This involves the feeding of varying dietary levels of a particular mineral and assessing thereby the point at which symptoms of deficiency appear.

The criterion of adequacy employed can be an important determinant of the selected minimum requirement of a mineral. As the amount available to the animal becomes insufficient for all the metabolic processes in which the element participates, as a result of inadequate intakes and the depletion of body reserves,

some of these metabolic processes fail or are adversely affected. Others remain unaffected initially. An example of this is that pigmentation and keratinization of wool is first affected by low copper status when no other effect is manifest. Thus if wool quality is the fundamental criterion of adequacy then the recommended copper requirements will be higher than if growth rate and blood haemoglobin were used as criteria. In addition to the prevention of all known deficiency symptoms, minimum mineral intakes must be sufficient to ensure the long term maintenace of the mineral reserves of the body tissues and the amounts of those minerals in the edible products of the animal. The animal body has the capacity to make some adjustment to suboptimal intakes by reducing the amount of mineral in its products, e.g. milk and eggs. Thus quality characteristics such as the shell strength of eggs, may be reduced in order to maintain quality.

The evaluation of feeds and feed supplements as sources of minerals depends not only on what the feed contains, but on how much of the total mineral can be absorbed from the gut and used by the animal's cells and tissues. This depends on the age and species of the animal; the intake of the mineral and its need; the chemical form in which the mineral is ingested; the amounts and proportions of other dietary components with which it interacts metabolically; and the

environmental factors such as accessibility.

In 1965 the Agricultural Research Council published an exhaustive list of the nutrient requirements of farm livestock. For the major mineral elements, the factorial approach was used in which the requirement was calculated from (i) the endogenous losses of the element in faeces and urine and from skin; (ii) the amount of the element stored during growth and in pregnancy; (iii) the amounts excreted in milk and wool; (iv) the extent to which the element in the ruminant's food is available to it. The factorial calculation is the most logical method of arriving at requirements, but it was not applicable in all cases to trace elements and vitamins.

The minimal requirement figures given in ARC (1965) have been generally accepted in the U.K. as the guidelines for nutritional, advisory and feed formulation work. The Agricultural Research Council have since revised and updated the work in 1980 in their publication "The Nutrient Requirements of Ruminant Livestock". This takes account of further research work carried out in the interim period. These modified proposals were critically appraised by a working party commissioned by ADAS in 1983.

Copper

At the time of publication of ruminant mineral requirements by ARC (1965), copper availability had not been fully researched and thus was not accurately known. Underwood (1962) estimated that 5-10% of dietary copper was absorbed and retained. Murty (1957b) was able to relate copper intake and copper retention in a regression equation and the maintenance requirement arising from his work was estimated to be 7 mg copper/kg DM intake. A similar requirement of 100 mg/d for an adult cow for maintenance was proposed by Chapman and Kidder (1958). Having weighed up all the available evidence it was decided that intake levels recommending 10 mg copper/kg DM for cattle and 5 mg copper/kg DM for sheep were unlikely to be associated with copper deficiency conditions.

In the revised and updated ARC (1980), the proposed copper requirements do not differ substantially from those of 1965. The influence of dietary antagonists on copper availability were proved to have a more potent effect than was originally realised although more research is required in order for this effect to be more fully quantified. Assuming that there are no competitive dietary effects, factorial estimates of the copper necessary for growth, pregnancy, lactation and wool production plus endogenous losses were totalled.

An absorption coefficient is needed to convert net requirements to gross requirements for copper. Values vary depending on many factors only some of which can be accounted for. Firstly at the preruminant stage there are age-dependent changes. Preruminant calves and lambs absorb copper much more efficiently than the mature animal, in a similar way to monogastric species. Suttle (1975) has shown that the copper availability in preruminant lambs maintained on a milk substitute diet decreased from an absorption coefficient of 0.8-0.9 shortly after birth to 0.2-0.3 at six weeks of age. If age related changes in availability occur in the calf, they are probably completed within the first four weeks of life. The development of a fully functional rumen is associated with a decline in the apparent absorption of copper and in one study with sheep (Suttle, 1975) the decline associated with developing rumination to 0.08 in the coefficient of absorption obscured the age related changes when lambs were maintained exclusively on a milk diet as described above.

The amount of copper stored in the liver is dependent on intake and where molybdenum and sulphur levels are low, the coefficient of absorption of stored copper is of the order of 0.06 ± 0.01. This figure is based on the work of Hill and Williams (1965), MacPherson and Hemingway (1965), Hogan et al (1968)

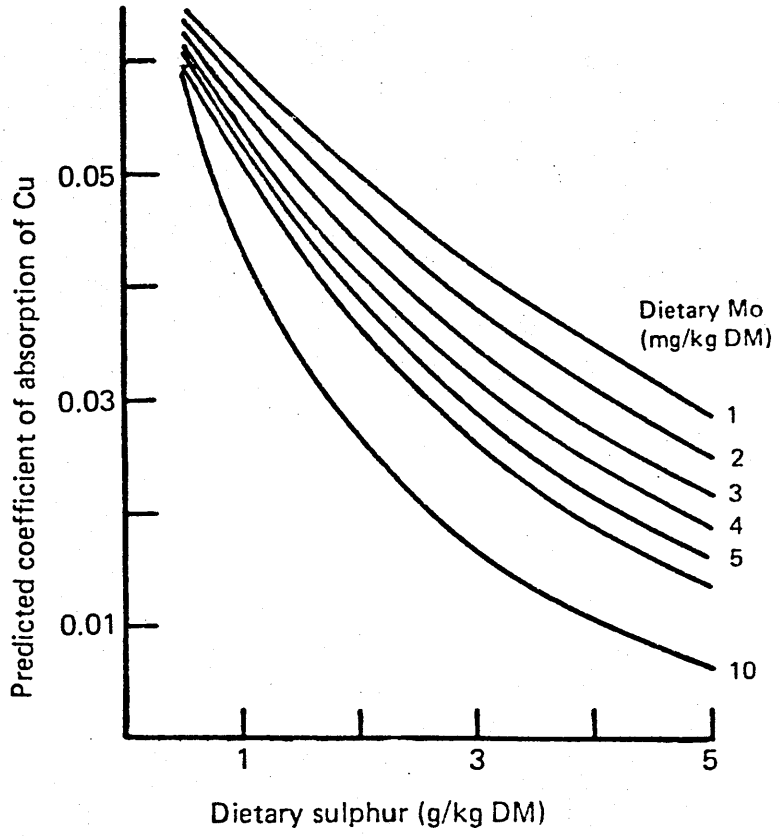
and Kline et al (1971). This value falls between the values obtained by isotope dilution technique (0.086; Smith et al, 1968) and by a repletion technique (0.057 ± 0.005) for eight semipurified diets with a low sulphur content of 1 g/kg (Suttle, 1974a, b).

The absorption coefficient of copper from fresh herbage suggests that it is less than for concentrate diets. Suttle and Price (1976) using a repletion technique obtained a value of 0.048 for sheep given cut herbage.

A highly complex interrelationship exists between copper, molybdenum and sulphur. Molybdenum has an adverse effect on the utilisation of copper by sheep, and inorganic sulphate potentiates this effect (Dick, 1953a, b), sulphate accelerates the clearance of molybdate (Dick, 1956) and molybdenum influences the ruminal metabolism of sulphide (Mills, 1960; Gawthorne and Nadar, 1976). Under conditions of increased molybdenum in the ruminal fluid, the reduction of sulphate to sulphide decreases in rate by 50%. Although the rate of sulphide production is slower, the concentration of sulphide in rumen contents is increased. A dual role for molybdate in the metabolism of sulphide in the rumen is suggested to explain these changes. Suttle and McLauchlan (1976) produced a graphical prediction for a range of dietary sulphur

and molybdenum contents on the resultant availability of copper (see Diagram 1).

DIAGRAM 1. Relationship for sheep of coefficient of absorption of dietary copper to dietary content of total sulphur and molybdenum (from Suttle and McLauchlan, 1976).



When the soil structure is poor and herbage short, soil constitutes more than 10% of the DM intake of ruminants (Field and Purves, 1964; Healy, 1967). Experimentally the inclusion of 10% of three diverse soils in the diet of sheep reduced the absorption coefficient of copper by at least 50% (Suttle et al, 1975). Known copper antagonists, zinc and molybdenum,

were present in certain soils but were not thought to have been present in sufficient quantities to interfere greatly with copper metabolism. Soil ingestion may be involved in the aetiology of hypocuprosis in cattle and swayback in sheep since it commonly constitutes 10% of the DM intake of animals grazing winter pasture.

Dick (1954) found that hepatic copper retention was reduced by calcium carbonate administration in cattle and sheep but other workers such as Hemingway (1962) were unable to verify this effect. Liver copper reserves of calves at pasture were shown to be depleted by the administration of iron hydroxide equivalent to a dietary concentration of 1.4 g/kg DM. Mills and Dalgarno (1972) found that cadmium at 7 mg/kg added to the diet of the pregnant ewe reduced the liver copper stores of her offspring. More recent work indicates that even 3 mg cadmium/kg adversely affects copper retention and growth in lambs on a semipurified diet (Mills, 1974). Consumption of rations containing 40, 220, or 420 mg zinc/kg reduced the fraction of dietary copper retained by the liver of grazing lambs during a four week period from 0.04 to 0.028 and 0.015 respectively (Bremner et al, 1976).

There are widespread differences between the absorption coefficients of different breeds of sheep and also between individuals of a similar age, breed

and physiological state in a common environment. Thus the absorption coefficient for copper varied from 0.042 to 0.112 in a group of 36 ewes on a semipurified diet (Suttle, 1974a). In the grazing sheep, Wiener and Field (1970) have reported hereditable differences among three breeds and their crosses in susceptibility to swayback and copper poisoning, the breed that was most susceptible to swayback being least susceptible to copper poisoning. Genetic differences in the absorption or hepatic retention of copper in bovine species have not been proven, although it is assumed that they exist.

The estimate of ARC (1965) for the appropriate copper requirement for sheep and cattle was broadly agreed to be correct when the most recent information available was taken into account in the compiling of the 1980 report. However, as a result of the existence of currently available research, more specific recommendations are included in the 1980 report which cover the variations in requirements necessary for a growing lamb gaining, for example, 0.075 kg daily, as compared with one gaining 0.15 kg daily.

Whether this degree of detail is of any practical application is debatable, especially when the range of individual variation in absorption in animals of the same breed is considered. ARC (1980) recommended the

following dietary levels:- a lamb of 5-10 kg requires 1 mg copper/kg DM, a growing lamb 1.7-5.1 mg copper/kg DM depending on its weight and rate of growth, and an adult of 50 kg, 4.6-7.4 mg copper/kg DM. For cattle, ARC (1980) recommended the following dietary concentrations:- a preruminant calf, 1.2 mg copper/kg DM, a growing bullock 8-15 mg copper/kg DM depending on weight and rate of gain, and an adult of 500 kg, 12-19 mg copper/kg DM. The working party commissioned by ADAS in 1983 critically appraised the ARC (1980) requirements in the light of existing knowledge and experience of members of the party. For specific detailed reasons, certain alterations were made to ARC (1980) recommendations. Categories of stock were also simplified and requirements quoted as a single value per kg/DM feed, rather than as a range of values. The ARC (1980) requirements for copper were accepted by ADAS (1983) and were published as follows: a preruminant calf 2 mg copper/kg DM, all other cattle 12 mg copper/kg DM. For sheep, a preruminant lamb 1 mg copper/kg DM, a growing lamb 3 mg copper/kg DM, and all other sheep 6 mg copper/kg dietary DM.

Zinc Requirements

The minimum zinc requirements of farm animals vary with the species and breed, the age and productive functions of the animal, and with the composition of the diet, particularly the amounts and proportions of

of those components, organic and inorganic, that affect zinc absorption and utilisation.

Insufficient data is available from which to calculate zinc requirements from factorial estimates. ARC (1980) however has reviewed the scant existing evidence to arrive at a recommended value.

The only estimate in cattle of endogenous faecal output was calculated by Hansard and Mohammed (1968) at 0.033 mg zinc/kg body weight in pregnant heifers. In sheep a similar endogenous measurement was calculated to be 0.053 mg zinc/kg body weight (N.D. Grace, unpublished data) cited by ARC (1980). Endogenous urinary losses of zinc were found to range from 0.004 to 0.019 mg/kg live weight/day for cattle (W.J. Miller et al, 1966, 1970) and 0.023 mg/kg live weight per day for sheep (Grace, 1975, unpublished data).

The ARC (1980) estimate for the zinc required for tissue growth is based on work by Kirchgessner and Neesse (1976) and Miller et al (1966, 1970), and more recently by Suttle (unpublished) cited by ARC (1980). Suttle's value of 24 mg zinc/kg live weight is considered to be the more appropriate figure.

A factorial estimate for wool growth of 115 mg

zinc/kg wool is quoted by ARC (1980) based on the work of Burns et al (1964) and Healy and Zieleman (1966). During pregnancy the requirement for zinc increases by a factor of six from mid to late gestation in both cows and sheep (Hansard and Mohammed, 1968). Values adopted by ARC (1980) are as follows: daily requirements are estimated to be 1.1 mg zinc/kg LW and 0.28 mg zinc/kg LW for cattle and sheep respectively and for late pregnancy correspondingly to be 6.3 mg zinc/kg LW and 1.5 mg zinc/kg LW. While adopting these levels, ARC point out that the demand for each foetal lamb may rise to 2.5 mg zinc/kg LW per day during the last days of intrauterine development (Williams and Bremner, 1976).

Estimates of the zinc content of cows' milk vary from approximately 3.4-5.8 mg/litre (Osis et al, 1972) and can be subject to alteration by dietary factors. ARC (1980) assumed a content of 4 mg zinc/litre of cows' milk and the higher figure of 7.2 mg zinc/litre of ewes' milk (Suttle, unpublished data, cited by ARC, 1980).

Fairly limited data is available on the efficiency of absorption of zinc. It is known that young ruminants absorb zinc very efficiently although this may be altered by dietary factors. Milk-fed calves of one week old are the most efficient absorbers of zinc with a coefficient of approximately 0.55. Net

absorption of zinc in one study in mature cows was found to be 0.12, and in calves 5-12 months old, 0.2 (Miller and Cragle, 1965). Calves fed on a milk-substitute which contains certain substances such as phytic acid, may experience a 50% reduction in absorption (Miller, 1967). Different zinc absorption coefficients of 0.31 and 0.25 respectively have been found for sheep grazing on fresh herbage or lucerne hay (Grace, 1975, unpublished data). In the light of this available evidence, ARC (1980) have adopted a value of 0.3 for the absorption coefficient of zinc for young growing ruminants and for mature animals, a value of 0.2.

Factorial estimates of zinc requirements, when totalled, result in the ARC recommended value of 20-51 mg zinc/kg dietary DM for sheep depending on the age stage of production and rate of growth. Thus a lamb of 5 kg requires 41 mg zinc/kg DM, a growing lamb 24-51 mg zinc/kg DM, and an adult of 50 kg, 30-48 mg zinc/kg DM. Requirements for cattle are estimated to be lower, at between 12 and 35 mg zinc/kg DM. By ARC (1980) calculation, a growing calf on a liquid diet requires 28 mg zinc/kg DM, a growing bullock requires 14-35 mg zinc/kg DM, and an adult requires 15-25 mg zinc/kg DM.

There are suspicions that ration type and absolute zinc content may modify the efficiency with which

dietary zinc can be used. It has been shown that calves and lambs fed on a semisynthetic diet in housed conditions appear to grow normally on dietary zinc levels as low as 8 mg zinc/kg DM (Mills et al, 1967; Miller et al, 1963). By contrast, at pasture, a high incidence of zinc-responsive foul of foot occurring solely in bull calves was reported by Bonomi (1964) in conditions providing less than 30 mg zinc/kg DM. Herbage containing 30-50 mg zinc/kg DM was regarded as marginal according to Bonomi.

When a semipurified diet containing low zinc levels was fed to a group of lambs receiving varying amounts of calcium carbonate, the most severe lesions due to hyperkeratosis were found in lambs receiving the greatest amount of calcium (Mills and Dalgarno, 1967), suggesting that some degree of antagonism exists between the two elements.

Phytic acid, which is contained in soya bean meal, reduces the availability of zinc. Therefore it is generally accepted that any ration which contains this antagonist should include enhanced zinc levels. As a consequence of this and other less specific effects such as increased susceptibility to foul of foot plus the rapidity in onset and severity of clinical zinc deficiency syndromes, ADAS (1983) recommended that the higher level of 40 mg zinc/kg DM for all stock was a

more suitable guideline than that of the ARC (1980). Preruminant calves and lambs on a liquid diet containing components rich in phytic acid should have 50 mg zinc/kg DM added. The figure of 40 mg zinc/kg DM stated by ADAS is closer to the recommendation of ARC (1965) of 50 mg zinc/kg DM. The ARC (1965) figure was based on a single trial in growing calves by Miller and Miller (1960).

Further research, as previously quoted, has shown that the ARC (1965) figure may be reduced. However, in the interests of safety, and taking into account between-animal variation and the fact that zinc even at ten times the recommended level is relatively non toxic, ADAS (1983) believe that the ARC (1980) requirements are minimal.

Manganese Requirements

The ARC (1965) recommendations for manganese levels are based almost exclusively on evidence from feeding trials. Little data at that time was available for estimating requirements from factorial calculations.

In a trial by Hawkins et al (1955), 1 mg manganese/kg dietary DM was found to be adequate for some growth in calves although serum manganese was depressed. Addition of calcium and phosphorus to the experimental diet increased the requirement for manganese. When a

diet containing 10 mg manganese/kg DM was given to heifers by Bentley and Phillips (1951), milk yield was not affected but oestrous was delayed and they were slower to conceive than animals given 40 mg manganese/kg DM. Sawhney and Kehar (1958) reported that haemoglobin and red cell counts were depressed by a diet containing 30 mg manganese/kg and that levels were restored to normal when the equivalent of an additional 5 mg/kg DM was administered daily. It was postulated by Hignett (1959) that increased growth rate depressed the fertility of heifers if dietary calcium and phosphorus was not balanced when the manganese level was low in the order of 1 mg manganese/kg body weight. However, Littlejohn and Lewis (1960) found no relationship between fertility and the calcium and phosphorus levels of the diet in an experiment in which the manganese intake was less than 1.2 mg manganese/kg body weight.

In one study the endogenous manganese loss in sheep was estimated (Murty, 1957) at 6 mg/d, and the net maintenance requirement at 33 mg manganese/day. The manganese content of milk was given as 0.02-0.03 mg manganese/kg by Vallee (1959). Having considered all the available evidence, ARC (1965) determined that 40 mg manganese/kg DM was an adequate level of manganese in the diet.

By 1980 suitable biochemical data for more precise estimation of the quantity of manganese required for ruminant production was still unavailable. Thus ARC (1980) recommendations are based on evidence from further feeding trials.

Manganese has a limited capacity for storage in the liver. The skeleton contains the major body reserves but it is not readily mobilised in times of deficiency. The coefficient of absorption for manganese is fairly constant at 0.01 over a wide range of intakes (Sansom et al, 1976).

Blood manganese levels are unsuitable as a monitor of physiological status for experimental work because of a lack of agreement between the analytical results of individual workers. Similarly, although the manganese content of hair and wool is related to dietary intake (Lassiter and Morton, 1968) variation between individuals on the same diets is too great. Differences related to the degree of pigmentation and hair length also confound this as a method of physiological manganese measurement (Hartmans, 1972). As a result, the ARC (1980) assessment of manganese requirements is derived from consideration of the reported effects of differing intakes on growth, performance, and the presence or absence of clinical defects.

The 1965 ARC manganese requirements were accounted for by estimating what level of manganese sustained healthy growth, fertility and milk yield, taking into account endogenous loss. Since 1965 more detailed studies have taken place on the effect of different manganese levels on skeletal parameters, growth and fertility.

These have been given consideration in the ARC (1980) recommendation which is 15-20 mg manganese/day DM less than the previous requirement of 40 mg manganese/day DM. However a criticism of the calculation of the 1980 requirement could be that there is no provision for the manganese content of milk, a factor which was formerly considered to be relevant.

Defects in joints, reduced tibia length and breaking strength were reported by Lassiter and Morton (1968) in lambs on a diet providing approximately 0.8 mg manganese/ kg DM. These defects did not appear in lambs receiving 30 mg manganese/kg DM.

Rojas et al (1965) offered low manganese diets (16-17 mg manganese/d) to cows during pregnancy. The calves exhibited enlarged joints and deformities of long bones. Abnormalities in gait were observed in calves born of dams receiving 13 or 14 mg manganese/kg diet, but not when 21 mg manganese/kg was provided

(Howes and Dyer, 1971). High dietary concentrations of calcium have been shown to decrease the availability of manganese and exacerbate the clinical signs of manganese deficiency in rats and chicks but there is no evidence that this interaction also takes place in ruminants.

The claim by Hawkins et al (1955) that 1 mg manganese/kg dietary DM was adequate for growth of young calves maintained on a liquid feed was disputed by Lassiter and Morton (1968) who fed lambs on a semisynthetic diet with a similar content of manganese and found it to be grossly inadequate. Pregnant heifers on a diet containing 13-17 mg manganese/kg DM were reported to have suffered no adverse effect on weight gain by Rojas et al (1965) and Howes and Dyer (1971) although in both cases the subsequent calves were debilitated. In Rojas' trial, all calves from cows fed 15-17 mg manganese/kg DM exhibited neonatal deformities. Pregnant and lactating cattle were maintained on a diet containing 16-21 mg manganese/kg DM by Hartmans (1972) without reducing the subsequent growth of their calves.

The manganese requirement for optimum fertility has been variously estimated. Delayed or irregular oestrous and poor conception rates resulted when heifers or mature cows received diets with the following

contents of manganese (mg/kg DM) before service: 11 to 16 (Munro, 1957), 16 or 17 (Rojas et al, 1965).

ARC (1980) have therefore designated 20-25 mg manganese/kg dietary DM as permitting optimum skeletal development, and rations providing 10 mg manganese/kg DM as meeting growth requirement.

The ADAS (1983) report is critical of these levels and believes that the ARC (1965) figure of 40 mg manganese/kg DM should be maintained. The figure quoted by ARC (1980) is, in their opinion, minimal when clinical symptoms are observable in calves fed less than 20 mg manganese/kg DM and impaired fertility has not been found in animals fed more than 20 mg manganese/kg DM. Although it has not been fully proven in ruminants, there is a possibility that an antagonistic effect which reduces the availability of manganese in the presence of calcium and phosphorus in monogastric species may occur, and several workers have claimed this interaction exists, notably Hignett (1959).

Milk production is estimated by ADAS (1983) to require 13 mg manganese/kg DM for 40 kg milk, but by recommending the higher level of 40 mg manganese/kg there is a sufficient safety margin to cover this. ARC (1980) has claimed that 20-25 mg manganese/kg DM

also covers milk production, but in a pregnant lactating cow it is conceivable that the calf *in utero* could be born with manganese deficiency symptoms. For all these reasons it seems more reasonable to recommend the 40 mg manganese/kg dietary DM as favoured by ADAS (1983) and reject the lower figure of 20-25 mg manganese/kg of ARC (1980).

Iodine Requirements

The sole function of iodine is for the synthesis of hormones which are involved in the control of oxidative metabolism. This was demonstrated by Munroe et al (1951) when they showed that 48 hours after radioactive iodine was injected into cows, almost the whole of the radioactivity was associated with thyroxine. The 1965 ARC requirements were calculated from fairly limited information dervied from feeding trials which included the administration of different levels of iodine. Estimates of the daily iodine requirement of a non-pregnant, non-lactating sheep varied from 1 µg iodine/kg live weight (Mitchell and McClure, 1937) to 3 µg iodine/kg (Albritton, 1954).

However the presence of goitrogens in the feed was known to affect the amount of iodine required. Goitrogens operate in two different ways. The cyanogenetic goitrogen is found mainly in leguminous plants such as white clover and acts to impair iodide

uptake by the thyroid gland. Generally its effects can be overcome by iodine supplementation. The second type is a thiouracil goitrogen which interferes biochemically with the formation of thyroxine and triiodothyronine and has been identified in brassica seeds (Astwood et al, 1949). The effects of this goitrogen are more difficult to reverse by iodine supplementation. A wide range of plant species contain goitrogens (Sinclair and Andrews, 1961; Josefsson, 1970), and there is evidence that the goitrogen content of many crops may be influenced by fertilisers. Thus it was discovered (Sinclair and Andrews, 1961) that 30 μg iodine/kg live weight was required to prevent iodine deficiency in pregnant sheep grazing kale.

ARC (1965) therefore recommended the following dietary levels: 0.8 mg iodine/kg DM and 0.12 mg iodine/kg DM for pregnant and lactating animals, and for other animals respectively. When the feed contains goitrogens it recommended that iodine intake should be increased by 1.2 mg iodine/kg DM.

Excessive intakes of iodine can have an adverse effect on production with weight loss and irregularity in reproduction. A safe upper limit of 8 mg iodine/kg dietary DM was recommended by ARC (1965).

When the ARC (1980) requirements for iodine were compiled, a more scientific approach was adopted to the methods used in assessing the adequacy of iodine intake. There are limitations to detecting suboptimal iodine levels via an increase in thyroid weight since the size of the gland can also increase when excess levels of iodine are administered (Newton et al, 1974). The assessment of iodine status and thyroid function by determination of the plasma protein-bound iodine concentration does not correlate with the flux of circulating iodine-containing hormones. Use of radioimmunoassay techniques when radioactive iodine has been administered and can subsequently be traced are used for a more accurate assessment of thyroid status and the influence of dietary iodine on thyroid hormone secretion rate. ARC (1980) requirements are therefore calculated from this basis.

In order to deduce the iodine requirement from the thyroid secretion rate, the proportion of circulating iodide taken up by the thyroid gland must be estimated. In iodine insufficiency situations, a greater proportion of iodide is accumulated by the thyroid so there is difficulty in defining the requirement when the normal percentage accumulation is not know. When dietary iodine is adequate the proportion accumulated is between 20 and 50% (Sørenson, 1958; Falconer and Robertson, 1961; Falconer, 1963) in sheep and cattle, so a value

of 33.3% was chosen since this results in the calculated requirement for iodine being double the thyroid secretion rate. Castration of males decreases the thyroid secretion rate (Singh et al, 1956; Sørenson, 1958) as does the ageing process in mature animals (Henneman et al, 1955; Falconer and Robertson, 1961).

Lactation has been shown to increase thyroid secretion rate in both cattle and sheep and Sørenson (1958) constructed an equation which shows that dietary iodine requirements are directly related to butterfat production in cows. Using this equation, Alderman and Stranks (1967), predicted that the iodine output in the milk for a cow producing between 10 and 25 litres of milk with 4% butterfat was a relatively insignificant 3 to 6% of the dietary iodine requirement. When cows are subjected to low dietary iodine intakes, milk iodine concentration falls before thyroid secretion rate is affected (Swanson, 1972). By contrast sheep's milk is much richer in iodine content and up to 50% of the animal's turnover is secreted in the milk. It has been suggested that the difference is due to the ability of the ewe's mammary gland to concentrate iodine up to fiftyfold unlike the cow's mammary gland which does not. Estimates of requirements for lactating ewes must be appreciative of this fact.

Investigations have not shown any increase in

thyroid secretion rate during pregnancy (Henneman et al, 1955), although this was previously thought to be the case. Thyroid secretion rate tends to be inversely related to environmental temperature so that a lower level of iodine intake in the summer is compatible with effecient production (Yousef and Johnson, 1966). Dietary requirements for iodine are increased by the intake of goitrogens, as was previously discussed. However ARC (1980) has considered it necessary to recommend a dietary level of 2 mg iodine/kg DM to all classes of ruminant consuming goitrogenic material. In the absence of goitrogens, and even during pregnancy and lactation, a concentration of 0.5 mg iodine/kg DM is thought to be adequate, and it is suggested that during summer this could be reduced to 0.15 mg iodine/kg DM. There is agreement with ARC (1965) that toxic effects can occur at dietary concentrations greater than 8 mg iodine/kg DM.

ADAS (1983) having considered the evidence, agrees with all the levels of dietary iodine recommended by ARC (1980), and also accepts the upper limit before toxicity as 8 mg iodine/kg DM.

Selenium Requirements

Vitamin E and selenium are closely and mutually involved in a variety of metabolic processes and it is often impractical to consider them in isolation when

defining nutrient requirements.

In 1965 there was much apprehension surrounding the use of selenium as a dietary supplement. ARC (1965) stated categorically: "Even when added selenium is beneficial, the margin between benefits and disaster to the livestock industry is too small to warrant its use". In conditions of known selenium insufficiency where clinical white muscle disease occurred, it was recommended that prophylactic treatment of calves and lambs should consist of the oral administration of vitamin E. In the event of this failing to avert the disease, only then should selenium administration be reluctantly considered.

Evidence however has accumulated in more recent years which shows that clinical and subclinical selenium responsive disorders are progressively more common in ruminants fed diets containing amounts of selenium from 0.08 mg selenium/kg DM downwards (Lindeberg and Jacobsson, 1970; Havre and Steinnes, 1968). A relationship between selenium deficiency and ill-thrift responsive to oral or subcutaneous administration was reported by Cousins and Cairney (1961) and Hartley and Grant (1961). A more recent report of Gleed et al (1983) on selenium supplementation in steers showed that there was a significant weight gain in animals receiving 0.15 mg selenium/kg body

weight as compared with control steers who had a clinically low selenium status (130-1050 mU/ml glutathione peroxide activity) but not showing any clinical deficiency symptoms.

When the selenium and vitamin E contents of the diet are known, and the vitamin E content is within the adequate range (15-28 mg/kg) an appreciable incidence of clinical or subclinical muscular dystrophy can arise in lambs or calves if the diet of the dam has contained less than about 0.025 mg selenium/kg DM (Jenkins et al, 1970; Mikkelsen and Hansen, 1967). At higher dietary contents of selenium, in the range 0.035-0.04 mg kg DM the reports of Schubert et al (1961), Mikkelsen and Hansen (1968) and Allen et al (1975) on the incidence of muscular dystrophy in lambs or delayed myopathy with myoglobinuria in growing cattle at differing vitamin E intakes, provide some indication that the severity of clinical or subclinical lesions may be inversely related to the dietary content of vitamin E.

Schubert et al (1961) proposed that an antagonistic effect exists between selenium and sulphur since lambs born of ewes fed mixed lucerne and grass hays gave birth to lambs with a high incidence of muscular dystrophy. Hidiroglou et al (1965) failed to detect any association between dietary sulphur content and the incidence of myopathic disorders. Other researchers

have failed to prove a relationship between selenium and sulphur levels (Paulson et al, 1966, Allaway and Hodgson, 1964) but it is possible that an additional variable influencing the effect of sulphur on selenium utilisation could be involved.

High dietary intakes of polyunsaturated fatty acids (PUFA) induce skeletal and cardiac myopathies in calves (Blaxter and McGill, 1955) and lambs (Boyd, 1973). Experiments demonstrating the prophylactic effectiveness of vitamin E when PUFA's are administered, indicate that there is antagonistic effect between the two. Selenium is ineffective in combatting myopathies which arise from high PUFA intakes (Blaxter, 1962).

In considering what level of dietary selenium should be recommended, ARC (1980) took into account the work of Mikkelsen and Hansen (1968), and Buchanan-Smith et al (1969) which implied that a dietary concentration of selenium of less than 0.03 mg selenium/kg DM was inadequate. They regard 0.03-0.05 mg selenium/kg as being in the marginal range, and above 0.05 mg selenium/kg DM as adequate.

ADAS (1983) however, disagrees with this value, and considers the figure of 0.1 mg selenium/kg DM as being more appropriate. The work of MacDonald et al (1976), Lewis et al (1978), NRC (1975) and NRC (1978) fully

support their claim that 0.05 mg selenium/kg DM is too close to marginal levels to be recommended as requirements.

Cobalt Requirements

Cobalt is required daily in the diet by the ruminant for the synthesis of vitamin B_{12} in the rumen. The recommendation of its dietary requirement in ARC (1980) has not changed from the 0.1 mg cobalt/kg DM estimated by ARC (1965) although the metabolic processes in which it is involve are more completely understood. It has been estimated that the minimum net requirement of growing sheep for vitamin B_{12} is 11 µg/day (Smith and Marston, 1970). When the cobalt content in rumen fluid falls below about 20 µg/litre the rate of vitamin B_{12} synthesis by rumen microorganisms fails to meet the demands of the host (Marston et al, 1961).

The efficiency of conversion of cobalt to vitamin B_{12} decreases with increasing levels of cobalt intake. When intake is adequate, only 3% of cobalt is converted to vitamin B_{12}, partly because a proportion is diverted to the production of an inactive analogue of vitamin B_{12} (Gawthorne, 1970). Smith and Marston (1970) reported $13 \pm 5\%$ conversion in sheep on a cobalt deficient diet. However the content of other unidentified dietary variables are known to influence the amount of active vitamin B_{12} produced. Hine and

Dawbarn (1954) and Dawbarn et al (1957) have shown that the rumen contents of pasture fed animals contain significantly higher proportions of vitamin B_{12} in a physiologically active form than the rumen contents of animals maintained on experimental diets based on chopped hay or straw with similar contents of cobalt. There is some evidence that rations containing high proportions of concentrates may cause diminished vitamin B_{12} production (Sutton and Elliot, 1972), and high dry matter intakes may similarly affect vitamin B_{12} levels (Hedrich et al, 1973).

The cobalt status of sheep is best derived from an assessment of the vitamin B_{12} content of the liver. Serum levels can also be used, although less effectively. It is suggested that serum vitamin B_{12} concentrations below 0.2 µg/litre are strongly indicative of cobalt deficiency and that 0.25-0.30 µg/litre may be marginal (Dawbarn et al, 1957; Somers and Gawthorne, 1969). From similar studies of the vitamin B_{12} content of liver, it has been concluded that less than 0.07 mg vitamin B_{12}/ kg fresh liver indicates severe deficiency; moderate deficiency is indicated by levels of 0.07-0.10; mild deficiency as 0.11-0.19 and adequate cobalt status is indicated at levels above 0.19. Maximum weight gain was achieved with a daily cobalt allowance in sheep of 0.11 mg/d. An allowance of 0.07 mg cobalt/kg dietary DM maintained the weight at a stable value (Marston,

1970) and it is suggested that it maintained serum vitamin B_{12} concentrations only just above the critical threshold.

Thus ARC (1980), while pointing out that 0.08-0.1 mg cobalt/kg dietary DM may apparently be sufficient to prevent clinical deficiency symptoms, their recommendations are for 0.11 mg cobalt/kg dietary DM because it is unlikely that the lower value will maintain a store of vitamin B_{12}. ADAS (1983) accepts that this level is the appropriate requirement, and suggests that when a high proportion of concentrates is being fed, the requirement should be increased to 0.2 mg cobalt/kg dietary DM.

4. Methods of Supplementing Trace Minerals

In recent years there has been a vast expansion in commercial involvement in methods which supply trace mineral supplements to ruminants. This is mainly the result of a growing body of evidence citing improved efficiency of production in animals adequately provided with all necessary trace minerals. Marginal subclinical mineral deficiencies of livestock in a particular area may only come to light when enhanced levels of the appropriate mineral are shown to be beneficial to the growth and production of the animal. Analysis of herbage and feeding would subsequently prove that the trace element intake had fallen short of the optimum level.

Traditional methods of supplementing minerals involving direct addition to feed or spraying onto pasture are presently being challenged by more sophisticated techniques such as intraruminal devices and slow release parenteral treatments which claim, perhaps unjustifiably, greater efficacy. In practice, novel methods of mineral supplementation are likely to take over the section of the market where no practical alternative treatment is possible and would probably be most significant in contributing to hill sheep and cattle farming husbandry.

Soil treatment

Having identified an area on which grazing animals are deficient in a particular trace element, the simplest and traditionally practised method of ensuring an adequate intake is to add the deficient mineral directly to the soil.

Stewart, Mitchell and Stewart (1941) proved that pining in sheep could be corrected by applying cobalt to the soil in order to increase concentrations in the herbage. In cobalt manuring experiments on cobalt deficient soils, typical differences in the average live weights of six month old lambs grazing treated plots compared to those grazing untreated plots was found to be 9 kg per animal (Mitchell, 1963). Extensive field investigations have examined the effects of different forms of applied cobalt, the persistence of treatments, the influence of soil type, and the effects of NPK fertilisers (Reith and Mitchell, 1964; Mitchell, 1972). A single application of 0.5 kg cobalt sulphate per hectare was effective over the period of a 3-4 year ley in raising the cobalt content of mixed herbage in an otherwise cobalt deficient area on three different types of soils (Burridge, Reith and Berrow, 1983). An equivalent quantity of cobalt added as an organic chelate was effective for less than two years. Elevated cobalt levels persisted in the herbage for at least five years on the peaty soil type. Freely

drained soils showed considerably lower herbage cobalt values immediately post treatment, and tended to decline more rapidly over three years, particularly on the soil derived from granites and gneiss. Plant yield is not affected by the addition of cobalt salts to the soil. Heavy liming reduces plant trace element uptake appreciably and should not be practised on soils whose cobalt status is low or marginal.

On hill-land sparsely stocked, soil treatment is unlikely to be practicable and direct veterinary orientated methods of supplementation may be required.

Where it is practical, application of copper to soils is an effective method of increasing the copper content of grass for grazing animals. Treatment with 22 kg/hectare of copper-sulphate has a prolonged beneficial action which is reported by Reith (1976) to persist for at least twenty years on acidic soils. On a marginally deficient soil, the addition of 10 kg copper sulphate/hectare can double the yield of the cultivated oat Avena sativa even after eight years post application. The yield of barley and mixed herbage is also increased, though not so dramatically as for oats. Alkaline conditions do not affect the availability of copper to plants, but vastly increase the availability of molybdenum. This can induce copper deficiency in animals. Therefore top dressing with copper sulphate

a pasture which has a high pH may be ineffective in raising the copper intake of grazing stock to an adequate level where molybdenum was also present. An increase in soil content may not affect all plant species similarly. When the available copper in a soil increases, the copper content in clover growing on that soil rises while the content in grasses in the same mixed herbage remains relatively constant (Mitchell et al, 1957b).

Manganese deficiency occurs mainly in soils in areas where several other trace elements such as copper and cobalt are also present in low amounts. Where manganese deficiency is encountered it is commonly associated with high soil pH which is a feature of soils on calcaleous shelly-sands or arise from over-liming. Soil manganese treatment of 20 kg/hectare is of limited effect in raising the content of herbage at maturity because added manganese seldom remains available in the soil except in very acid conditions (Burridge, Reith and Berrow, 1983). The occurrence of manganese deficiency where associated with high soil pH is best treated by spraying, or to some extent, by drilling with the seed but on such soils there is a considerable danger of rapid immobilisation of the manganese.

Direct application of selenium to soil is considered impractical because selenium is poorly

absorbed by most plants, especially from acid soils (Allaway et al, 1967). Furthermore, high values can occur immediately after application and pose a toxic hazard. Such high values are due to initial surface contamination as well as to root and foliar uptake. Nevertheless, Watkinson and Davies (1967) maintain that "with proper precautions to minimise pasture contamination" 1 oz/acre and possibly 2 oz/acre (as sodium selenite) should present no hazard "at least for a few years".

Iodine in the form of iodate or iodide was experimentally applied to deficient soils by Whitehead (1975). However this method was found to be inefficient because of the low uptake of the element into the herbage although added iodate is much better absorbed than iodide especially where liming is practised.

Zinc deficiency may be prevented and controlled by soil treatment in the order of 5-7 kg/hectare of zinc sulphate every 2-3 years. Under more extensive range conditions such application is obviously impractical or uneconomic and alternative methods must be pursued.

Direct methods of supplementing trace minerals to ruminants

(a) Parenteral methods

Dietary manipulations or other oral treatments are, in many cases, the most efficient solution to the problem of trace element deficiency diseases in farm animals. Such approaches are particularly effective against chronic primary deficiencies where there is simply not enough of the required element in the diet to satisfy the animal's needs. In some situations, however, notably severe and acute deficiencies, parenteral injections of a supplement provide the best methods of restoring normal mineral concentrations in the deficient animal's tissues. Parenteral supplements are also particularly effective against many secondary deficiencies in which factors in the diet convert apparently adequate dietary concentrations of the required element into an unavailable form: their effectiveness arises because absorption from the alimentary canal is bypassed. Injectable supplements of some trace elements have also proved useful for short term supplementation during periods when there is a great demand for the element, for instance, during pregnancy. One further advantage of the parenteral rate of supplementation is that a known amount of the element is introduced into the animal's system.

To avoid the necessity of repeated dosing, which would be both costly and inconvenient, especially while the animals are at pasture, the supplement must either itself provide a source of the element over a long period, or increase the animal's physiological stores of the element, from which it can draw if needed. To accomplish either of these ends, a relatively large amount of the element must be given in a single dose and in a form which is not toxic. Rather than being in a biologically active form which may be toxic, the element must be constrained either chemically or physically in a biologically inactive form which breaks down after injection to release the active supplement at a controlled rate. This requirement greatly restricts the range of compounds which are suitable and safe for use in parenteral administration. Trace elements should therefore be administered parenterally only when it is necessary to correct a confirmed deficiency or when prophylaxis is required in circumstances which are likely to lead to deficiency.

The provision of supplementary copper to farm animals has been particularly well studied. Deficiencies of copper occur in many areas of the world and in sheep and cattle these deficiencies are often the result of interactions with

molybdenum and sulphur in the diet (Suttle, 1974a). More than eighty different copper compounds have been examined for their potential parental use against copper deficiency (Camargo, Lee and Dewey, 1962). The criteria by which suitable compounds were judged was as follows: (i) Minimal damage at the site of infection: (ii) satisfactory liver storage (90-100% of the administered dose) (iii) a safe margin between therapeutic and toxic doses.

A 50 mg dose of copper glycinate was considered by Harvey and Sutherland (1953) to be most satisfactory for sheep. This is the equivalent of half of the toxic dose, there were no significant lesions at the injection site, and virtually all the copper was stored in the liver. In 1962, Camargo et al examined some more copper complexes in adult sheep. As well as copper glycinate, they studied copper calcium EDTA and copper methionate. They found that 100 mg copper as copper calcium EDTA gave satisfactory storage of copper in the liver more rapidly than did 90mg copper as copper glycinate.

However, many fatalities as a result of parenteral copper treatment have been reported in sheep (Sutherland, Moule and Harvey, 1955; Ishmael,

Howell and Treeby, 1969; Weiner and Macleod, 1970) following the use of these and other copper compounds. Further evaluation of copper containing compounds has taken place more recently. Suttle (1981a) has confirmed that the safest compound in terms of being least likely to cause toxicity was the least effective in alleviating hypocupraemia in both calves and sheep, while the most effective compound in sheep, copper diethylamine oxyquinoline sulphonate, was also the most toxic. Mallinson, Sansom and Drake (1980) demonstrated that of the commercially available compounds which they examined, only copper calcium EDTA resulted in a sustained increase in liver copper stores of sheep sixteen weeks after treatment when administered at the manufacturer's recommended dose. Cunningham (unpublished observations, cited by Deland, Cunningham, Milne and Dewey, 1979) pointed out that four subcutaneous injections of copper glycinate which were necessary to prevent hypocupraemia in cattle grazing deficient pastures in Southern Australia caused significant carcase damage. Gleed et al (1983) demonstrated that copper calcium EDTA injections which provided 200 mg copper afforded growing beef steers protection against hypocupraemia for only 3-4 months while the animals were at pasture, so that a repeat dose was necessary to prevent deficiency

occurring during the grazing season.

Parenteral administration of selenium has been commonly used in sheep and cattle for the prevention and treatment of selenium deficiency since the original observations on deficient sheep of Drake et al (1960). Similar restrictions in use as were found with parenteral copper supplements also apply. Two treatments may be required to cover the grazing season, and reactions at the site of injection are common (Herigstad and Whitehair, 1974). Most therapeutic selenium preparations provide only 0.05 mg selenium/kg body weight, selenium having a low "therapeutic index". However, there is little evidence that subcutaneous injections of up to 0.35 mg selenium/kg body weight as readily soluble sodium selenite or sodium selenate are lethal. Caravaggi et al (1970) reported 0.45 mg seleneium as sodium selenite/kg body weight as an LD50 dose in sheep. The use of selenium injections or doses in the prescribed amounts does not result in excessive or dangerous concentrations in the edible tissues of the treated animals (Cousins and Cairney, 1961; Doornenbal, 1975).

A single 1 ml intramuscular injection of iodised poppyseed oil containing 40% by weight of bound iodine and administered to ewes 7-9 weeks before

lambing is as effective as oral dosing in preventing iodine deficiency dependant goitre (Sinclair and Andrews, 1958, 1961).

Parenteral injections of cobalt salts are not effective because insufficient of the cobalt entering the animal by this route reaches the rumen where microbial synthesis of vitamin B_{12} takes place. However, injections of vitamin B_{12} if sufficiently large and frequent, represent an effective means of preventing or overcoming cobalt deficiency in ruminants. The high cost of this method of supplementation relative to the cost of orally administering cobalt makes it impractical as a regular husbandry technique. Intramuscular injections of vitamin B_{12} at a rate of 100 μg/week or of 150 μg/fortnight produce a rapid remission of all signs of deficiency in lambs and are just as effective as cobalt administered orally at the rate of 7 mg/week (Andrews and Anderson, 1954).

Zinc and manganese are not commonly supplemented by injection, and oral treatments are considered to be more desirable.

(b) Depots

An alternative method of providing trace elements

parenterally is by the placement of implants and depots under the skin of animals from which the mineral element gradually elutes over a prolonged period of time. Several methods of physically constraining elements in different media have been attempted and mainly applied to selenium and copper supplementation.

In sheep, Hartley (1967) described the use of 5 mg or 10 mg selenium as barium selenate (Ba_2SeO_4) suspended in an oil-beeswax. A similar oil-based parenteral treatment containing barium selenate is presently available (Deposel: Rycovet Ltd.) and claims efficacy lasting up to 12 months or more with little localised site reaction (Cawley and McPhee, 1984; MacPherson and Chalmers, 1984). However, Allen and Mallinson (1984) have observed a large concentration of between 76 and 97% of the administered barium selenate in the carcase after 119 days, and the presence of site reactions which is clearly undesirable because of both carcase spoilage and possible public health risk. Evidence is presently in conflict and the true picture is uncertain.

Hidiroglou et al (1971) used pellets formed from 20 mg selenium either as sodium selenite and stearic acid with silastic glue or as sodium selenite with hydrogenated peanut oil and

magnesium stearate. These were tested in sheep and calves and found to be moderately successful although there was some loss of pellets, and swelling at the implantation site. At present more research is required to minimise hypersensitivity reactions and improve the efficiency of existing parenteral treatments.

A more novel method of slow release parenteral supplementation has been introduced by the development of soluble "controlled release glass" (CRG) (Drake, 1978). Using CRG, the rate at which the recipient animal's tissues are exposed to the active element is governed by the rate of solution of the glass after it had been implanted subcutaneously. Mallinson et al (1980) demonstrated that implantation into sheep of CRG containing either 33 mg or 65 mg copper resulted in an increase in copper liver stores as great as that following the injection of 50 mg copper as copper calcium EDTA. Moore et al (1982) have shown that up to 80% of a 100 mg dose of copper from CRG can be stored in the liver of sheep within one week of its administration. In common with many other parenteral copper injections, CRG, gave rise to reactions at the site of implantation which appeared to be in response to the local concentrations of copper

present before the glass had dissolved completely. Such reactions may degrade the quality of the carcase. Selenium supplements provided by CRG however caused no apparent reactions and were not rejected after subcutaneous or intramuscular implantation into sheep and they increased blood selenium concentrations.

Slow release injections are not commonly available for any other mineral.

(c) Oral treatments

The oral treatment of trace element deficiency can take many forms. The simplest most direct method involves the administration of a salt solution in water by oral drenching. This is suitable in cases where the element can be stored in the body as with copper. A general recommendation for the prevention of swayback was to dose the ewes twice in late pregnancy with 1 g of copper sulphate and at eight and four weeks prior to partuition.

If massive doses of cobalt which are in great excess of requirements are given at monthly intervals, it has been shown that LWG are almost equivalent to those in daily or weekly dosed lambs (Stewart, Mitchell and Young, 1955). However, Russel et al (1975) have shown more

recently that 200 mg drenches of cobalt remain effective for no more than three weeks when assessed by serum vitamin B_{12} concentrations. Thus, frequent handling would be required to ensure optimum cobalt intakes.

Iodine is also effective when orally administered but again frequent handling is required when there is persistent deficiency. Two oral doses of 280 mg potassium iodide or 360 mg potassium iodate, one given at the beginning of the fourth month and one at the beginning of the fifth month of pregnancy, have been found satisfactory for the prevention of neonatal mortality and associated goitre in lambs which develop when ewes are wintered on goitrogenic kale.

There have been varying reports on the suitability of administering selenium by mouth. The rapid disappearance of orally administered selenium in ewes was confirmed by Hidiroglou, Jenkin, Carson and MacKay (1968) when they reported that 80% of a single dose of $H_2{}^{75}SeO_2$ was excreted in the faeces within three days. Slower rates of disappearance were reported by Jacobson (1966) following subcutaneous injection and intraruminal dosing when 64% and 75% respectively were excreted in two weeks. However McDonald (1975) claimed

that unthriftiness in young Merino sheep in eastern Australia could be controlled if the ewes and lambs were orally treated with a dose of sodium selenite (0.1 mg/kg body weight) at lamb marking, and again four months later.

Zinc, unlike copper, is not stored in the tissues in amounts and forms that can be readily used in times of need. The bones and skin contain a high proportion of total body zinc, but this zinc is not in dynamic equilibrium with the zinc in the fluids and tissues of the rest of the body. Oral drenching with zinc sulphate is effective (Legg and Sears, 1960) in cattle but the technique is costly in time and labour unless combined with drenching procedures required for other purposes.

Manganese may also be administered by oral drenching, although literature on the subject is scarce.

Where supplementary feeding is being administered, a relatively simple and effective way of increasing the dietary mineral intake is to add enhanced levels of the lacking element directly to the food. This is appropriate for all elements and is an ideal method of ensuring an adequate intake where individual feeding is practised. However, in

group feeding situations there can be considerable variation in intake, even when it takes place indoors. McEleney (1985) has noted that in an indoors group feeding situation, thirty-four ewes fed a pelletted concentrate in late pregnancy had an average intake of 0.29 ± 0.0704 kg which gives a coefficient variation of 24.6%. At grass much higher coefficients of variation have been recorded. A group of twenty-seven suckler cows at grass in autumn provided with 2 kg pelletted concentrate containing supplementary magnesium had a faecal chromium output variation of 61% which therefore reflects wide variation in intake (McEleney, 1985). Three of the twenty-seven cows consumed virtually none of the supplementary food provided and were in danger of succumbing to hypomagnesaemia.

When frequent animal handling is not possible at grass, or in extensive range conditions as in many sheep farming areas, the provision of supplementary mineral becomes of major concern. Soil treatment with mineral is impractical in view of the expense and labour involved.

This has led to the practice of providing mineral supplements in large quantities on a self-help basis where other methods of increasing the

dietary intake of particular minerals appears untenable.

Basically self-help minerals are presented in three forms, that is (a) as a loose powder; (b) as a salt lick; (c) as a solid, usually mixed with high energy materials such as molasses and fats.

Salt licks have been used in many countries as a vehicle for mineral supplementation of livestock (Paulson et al, 1968; Jenkins et al, 1974). The intake of minerals is regulated because animals apparently regulate their intake of salt. The major disadvantage of licks, as with all self-help systems, is the variable intake between animals. In one study, 35% of sheep in a flock failed to consume supplement (Wheeler et al, 1980). Incorporation of 4 or 8% molasses in salt licks has been shown to improve acceptability (Rocks et al, 1980) but in other experiments non-consumers have been detected in flocks and herds fed supplements containing molasses (Nolan et al, 1974; Lobato and Pearce, 1978).

Although manufacturers claim that the voluntary intake of mineral containing feedblocks is sufficient to prevent deficiencies arising, in a definitive study of feedblock consumption (Kendall, 1977) an

entirely different picture emerged. On upland/ hill farms, 19% of 2931 ewes sampled had not eaten any of the various feedblocks which had been continuously on offer, and around 36% had eaten little or no feedblock (Ducker, Kendall, Hemingway and McClelland, 1981). Obviously this result is most unsatisfactory and highlights the possibility of large numbers of animals succumbing to deficiency diseases. Kendall (1977) has also dispelled the myth that deficient animals are driven by instinct to supplement their diet when a specific element is lacking by proving that no such behaviour exists. Cold weather was also found to adversely affect consumption (Ducker and Fraser, 1975). It is anticipated that a similar response would be found when pow dered supplements are provided, and an experiment to test this supposition was performed in the experimental programme of this thesis.

Novel methods of mineral supplementation have been introduced recently which involve treatment of the drinking water. Basically there are two methods, one of which is a trace element metering device which allows the maintenance of a particular concentration of an element in the drinking water supply (Rowett Water Proportioning Device, Patent No. 16784/78). A reservoir of minerals are added at a prescribed rate to the flow of water

to the trough. This method has proved its success at the West of Scotland College of Agriculture (MacPherson, 1983) in indoor trials.

The other method is the production of slow release pellets which are placed in a filter tube in the water trough where they slowly dissolve, and, allegedly because of their solubility product, maintain a desired trace element concentration ("Aquatrace": Comac Agrochemicals, Widnes, Cheshire). Variable results were noted by MacPherson (1983) in the pilot testing of copper and cobalt Aquatrace. Levels in the drinking water were not sufficient to maintain an adequate supply of these minerals to cattle in indoor and outdoor experimental situations. After some modification to the selenium containing Aquatrace, it proved to be an effective way of increasing the selenium content of drinking water to ensure an adequate mineral intake.

These methods may be practised where there is a restricted water supply and is obviously unsuitable when there are natural water sources, which again rules out most hill sheep farming situations.

In recent years the most exciting breakthrough in copper supplementation for all classes of livestock

has been through the direct oral administration of copper oxide needles. In 1960, Lassiter and Bell reported that copper availability can be influenced by the chemical and physical form of the copper. They observed copper in copper oxide to be less available over ninety-six hours than in a water soluble form or as a carbonate. Their data indicated some retention of copper oxide needles in the alimentary tract. In 1977 Dewey followed this work by demonstrating that ewes receiving 10 g copper oxide needles had a very much greater concentration of copper in their liver after sixty-four days than control animals. Post mortem analysis showed that copper oxide needles were recovered from the abomasum up to thirty-two days after treatment. Throughout, the abomasum held the greatest proportion of needles with a small amount lying in the rumen/omasum and small intestine. The efficacy of oral administration of copper oxide needles compared to parenteral treatments in sheep and cattle was demonstrated respectively by Whitelaw et al (1980) and MacPherson (1984). Whitelaw observed that a single 2 g dose of copper oxide needles to five week old lambs maintained a plasma copper concentration above that of lambs given a series of four copper calcium EDTA injections over a period of approximately four months. The

possibility of copper toxicity in sheep must always be given serious consideration. Dewey (1977) originally recommended that 10 g copper oxide needles was an appropriate dose for a ewe and that 2.5 or 5.0 g was less effective. Suttle (1981b) was in agreement with a dosage of 10 g for ewes and 50 g for calves, but was of the opinion that this would lead to a lifetime protection from copper deficiency. The evidence from Whitelaw (1980) showed no significant difference in the plasma levels when 4 or 8 g were administered, and after dosing, only half of the copper oxide needles could be recovered after four weeks (Judson et al, 1982), the remainder not being absorbed and being passed out in the faeces. At fifty weeks treated animals still had a significantly greater ($p < 0.01$) liver copper concentration. At the higher doses of oxide (10 g and 20 g) raised liver concentrations close to those recorded in cases of copper poisoning were noted.

The size of dose required to attempt lifetime protection from hypocupraemica in sheep is in danger of causing toxicity. For safety and efficacy it is recommended that sheep should be dosed yearly or twice yearly at a lower rate. A commercial preparation has now been marketed which encapsulates 2 g of copper oxide needles as

a suitable dose for lambs and 4 g for ewes (Copprite Beecham Animal Health).

For cattle, the recommended dose of copper oxide needles is 20 g for adults, 10 g for yearlings and 5 g for calves, which should impart approximately six months' protection from hypocupraemia (MacPherson, 1984). When copper oxide needle doses of 20-60 g were administered to suckler cows, MacPherson (1984) calculated that the number of days of maintenance of serum copper in the adequate range was 330 days for 20 g, 360 days for 40 g and 396 days for 60 g (by extrapolation). It is therefore most efficient to dose in 20 g amounts and this amount is commercially available in gelatin capsules (Copprite: Beecham Animal Health).

(d) Intraruminal supplementation

The property of the rumenoreticulum to retain dense objects which have been swallowed was first reported by Schalk and Amadon (1928). Small and comparatively light non-food objects may possibly be eliminated during rumination by expulsion from the mouth, but a more dense 50 g bolt remained in the reticulum of one cow for three months before being removed manually.

Practical exploitation of this property was

pursued thirty years later by Dewey et al (1958) when a dense compacted and baked cobalt "bullet" was produced which was designed to lie intraruminally, and release cobalt over a long period of time. The bullets were originally composed of a mixture of 75% cobaltic oxide and 25% china clay shaped as a ½" x ½" cylinder of density 3.5 gcm^{-3} and were expected to last about two years. Although results were promising in extensive trials, there was some incidence of regurgitation of boluses, and thereafter density was increased to 4.1 gcm^{-3} by increasing the proportion of cobaltic oxide to 90% which resulted in improved retention. Interestingly, boluses with a density of 7.7 gcm^{-3} had a higher rejection rate than those with 4.0 gcm^{-3}. There was also a tendency for a few boluses to become coated entirely in calcium phosphate, thus preventing cobalt elution. The ingestion of calcium containing compounds in the presence of a relatively high pH was thought to be responsible for the deposition of calcium phosphate.

Following research by Millar and Andrews (1964) with radioactive cobaltic oxide bullets in sheep, the rate of loss in a sixteen month period in ewes and lambs was calculated at 33%. Of the eighteen pellets not accounted for, nine were located in the paddocks not associated with dung patches,

implying losses occur via the oesophagus during rumination. A number of recovered bullets were coated, to varying degrees, with calcium phosphate. In more recent years improved efficacy of cobalt bullets in sheep (Permaco-S - Tasman Vaccine Laboratory (UK) Ltd.) has led to claims that protection from cobalt deficiency can last up to three years (Whitelaw, 1979).

In the early 1960s, intraruminal therapy was further exploited in the development of a bolus treatment for cattle to help prevent hypomagnesaemic tetany in livestock (Ritchie, 1966). The intraruminal magnesium treatment consists of a cast Mg alloy cylinder of 86% magnesium, 12% aluminium, 2% copper with particles of iron shot included in the matrix to increase the density and assist retention. The design of the bolus is such that it completely corrodes over the experimental period and no residue remains. The release rate is approximately 1 g magnesium per day, and it has been shown to have a 98% retention, and act to prevent hypomagnesaemic tetany in spring grazing cattle when two or four boluses are administered (Ritchie and Hemingway, 1968; Hemingway and Ritchie, 1969; Smyth, 1969).

Prevention of tetany in lactating ewes has been

reported when one magnesium bolus was administered (Davey, 1968; Egan, 1969) but later experience has shown that, on occasion, many of these bullets are regurgitated and lost at pasture (Kelly, 1979).

Selenium supplementation of sheep is necessary for optimal production over large areas of Australia (Gardiner, 1969) and New Zealand (Andrews et al, 1968). The need to supply a continuous low dosage of selenium to sheep has resulted in the development of a ruminal pellet containing 5% elemental selenium (Kuchel and Buckley, 1969) which is now commercially available in Australia and U.K. (Penmasel-Tasman Vaccine Laboratory (U.K.) Ltd. It has been claimed that pellets will maintain adequate blood selenium levels for three to four years in animals grazing deficient pastures. Several experiments have demonstrated however that protection from selenium deficiency on administration of a bolus in fact lasts only twelve months (Wilkins and Hamilton, 1980; Andrews et al, 1974). Recent Australian work has shown that the grain size of the elemental selenium may be critical in determining their effectiveness (Hudson, Hunter and Peter, 1981; Peter, Hunter and Hudson, 1981) and improvements are currently being made to the commercial product.

Selenium pellets are also available for cattle and are similar to sheep pellets except that they contain 10% elemental selenium and are three times as heavy (30 g). In trials, the administration of a single bolus has not been effective in raising and maintaining GSH-Px concentrations in cows, pregnant dairy heifers and rearing dairy youngstock for 4-6 months (MacPherson, 1981). With suckling calves, the rise in GSH-Px was not so marked as with older animals, and rapidly reached a plateau. Thus, although the levels were still significantly above those of untreated calves at four months after treatment, this may not have been maintained for much longer. Selenium pellets may be effective for up to one year in adult stock while in calves the period of protection may not exceed six months.

The most recently commercially available intraruminal mineral supplement device is quite different from any previously manufactured, being composed of a type of soluble glass containing copper, cobalt and selenium (Cosecure: Chance Pilkington Ltd.) Manufacturer's literature claims that sufficient quantities of copper, cobalt and selenium are released over 365 days to prevent any deficiency in these elements in cattle and sheep when dosed with the appropriate size and number of boluses (two large size for cattle, one small size for

sheep). In the literature however, blood GSH-Px levels are shown to have declined to within the marginal range at around 250 days in both cattle and sheep.

Presently, feedback information from the field is limited by the novelty of the product, although MacPherson (1985) reported that in a situation where high sulphur levels were prevalent, a single bolus administered to sheep as directed was ineffective in maintaining normocupraemia in the blood. Spot checks of flocks treated with the glass bolus have shown approximately 10 to 100% of the ewes to be hyprocupraemic. A higher dosage level of two boluses per animal was subsequently recommended by the manufacturer in response to these circumstances. Prototype glass boluses containing copper alone have been tested by the inventors of the device (Allen et al, 1984) and have been shown only to contribute to the copper requirements, but not to satisfy them.

A number of other diverse types of intraruminal devices have been patented but are not yet commercially available.

A bolus to provide zinc to sheep has been developed in Australia in recent years, composed of 50% zinc

shot and 50% iron filings (Masters and Moir, 1980). The pellet functions as a shortened voltaic cell in the rumen and is completely degradable. In tests, the effective life of the bolus was found to be about seven weeks, but the level of zinc in the faeces indicates that the pellets release approximately 15 mg zinc/day in the first week, 9 mg/day in the third, and only 3 mg/day after seven weeks, which falls far short of the daily requirements of 40 mg/day 1 kg DM intake. Thus at present, this bolus is of limited application but may be useful in overcoming short term or seasonal suboptimal zinc intakes as described by Masters and Somers (1980).

A variety of intraruminal devices have recently been patented in Australia by Laby (1974), initially for the purpose of supplying iodine to pregnant ewes. The iodine containing device consists of a plastic cylinder of 8 mm in length to which 1 g iodine is inserted as a pellet, and the filling cap secured by a heat welding process. The solid iodine is in equilibrium with iodine vapour, which diffuses through the plastic at a constant rate. The plastic offers sufficient resistance to the diffusion of the vapour, which is the driving force, to restrict its escape to an amount sufficient to meet nutritional

requirements and make up for any possible anti-thyroid or goitrogenic substances in the pasture. The arms of the capsule are restrained during administration by stomach tube. Once in the rumen, they spring out, making the device too large to pass back up the oesophagus or out of the rumen. In field trials, retention of the device exceeded 99.5% (Ellis, George and Laby, 1983) in ewes, and the effective life of the device was estimated to be three years.

A more general purpose spring loaded device has also been invented by Laby. This type of capsule is thought to be suitable for slow release drug administration. The plastic housing of the capsule is like a syringe with an opening at one end where the matrix is exposed to rumen fluids. A spring and loose plunger in the base of the syringe exert sufficient pressure behind the solid core to keep it moving so that the dissolving surface remains flush with the opening until the core has been used up, and all the drug has been supplied to the animal. In a trial in which monensin (Eli Lilly & Co.) was administered by capsule (Watson and Laby, 1978) on two occasions over an 8-9 week period, impressive weight gains were noted in treated cattle.

The main disadvantage of such non-degradable Laby

devices is that several may have to be administered in the case of monensin supplementation (one per month) and could conceivably cause digestive obstructions, as well as involving considerable stock mustering. Attempts are presently continuing in Australia to fully adapt the device to administer oxfendazole to control parasitism at pasture in ruminants. Initial trials have been promising (Anderson et al, 1980) but a capsule lasting 150 days to cover the grazing season is being researched. The equivalent effective dose of anthelmintic when continuously administered is about one tenth of the single oral dose.

Pfizer Ltd. in this country have successfully marketed a commercial slow release anthelmintic device for cattle under the name of Paratect containing morantel tartrate designed to control parasitic gastroententeritis due to Ostertagia and Cooperia species. The bolus is comprised of a stainless steel tube (9.1 cm in length and 2.7 cm in diameter) filled with morantel tartrate in a base mixture and enclosed at both ends by a semipermeable membrane. The bolus was reported by Jones (1983) to have been designed to release "approximately 90 mg of base/day for an extended period (at least sixty days)". Experimental results however from the same publication in fact

show a mean release rate of 170 mg/d for days 0-30, 75 mg/d for days 30-60 and only 47 mg/d for days 0-90. A residue of morantel of 4.69 g was recorded as still being present in the bolus after 90 days.

Various field trials have shown superior weight gains in young beef calves turned out in spring for the first time, dosed with Paratect compared to control animals (Entrocasso, 1984; Armour et al, 1981; Tharaldsen and Helle, 1982). Entrocasso also observed an improvement in carcase quality in treated animals. Morantel administered in this way is not capable of reducing the intestinal parasite counts to anything approaching zero although it does control intestinal parasitism.

Conventional anthelmintic treatment regimes fall into three main categories: (i) tactical - i.e. treating parasitic gastroenteritis once clinical signs of the disease appear: (ii) strategic - treat cattle before clinical signs of the disease appear: (iii) the use of an anthelmintic strategically during the first part of the grazing season in order to prevent the midsummer rise in parasite contamination of the pasture and thus provide safe grazing for susceptible calves.

In a study by Jones and Bliss (1983), Paratect treated calves were compared with animals subjected to these three conventional anthelmintic therapies. Compared with control animals, significant reductions in faecal worm egg output of bolus-treated animals was recorded. Subsequent reductions in herbage/larvalcontamination developed on pastures grazed by bolus treated animals compared with control pastures so that, overall, the bolus-treated animals outperformed the control animals in all trials. Labour and management costs were substantially reduced in bolus-treated animals compared with animals receiving tactical or strategic anthelmintic treatment.

One disadvantage of Paratect is that the residues of the steel tube casing have been known to cause problems when the animals are slaughtered by interfering with the machinery at abattoirs during carcase processing (Parkins, personal communication).

Paratect boluses have also been found to be incompatible with the use of the large erodable magnesium alloy bullets. Once the magnesium bullet erodes to a diameter of less than the Paratect steel tube casing, it is capable of rupturing the semipermeable membrane, penetrating the tube and thereby forcing out the morantel

tartrate matrix.

The object of this project was primarily to produce a completely erodable intraruminal device capable of supplementing the mineral requirements of ruminants with the six major micronutrients for a prolonged period. Therefore it was aimed to supply copper, cobalt, selenium, manganese, iodine and zinc plus vitamins A, D and E.

Assuming that a suitable steady release rate could be developed, it was anticipated that the device could be used as a carrier vehicle for growth promoters or anthelmintics.

SECTION II

1. Development and Production of a Mineral Bolus

INTRODUCTION

The physiological benefits of intraruminal supplementation plus the labour-saving advantages of a long term slow release system prompted the decision to undertake a programme of development of a dense slowly dissolving intraruminal device capable of supplementing trace minerals over a prolonged period. Cobalt and selenium boluses were already in popular commercial use, but it is not uncommon for more than one trace element deficiency ultimately relating to soil type to occur simultaneously in an area. For example, cobalt, copper and selenium often occur in combination.

Certain priorities were defined when an attempt was made to formulate a combined trace mineral bolus. The bolus should be completely degradable and leave no residue and be dense enough to lie in the reticulum and should contain sufficient material to supply the trace mineral requirements of an adult cow or sheep for at least thirty days.

Materials should be cheap and readily commercially available. Sophisticated machinery or technology should

preferably not be required in bolus production, and it must be possible to produce the device on a laboratory scale.

The first step was to define which trace minerals should be incorporated into the supplement. Copper, cobalt, selenium, zinc, iron, iodine and manganese are the essential trace minerals. Although frequency of cases of deficiency of, for example, cobalt, is more common than manganese deficiency, it was decided, that for the sake of completeness, the nutritional requirements for each element should be met by the bolus. In addition, a percentage of the recommended daily allowance of vitamins A, D and E were also to be included.

Table 3 lists the quantities of each element and vitamin which would be required to provide the total trace element requirements for a thirty day period in accordance with ARC (1965).

The simplest method of manufacturing a bolus would be by compressing mineral salts at high pressure into an appropriate shape. Existing mineral boluses are cylindrical in shape so in order to fit available balling guns and with a view to commercial acceptability, the cylindrical shape was determined as one of the basic features of the bolus. It was known from various

workers (originally Schalk and Amadon, 1928) that dense objects tend to lodge in the reticulum of cattle. Small particles of a metal mixed with the mineral salts in proportions to increase the density of the compressed object was thought to be desirable, particularly if the metal was one of the trace minerals required, since mineral salts alone would be unlikely to attain sufficient density on compression. The density required for an object to remain in the reticulum of cattle on administration has been investigated by various workers. Riner (1981) observed that objects placed in the reticulum of a density $\geqslant 2.2$ gcm^3 could be recovered from the reticulum or the anterior dorsal sac of the rumen in all cases. To ensure retention in the reticulum, a density of $\geqslant 2.7$ gcm^3 was aimed for.

TABLE 3. /

TABLE 3. 1 day and 30 day quantities of trace elements and vitamins required to be provided for an adult 500 Kg LW cow

	Daily Levels of Supplementation	Absolute Requirement for 30 days
copper	100 mg	3 g
cobalt	1 mg	30 mg
selenium	1 mg	30 mg
iodine	5 mg	150 mg
zinc	266 mg	8.0 g
manganese	200 mg	6.0 g
vitamin A	90 mg (500,000 iu/g)	2.7 g
vitamin D	25 mg (500,000 iu/g)	0.75 g
vitamin E	150 mg (∝ dl tocopherol acetate)	4.5 g

TABLE 4. The % formulation of mineral salt mix to provide trace elements in the proportional quantities given in Table 3.

Mineral Compound	% of Compound
$CuSO_4$ $5H_2O$	24
$CoSO_4$ $7H_2O$	0.36
$MnSO_4$ H_2O	42.9
ZnO	21.28
KlO_3	0.58
Na_2SeO_3	0.16
vitamin A powder	4.5
vitamin D powder	0.75
vitamin E powder	3.62

A cattle pellet should contain 44 g mix plus weighting

A sheep pellet should contain 8.5 g mix plus weighting

The following experiments in this section describe the steps taken towards production of a viable slow release mineral bolus. Although the work has been segregated into discrete sections, in practise, development was continuous and frequently several factors were simultaneously investigated. Therefore, a certain degree of overlap exists between succeeding experiments due to the nature of the investigations.

MATERIALS AND METHODS

i) Mineral mix

The mineral mix was formulated from the requirements recommended by ARC (1965) and listed in Table 3. Table 4 lists the formulation in terms of the percentage content of the various mineral salts required to fulfil the levels shown on Table 3. A single cattle sized bolus should contain 44 g of this mixture plus weighting per 30 day supplement period. For sheep, the equivalent would be 8.5 g. The density of compacted powder alone was 1.75 gcm^{-3} and it was anticipated that approximately 50% of the bolus should be composed of iron shot or iron filings in order to increase the density to ≥ 2.7 gcm^{3}.

The mineral mix was prepared in a bulk quantity of about 1.5 kg by the following method. Individual ingredients were weighed out separately in

accurate amounts. Ingredients present in small quantities such as sodium selenite were bulked up by the addition of part of the manganese sulphate component and thoroughly mixed. Gradually all the ingredients were mixed together in a large plastic bucket and thoroughly hand mixed for 10-15 minutes.

(ii) Methods of compaction

Two basic methods were tested at this stage, and material was initially experimentally compacted using both systems. A large hydraulic press at Glasgow University Engineering Workshop was capable of pressing our approximately 2.5 cm dia x 2.5 cm and 2.0 cm dia x 4.0 cm length cylindrical boluses in an end on compaction procedure.

Horizontally compacted boluses of a lozenge shape were produced at Thomson and Capper, Runcorn, Cheshire, a specialist tabletting firm, when a quantity of material was sent to them for experimental pressing.

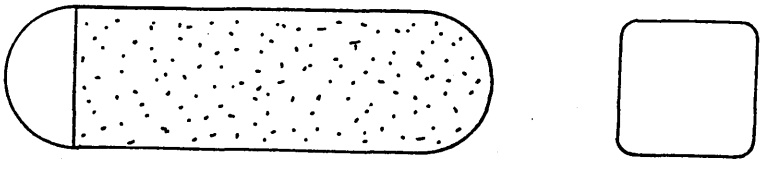
(iii) Boluses

(a) Pressed at Glasgow Engineering Workshop - Boluses of 50% mineral and 50% iron shot were compacted to a pressure of 4 tons psi. Boluses were of the following dimensions: 2 cm dia x 4 cm length cylinder,

2.0 cm dia x 3.1 cm length cylinder and 2.5 cm dia x 2.7 cm length cylinder. They weighed 46.14 g, 32.30 g and 40.41 g respectively. However, although in all cases their densities were in excess of 3.0 gcm^{-3} they had a very crumbly texture which was thought to be unsuited to further testing.

(b) Pressed at Thomson and Capper Ltd. - They produced horizontally compressed lozenge shaped tablets 5.6 x 1.5 x 1.5 cm (see Diagram 2) of density 3.1 gcm^{-3}, composed of 50% 0.7 mm iron filings and 50% mineral mix and weighing a total of approximately 40 g per bolus on average. They were well compacted with a firm texture. Fifty per cent of the boluses had the binder Calgon incorporated at a rate of 7% of the total. To meet the dosage recommended on Table 3, two boluses were administered per animal.

DIAGRAM 2. Schematic representation of bolus compacted at Thomson and Capper



Thomson and Capper lozenge shaped bolus.
Shading indicates coating.

Scale 1:1

(iv) Coating

It was anticipated that some sort of protection would be required on the bolus against the rumen environment lest digestion or rumination action quickly break the bolus down. It was determined that a skin should surround the bolus such that only an area equivalent to the cross section would be exposed as is shown on Diagram 2. As the bolus dissolved it was hoped to maintain a uniformly exposed surface. It was thought that a polyurethane varnish type of coat because of its flexibility, would retract as the bolus eroded, thereby maintaining the constant surface area. Therefore Vycoat ACA 60 PVC coating (Plastic Coating Systems Ltd.) was chosen as a coating agent.

Boluses were coated as follows: A retort stand was set up which held small clamps extending outwards. The topmost 0.5 cm of each bolus was grasped firmly in the clamp and a beaker filled with varnish was brought up to the bolus so that all but the top portion was immersed. Excess material was allowed to drip off into draining trays before the varnish hardened to a thin shiny lacquer (a process which took approximately 30 minutes). One coat only was applied.

(v) Fistulated cows

Cows on whom rumen fistulation had been performed were determined as the primary test animal for intraruminal bolus erosion data.

Fistulated cows had been operated upon so that there was an incision on the left flank and the rumen wall was fixed to the outer skin. A rubber cannula was inserted at this point, the central diameter of which was large enough to allow a person's arm to be inserted. A rubber bung with an inflatable valve was tightly fitted into the cannula and only removed when access was required. Plates 1(a), 1(b) and 1(c) show respectively a fistulated cow with the bung in place, with the bung removed, and with a researcher demonstrating the normal procedure for placement and removal of boluses

Boluses were placed into the reticulum by pushing one's right arm through the rumen contents anteriorly and downwards until the reticulo-rumen junction was felt with the fingers as a division between compartments. After further pushing in this direction, the honeycomb interior structure of the reticulum could be traced with the fingers. Boluses were then let drop onto the base of the reticulum. Retrieval of boluses involved

PLATE 1(a) Fistulated cow with bung in place.

PLATE 1(b) Fistulated cow with bung removed.

PLATE 1(c) Researcher retrieving bolus from fistulated cow.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reaching to the base of the reticulum to retrieve the bolus residues.

After removal, the boluses were washed in cold water, carefully blotted dry on paper towel and quickly weighed and replaced within as short a period as possible (no more than 30 minutes). Fistulated cows were maintained indoors throughout on a hay and concentrate diet unless otherwise specified. Periods spent in the byre were alternated with periods in loose housed pens, and some time was spent at grass during the grazing season.

(vi) Experimental design

Six coated boluses, numbers 1 to 6, weighing 39-42 g manufactured by Thomson and Capper were administered to three fistulated cows, two per cow, and were weighed and examined at frequent intervals. Bolus numbers 1-3 did not have Calgon, and 4-6 included Calgon at 7%. Four uncoated boluses (Thomson and Capper manufactured) containing Calgon were administered to two fistulated cows in a later experiment.

RESULTS AND DISCUSSION

The pattern of erosion of the coated mineral boluses is shown on Figure 1. After 24 hours there

was a reasonable weight loss, but thereafter rusty deposits were found on the exposed surface of the boluses, the build up of which was associated with a gain in weight. The deposits were thought to consist of oxidised iron filings. Water seepage under the skin at the open ends became apparent after three days, and some swelling was also noted in this area. The flexible skin then began to curl over the exposed surface as illustrated on Plate 2. No apparent difference in weight or behaviour was found between boluses containing Calgon at 7% and those with none. Since there were no further significant weight losses between days 1-8, these boluses were removed.

Uncoated boluses eroded initially at a steady rate between days 0-14, but thereafter, the release rate slowed down between days 14 and 18, as shown on Figure 2. Iron oxide deposits were present on all surfaces of the bolus. A similar trial was repeated with similar arresting of erosion at around two to three weeks.

However, the mere fact that a compressed mineral bolus could be administered to a ruminant with or without a protective casing, and be recovered partly worn over a period of several weeks was a significant discovery.

PLATE 2. Partially worn Vycoat covered bolus.


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8
9

An ideal bolus would erode or dissolve at a constant rate over a period of about thirty days. To this end it was thought that a protective shell ought to be a permanent feature of the design since it should ensure that a constant surface area was exposed. The mineral compound tested in this experiment tended to rust over in the rumen environment, and when the bolus was coated, the release rate was virtually zero. An improved bolus type, if it was to be encased and have a constant area exposed, must then be composed of a different mineral mix, preferably excluding iron filings which were oxidised in the rumen environment.

FIGURE 1. The erosion pattern of lozenge shaped boluses with 50% mineral and 50% iron filings, coated with "Vycoat" polyurethane varnish, tested in pairs in fistulated cows.

FIGURE 2. The erosion pattern of similar boluses which were left uncoated and tested in the same way.

FIG.1 "Vycoat" coated boluses

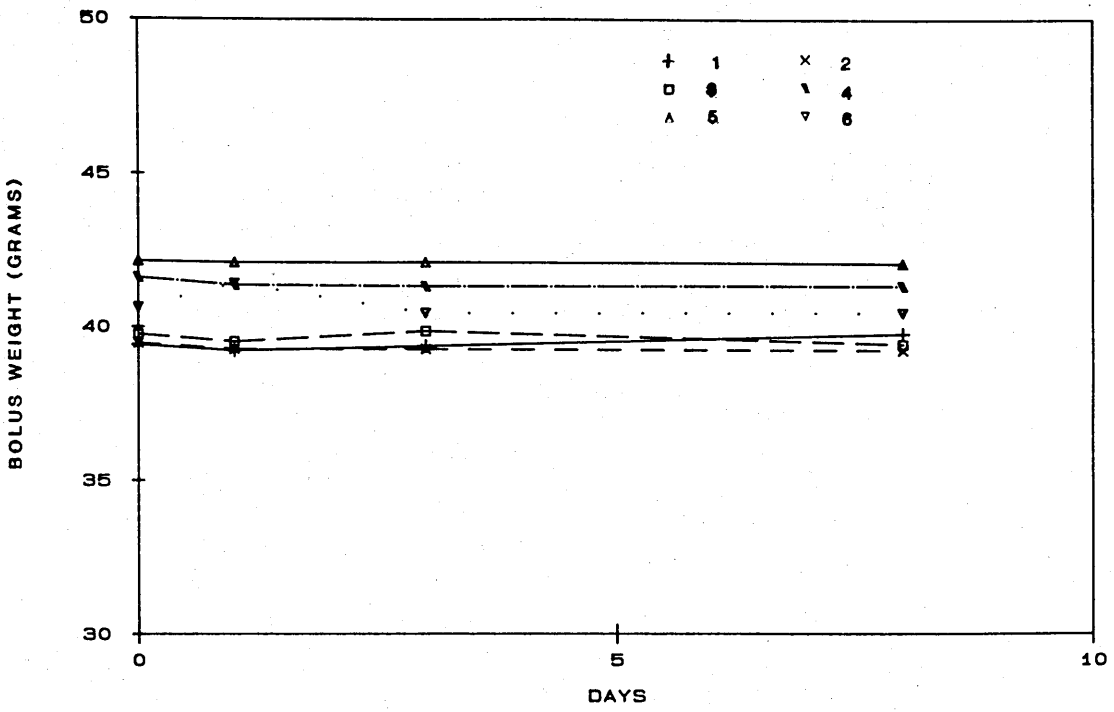
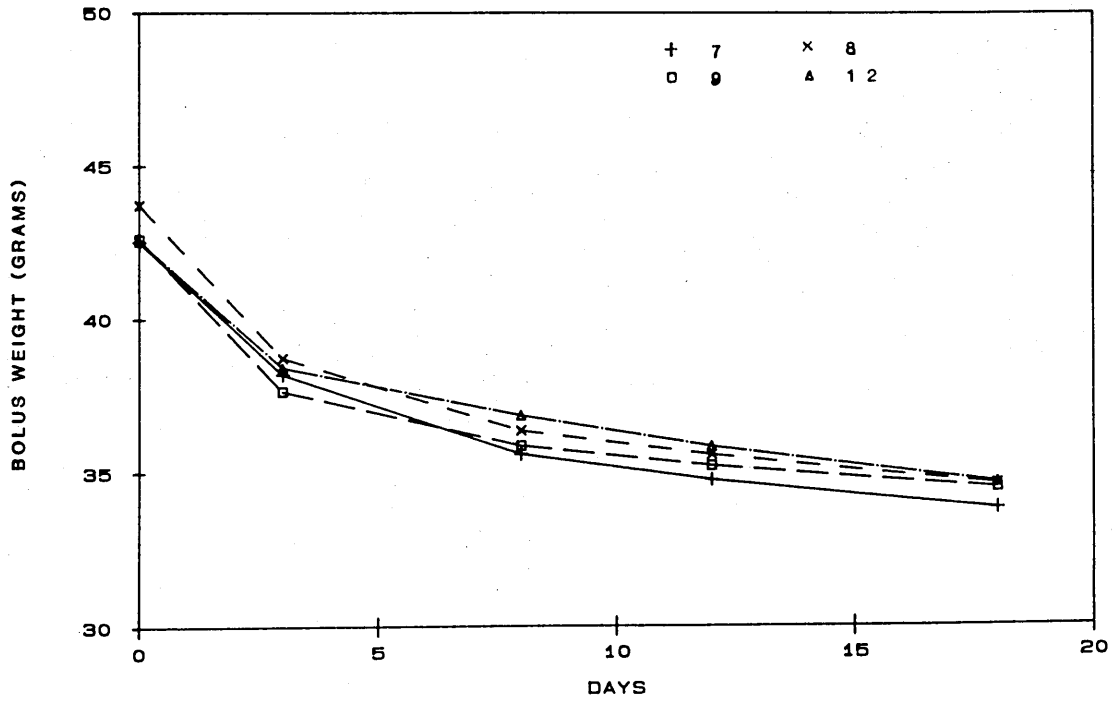


FIG.2 Uncoated boluses



2. Mineral Mix and Laboratory Bolus Production

INTRODUCTION

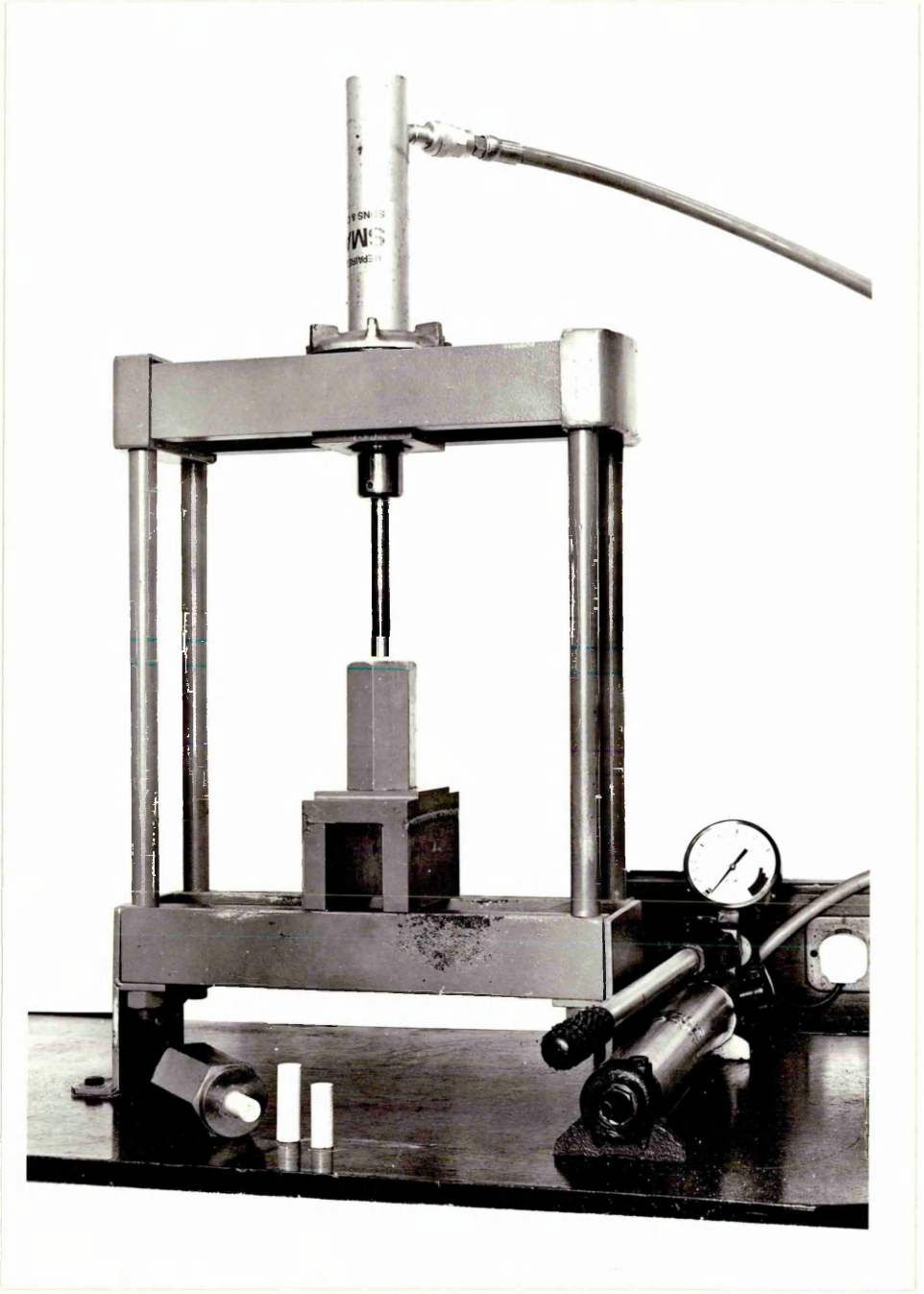
Oxidation of the iron filings component of the mineral mix was thought to be partly responsible for the failure of the previous bolus to lose sufficient weight. Rusty deposits were visible on the surface after two days, which implied that erosion was coming to a halt. Therefore it was decided to formulate a new mineral mix from ARC (1965) requirements from which iron was completely discarded. Cases of iron deficiency are relatively rare in grazing ruminants since a certain amount of natural ingestion of soil occurs during grazing, and soil normally contains a sufficiently high concentration of iron to prevent deficiency developing. However, a weighting agent to increase the density of the compacted mineral to at least 2.7 gcm^{-3} was essential to ensure that the bolus would be retained in the reticulum. At lower densities than 1.8 gcm^{-3} in cattle objects could pass into the rumen, be excreted, or be regurgitated (Riner, 1981). It was decided that it would be preferable to replace the copper sulphate component of the previous mineral mix with copper oxide needles 2-4 mm in length, which being dense (specific gravity 6.3) could act both as a weighting medium and copper source. It has been shown that copper oxide needles

when administered in a gelatin capsule lie in the gastrointestinal tract and dissolve slowly over a prolonged period (Dewey, 1977). Therefore this material seemed ideal for administration in a slow release bolus.

At about the same time as the mineral formulation was being altered, a small manually operated hydraulic bench press (Tangye Hydraulics Ltd: type PRE 80B) with an 8 tons ram was installed in the laboratory (see Plate 3). Two stainless steel cylindrical dies and plungers were manufactured to fit the press by Glasgow University Mechanical Engineering Department. Each mould was 4 inches tall and was bored out in the centre to $\frac{5}{8}$ inch diameter cylinder, and the other to a 1 inch diameter cylinder. Plungers corresponded in diameter and were about half an inch longer although they were slightly reduced in width so that they could travel the length of the die freely. The facility to produce boluses with this equipment in the laboratory, allowed the field of experimentation to be considerably expanded.

Since it was initially thought that the presence of a binder was a necessary feature of the bolus, four different binders were tested in boluses at small inclusion rates as follows: Bentonite 1%, Chapmans 0.5%, Dihard 0.2% and KelFlo at 0.25%. Binders are known to act by enhancing cohesion between particles.

PLATE 3. Hand operated bench hydraulic press.



(i) Mineral mix

The mineral mix was composed of the ingredients as detailed in Table 5. All the minerals were of a commercially available grade and were supplied by Agrimin Ltd., Grimsby. A bulk amount of material of 1.5 kg was made as one batch by the following method:

Each ingredient was individually weighed on an electronic balance where the quantity was less than 150 g, or on a large weigh scale where materials were required in greater quantities. Minor ingredients were bulked up by the gradual addition of one of the major ingredients and then all the components were incorporated together little by little and mixed very thoroughly for a long period by hand. Unused mineral mix was stored in a covered plastic bucket in a dry cupboard, and prior to being used again, it was thoroughly remixed and examined for any evidence of deterioration or discolouration.

(ii) Copper oxide needles

The copper oxide needles used throughout these experiments were of reagent grade 2-4 mm in length, product number 11005 supplied by

BDH Chemicals Ltd., Poole. Their specific gravity was 6.3 and they were of approximately 28 swg.

(iii) Method of bolus manufacture

The mineral mix and copper oxide needles component of each bolus was weighed on an electronic balance and then thoroughly mixed in a small plastic container with a spatula until there was a uniform distribution of needles. The base of the die was stoppered with a machined stainless steel insert. Using a 15 x 15 cm piece of folded paper as a slide to funnel the material, the mix was tipped into the mould, ensuring always that the powder and copper oxide needles remained homogeneously mixed. The plunger piston was then lowered into the mouth of the mould using the hydraulic pump and the compaction pressure taken to 3 tons per square inch, held there for approximately ten seconds before being released and raised above the cylinder. The steel insert was then removed from the base of the die, the plunger lowered again, and the compacted bolus pushed out of the die. Plate 3 shows a bolus which has been half pushed out of the cylinder. Boluses were then taken and the exterior dampened under a very slowly dripping tap and allowed to dry for two

hours. This damping treatment improves the future resilience of the bolus. Before coating boluses could be numbered and labelled with indelible ink markers which showed through the coating and facilitated identification.

After pressing some experimental boluses, it was found that a mixture of 50% mineral salts and 50% copper oxide needles produced a bolus with a compacted density of 2.95 gcm^{-3}. The compaction ratio of material was found to be approximately 2:1. When the $\frac{5}{8}$ inch diameter mould was filled with 33 g of 50% mineral, 50% needles mix, it measured 10.2 cm before compaction and produced a pressed bolus 4.7 cm in length. The one inch diameter mould had the same non-compacted length, and a pressed height of 5.1 cm to produce a 70 g bolus.

(iv) Coating

Boluses were suspended by being clamped gently by the top 0.5 cm to a retort stand. A beaker containing Vycoat (Plastic Coating Systems Ltd.) was then brought up to the bolus and it was immersed, apart from the top 0.5 cm, for a few seconds then allowed to drip onto a drainage tray until it was dry.

(v) Experimental

Eight boluses of 33 g were manufactured, each containing 50% mineral mix. Four types of binders were included in the eight boluses, two boluses per binder at the following rate, 1% Bentonite 0.5% Chapmans, 0.2% Dihard and 0.25% KelFlo. The remaining percentages were composed of copper oxide needles. One of each type of bolus was coated with one coat of Vycoat leaving the full diameter of one cylindrical face completely exposed. Two boluses were administered per animal, one coated, one uncoated.

TABLE 5. Constituents of mineral mix and quantity of element supplied in a 30 day cattle bolus

Compound Supplied	Element In	Absolute Amount of Element for 30 day Bolus	% of Each Compound
$CoSO_4\ 7H_2O$		0.14 g	0.434
$Na_2\ SeO_3$		0.066 g	0.205
Kl		0.25 g	0.775
ZnO		9.336 g	28.956
$MnSO_4H_2O$		18.45 g	57.223
vitamin A powder (500,000 i /g)			
vitamin D (included in vitamin A powder at a ratio of 5:1)		2.0 g	6.203
vitamin E		2.0 g	6.203

RESULTS AND DISCUSSION

Each uncoated bolus dissolved within twenty-four hours irrespective of the binding agent used. The remaining coated boluses were then doubled up to two per animal. After fifteen days the total weight loss was as shown on Table 6.

TABLE 6. Weight loss of boluses at day 15

	Binder			
	Bentonite	KelFlo	Chapmans	Dihard
average weight loss (g)	1.796	5.587	13.845	22.349

Although superficially the figures looked promising, the disintegration curve showed that in all, apart from the Dihard inclusion bolus, there was little weight lost after the fourth day. The Dihard bolus began to slow up after day seven. The main reason for this occurrence was thought to be due to the flexible coating tending to close over the eroding surface of the bolus. With prolonged exposure to the rumen environment, Vycoat appeared to become progressively more inflexible. It would appear that when the bolus was first introduced into the rumen, the mineral core was fairly soluble and the coat flexible, allowing the rapid passage of material out of the bolus. However

the flexibility of the coat appeared to decrease with time so that eventually it became rigidly fixed in a position which tended to occlude the open end of the bolus.

Binders included in the formulation were found to affect the rate of erosion, with those containing bentonite, Chapmans and KelFlo losing little weight after four days, compared to seven days for boluses containing Dihard binder. The magnitude of weight loss also varied, depending on the binder. Bentonite appeared to be the strongest, followed by KelFlo, Chapmans and Dihard. Since there were problems in trying to produce a bolus with a prolonged sustained release, the necessity of utilising binders at all was questionable, and it was thought possible that they were an unnecessary component of the mineral matrix.

3. Coating

INTRODUCTION

A new coating agent was sought, which while protecting the mineral core of the bolus from the rumen environment, would allow a constant surface area to be exposed. Vycoat was capable of deforming and thereby changing the amount of mineral core in contact with the rumen environment. A rigid type of casing into which the mineral core was inserted would possibly leave a residue when the mineral dissolved depending on the type of casing material. A more preferable coating would be initially rigid and yet be degradable so that no residue remained.

One suitable type of material was thought to be a liquid glass fibre resin. Once hardener and curing agent was added to the liquid resin component, the resin was known to set very hard. It was thought that a thin shell of this material applied to the exterior of the bolus would impart the desirable properties of rigidity with brittleness.

The type of resins investigated were polyesters which contained styrene monomers. The curing agent and hardener catalysed the polymerization to a rigid cross linked solid.

(i) Boluses

Boluses were composed as follows: Number 27A contained 15 g of mineral mix (as described in Section 2:2), 15 g copper oxide needles, 30 mg bentonite. Numbers 24A and 30A contained 15 g mineral mix, 15 g copper oxide needles and 6 mg Dihard. Number 32A contained 15 g mineral mix, 15 g copper oxide needles and no binder.

Boluses were compacted as described in Section 2:2.

(ii) Coating

Boluses 27A, 30A and 32A were coated as previously described with Vycoat polyurethane varnish. Two small holes of 8 mm diameter were made in the Vycoat skin at each end of the bolus. Bolus 24A however was coated with David's Isopon glass fibre resin by the following method.

The bolus was identified by numbering with a felt tip pen directly onto the mineral matrix core, then lightly sanded with the finest grade of sandpaper so that the surface was smooth. If the basewas to be enclosed in resin leaving one exposed face, the base edges were lightly rounded off. A quantity of liquid resin (10-20 ml) was

poured into a plastic pot and combined curing agent and hardener was added in the form of paste at the rate of 0.5-1% by weight.

The hardening paste was thoroughly mixed into the liquid. The bolus was stood end-on on a plastic sheet and a small artist's paintbrush used to apply a thin coat of the liquid to the bolus. With careful application there was only a small excess which collected at the base of the bolus. The resin remained liquid for about twenty minutes, then began to polymerize exothermically and swiftly set to a rigid solid to produce a thin hard shell on the bolus surface. Second and subsequent coats of resin could be applied after drying in the same way as described, though the bolus was turned between coats to stand on its opposite end to ensure a more uniform cover.

Once the coating was dry, excess material could be scraped off the exposed surface with a scalpel blade and any excess which had collected at the base could be sanded off with coarse sandpaper.

Bolus 24A was given only one coat of glass fibre resin at the outset to test the behaviour of this coat in the rumen environment.

(iii) Experimental

Boluses were administered in pairs to fistulated cows indoors by placing directly into the reticulum by hand. They were removed damp-dried on paper towelling, weighed and subsequently replaced on days 1, 4, 7, 14 and day 18 of the experiment although some of the boluses did not last for the full period.

RESULTS AND DISCUSSION

The results are shown in Figure 3 which gives the bolus weights plotted against the eighteen day time period of the experiment. In two of the three boluses coated with "Vycoat" (30A and 32A), erosion was rapid, and it was estimated that 32A would not last for more than ten days, and 30A was not recovered after four days. The "Vycoat" bolus with 1% bentonite which was known to be a strong binder (27A) lost a negligible amount of weight over fourteen days. However bolus 24A which had one coat of glass fibre resin and Dihard binder showed a much steadier rate of erosion over an eighteen day period. What was particularly impressive was that resin was found adhering to the mineral core even when only a small fragment of the bolus remained.

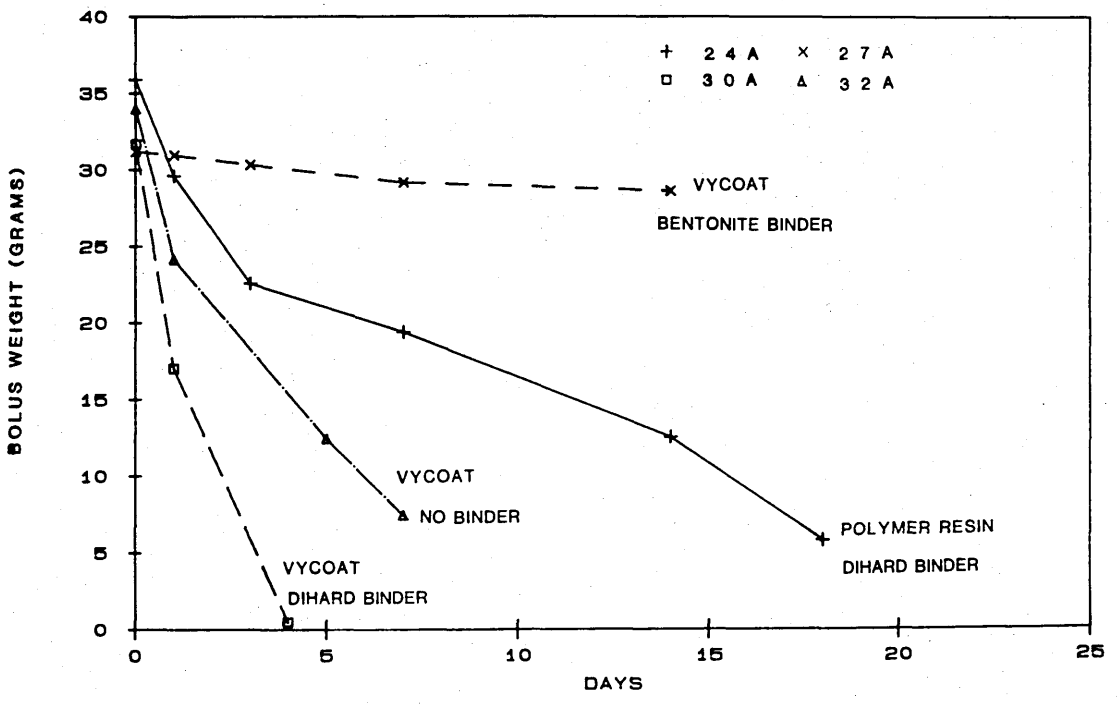
The resin coating seemed capable of performing the function for which it was intended. It protected

the mineral core, adhering to it closely, and only broke off in flakes at the exposed edge once the underlying core material had been removed by dissolution or erosion. The bolus therefore gradually shortened in length. Bolus 24A became sparsely coated however at 7-14 days, and it appeared that the resin layer was a little too thin to withstand the stress of the rumen environment and remain intact. It was thought that if the coating was thicker, it may allow a fixed area to be constantly exposed, and thereby exert a control on erosion so that it would be more uniform. It was decided therefore that further work on glass fibre resin was necessary, and that it would be a more suitable coating than "Vycoat".

The Dihard binder did not appear to be performing any useful function in the bolus in that number 30A eroded more quickly than 32A which had no binder. Other binders tested were thought to be too strong. Therefore the decision was taken that future formulations should not contain binder since they appeared to be a non-essential component.

FIGURE 3. The erosion pattern of boluses 24A, 27A, 30A and 32A tested in fistulated cows. Each bolus was composed of mineral and copper oxide needles and all, apart from number 32A, contained Bentonite or Dihard binders. Bolus 24A was coated with a polymer resin while the remainder were coated with Vycoat polyurethane varnish.

FIG.3 Comparison of Vycoat and resin coatings



4. Alterations to the Mineral Base

INTRODUCTION

Glass fibre resin appeared from the previous trial to be a more suitable coating agent for the slow release bolus than the formerly used Vycoat. Many more boluses were made to the same formula as 24A but with three coats of resin for extra protection and with this tougher type of coat, the surface area remained relatively constant throughout the tests in fistulated cows. As the boluses eroded and abraided against each other, small flakes of resin coating became detached to gradually expose new material.

Plate 4 shows boluses A to E which demonstrate the type of erosion observed with a glass fibre resin coating. The bolus shortened as it eroded yet the surface area remained relatively constant. Bolus A is a fresh bolus prior to administration, and B to E show progressive erosion. Bolus D had a section of the residue removed to show that underneath the darkened exposed surface, the mineral matrix and copper oxide needles were of similar appearance to untested material, implying that constantly fresh material was exposed during erosion.

Figure 4 shows the typical erosion pattern of boluses composed of 50% mineral, 50% copper oxide needles, coated with glass fibre resin with one exposed face. Although the release rate was reasonably steady, it was slower than that being sought after (ideally 1 g/d for thirty days between two boluses).

Different methods of improving the design of the bolus were investigated. Additives were sought of materials required in small quantities which would act to regularise the dissolution. Also, in some boluses the outer circumference of the exposed face was experimentally covered with one thin layer of resin coating. This layer was intended to withstand the initial period in the reticulum during which the dissolution rate of the initially exposed area was usually more rapid (plot 34A Figure 4 demonstrates this). It was hoped that the resin surround would then flake, exposing the entire surface area. It was hoped that this would then lead to a more gradual sustained release.

The first alteration tested was the addition of copper sulphate. It is highly soluble in water, and copper was already a component of the mix. Copper sulphate was incorporated in quantities of 5% of the standard mineral mix excluding weighting.

PLATE 4. Mineral boluses coated with glass fibre resin showing progressive erosion A to E.

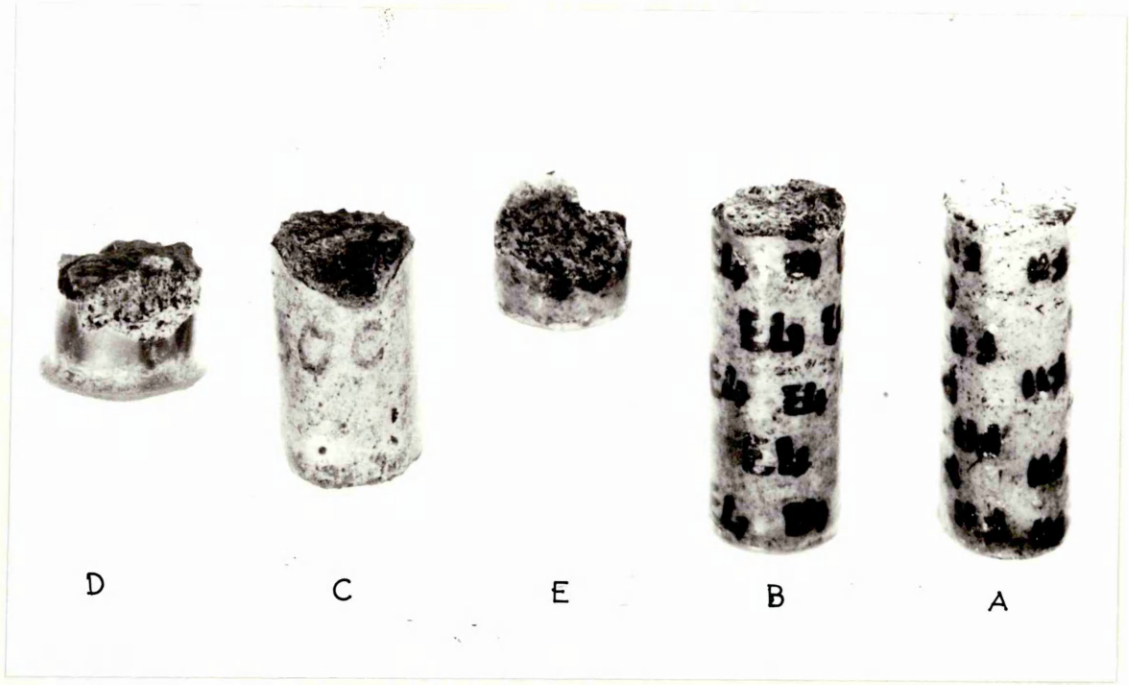
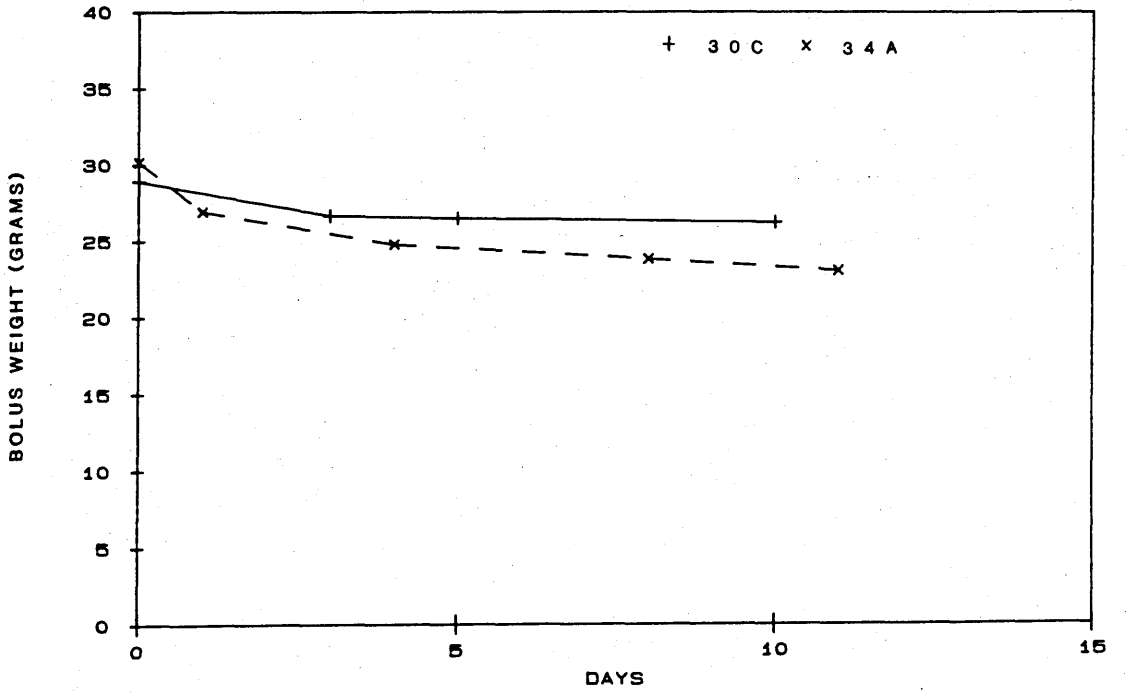

D
C
E
B
A

FIGURE 4. Typical erosion pattern of a pair of boluses, 30C and 34A, which were composed of 50% copper oxide needles and 50% mineral mix with one exposed face, tested in fistulated cows.

FIG.4 Mineral mix boluses with glass fibre resin



Soluble readily available agents such as salt and sugar were also included in varying proportions in the mineral bolus.

MATERIALS AND METHODS

(i) Boluses 44A, 44B, 57A and 57B contained 50% mineral mix, 45% copper oxide needles and 5% copper sulphate. A 9 mm diameter surface opening was made in one end of boluses 44A and 44B, while 57A and 57B had a 10 mm diameter opening. All boluses were coated three times with glass fibre resin (David's Isopon).

Three sugar containing boluses, 49, 56A and 56B were manufactured of identical formula as follows: 50% copper oxide needles, 49.15% mineral mix and 0.85% sugar. Each had a 10 mm diameter surface opening.

Boluses containing common salt in various quantities were made as follows: No. 46A had 46.88% mineral mix, 46.88% copper oxide needles, 6.3% salt and an 11 mm surface diameter opening; No. 50 had 50% mineral mix, 49.3% copper oxide needles and 0.7% of salt and an 11 mm diameter opening; No. 51 had 50% mineral mix, 49.17% copper oxide needles and 0.83% salt with the

same size of opened diameter. Each bolus was coated as above.

(ii) Experimental

Boluses were tested in pairs in fistulated cows indoors by placing them in the reticulum by hand and removing them for weighing and subsequent replacement at regular intervals over test periods of up to twenty-six days.

RESULTS AND DISCUSSION

Results of the boluses containing 5% copper sulphate are plotted on Figure 5. The erosion rate was found to be variable between boluses despite their almost identical design. Bolus 44A had a 9 mm diameter exposed surface which did not begin to wear fully, and thus weight loss was negligible. Although bolus 44B dissolved at a reasonable rate, replicates 57A and 57B with a 10 mm diameter exposed surface had a good initial erosion but then quickly reached a level when almost nothing was lost.

Sugar boluses (Figure 6) were also highly variable in behaviour despite uniformity in testing conditions. Bolus 56B lost three quarters of its weight within twenty-four hours while 56A lost almost half its weight over the first twenty-four hours but then lost very

little weight over the following seven days. A reasonable average erosion rate was shown by bolus 49 over nineteen days although it was not a particularly steady pattern.

Salt in percentages of less than 1% appeared to have a small retarding effect on the bolus release rate but when the amount was increased to approximately 6%, erosion became very rapid and the bolus was not recovered after three days. Bolus No. 50 (0.7% salt) lost 1.845 g in forty-eight hours and No. 51 (0.83% salt) lost 3.412 g in the same period. In the following sixteen days, bolus 50 lost a total of only 0.253 g, and bolus 51 a total of only 1.04 g.

The experimental models tested in this section were rejected from future development work. There was too high a degree of variability between boluses of the same formula, particularly copper sulphate and sugar containing boluses. The type of additive desired would give preferably a linear increase in erosion rate with increasing amounts of material incorporated into the bolus. Salt was found to have virtually an adverse effect on erosion in small quantities and too extreme an effect at slightly higher concentrations.

The method of making small openings on the surface of boluses was also rejected. In some cases (for

example 44A), the surface failed to flake off so that little erosion took place. It was felt in view of the results that this step introduced a further predictable factor. Further erosion control trials would be carried out with the whole cylinder diameter exposed from the outset.

FIGURE 5. The erosion pattern of boluses 44A, 44B, 57A and 57B which contained 50% mineral mix, 45% copper oxide needles and 5% copper sulphate. 44A and 44B had a 9 mm diameter surface opening while 57A and 57B had a 10 mm diameter opening. All were coated three times with polymer resin.

FIGURE 6. The erosion pattern of boluses 49, 56A and 56B which contained 50% copper oxide needles, 49.15% mineral mix and 0.85% ground sugar. All were coated three times with polymer resin with a 10 mm diameter exposed surface area.

FIG.5 Boluses containing copper sulphate (5%)

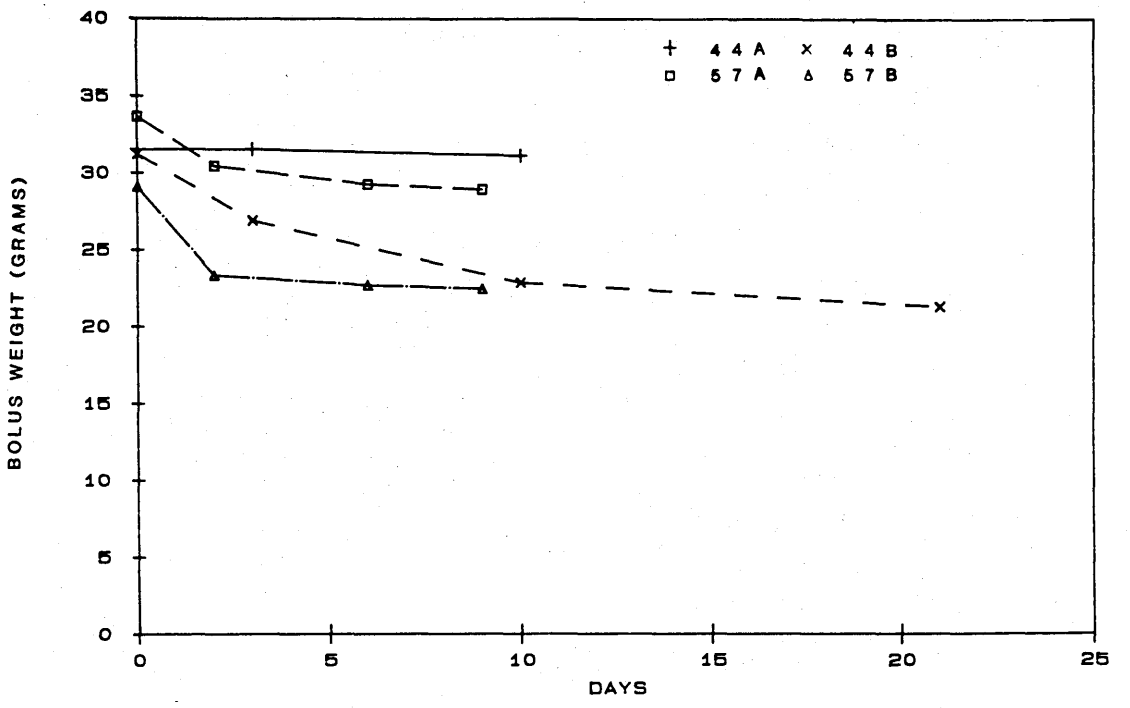
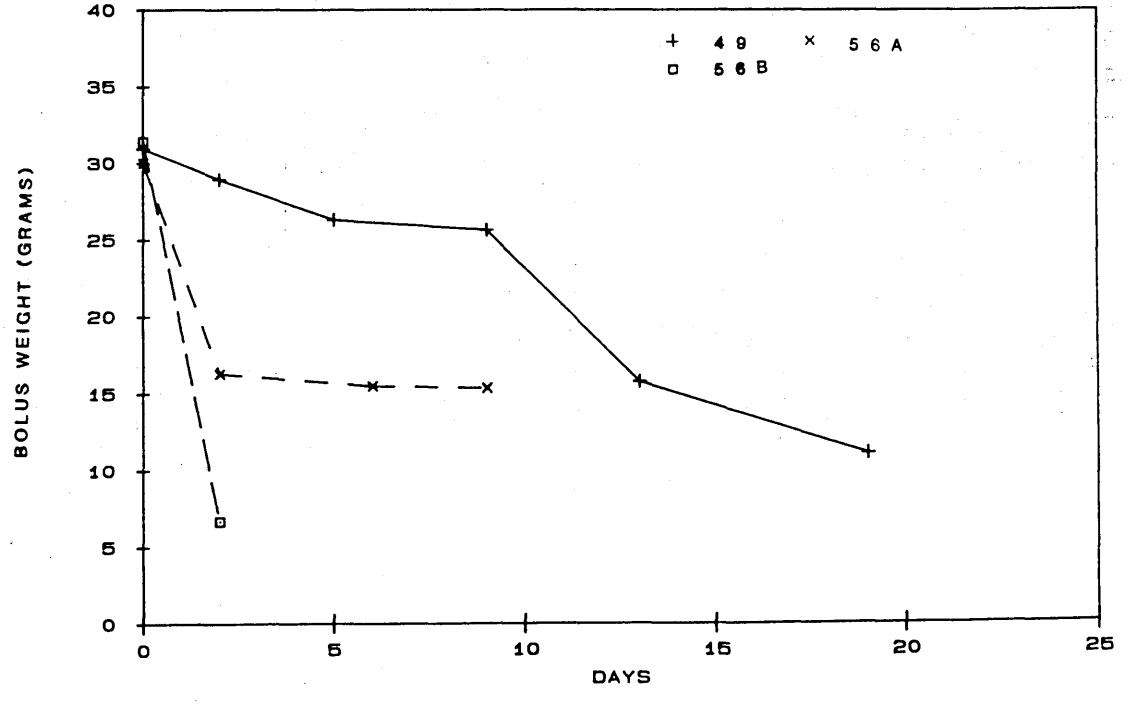


FIG.6 Boluses containing 0.85% finely ground sugar



5. The Addition of Zinc Sulphate Heptahydrate

INTRODUCTION

A range of substances were examined as prospective additives which might increase and regularise the erosion rate of the mineral bolus. Previous work had shown that, with the mineral formulation used to date, the bolus lost a reasonable amount of weight within the first few days, but subsequently became very slow to erode possibly because of a tempering process occurring in the rumen environment. An additive which might alter this property of the mineral mix was sought.

One commonly available material, zinc sulphate heptahydrate, a white crystalline salt, was found to be highly soluble in water. This type of material was considered to be ideal because zinc was already a component of the mix, and if it was found to be a successful regulator, some of the zinc oxide in the mix could be replaced if necessary with zinc sulphate.

Two approaches along these lines were therefore adopted. One option was to replace some of the existing zinc oxide in the basic mineral mix with zinc sulphate heptahydrate. The other was to add zinc sulphate to the existing mineral composition. Zinc

oxide is known to be relatively water insoluble so it was likely that alterations in its proportions would affect the properties of the bolus.

MATERIALS AND METHODS

(i) Boluses

Bolus 62 was 25 mm in diameter and composed of 30 g mineral mix (as Section 2:2), 30 g copper oxide needles and 2 g of zinc sulphate heptahydrate. The bolus was coated three times with glass fibre resin (Plastic Padding Ltd.) leaving a 14 mm diameter surface exposed.

For comparison with bolus 62, two other large sized (25 mm) diameter boluses were manufactured as follows: Bolus 61 was composed of 0.4 g finely ground sugar, 30 g mineral mix (as Section 2:2) and 30 g copper oxide needles and was coated in the same way as No. 62. Bolus 63 contained 0.4 g of sodium bicarbonate, 30 g of mineral mix (as Section 2:2) and 30 g of copper oxide needles and was coated as already described.

Small sized (17 mm diameter) boluses were made from the mineral formulae shown on Table 7. Boluses 81 and 87 contained 15 g of mix B and 15 g of copper oxide needles, and were coated

three times with glass fibre resin (Plastic Padding Ltd.) leaving one exposed face. Boluses 100 and 102 were composed of 15 g mix C and 15 g copper oxide needles with three coats of glass fibre resin (Plastic Padding Ltd.) and one exposed face. The major difference between mixes B and C was that mix B had 13.63% of zinc oxide and 23.66% zinc sulphate whereas mix C had 17.64% zinc oxide and 17.45% zinc sulphate.

TABLE 7. Percentage of mineral constituents present in three experimental mixes

Compound	Mix A %	Mix B %	Mix C %
$CoSO_4 7H_2O$	.36	.38	.39
Na_2SeO_3	.17	.18	.19
Kl	.65	.69	.71
$MnSO_4 H_2O$	47.68	50.51	52.29
vits A & D	5.17	5.48	5.67
vit E	5.17	5.48	5.67
ZnO	24.13	13.63	17.64
$ZnSO_4 7H_2O$	16.67	23.66	17.45

Boluses 118, 134, 138, 142, 143, 146, 147, 148, 150 were composed of the 15 g of the original mix (Section 2:2) plus 16.67% (3 g) of the mineral mix as zinc sulphate heptahydrate as shown by mix A on Table 7 plus 15 g of copper oxide needles. Bolus No. 90 was composed of 15 g of the standard mineral mix (Section 2:2) plus 4 g of zinc sulphate heptahydrate plus 15 g of copper oxide needles. It was coated three times with glass fibre resin (Plastic Padding Ltd.) leaving one exposed face. Boluses 118-150 already mentioned were coated three times with glass fibre resin with both faces exposed.

(ii) Experimental

All boluses were administered in pairs to fistulated cows. Numbers 142-150 were administered to grazing fistulated cows outdoors while the remainder of the boluses (61, 62, 63, 81, 87, 100, 102, 90, 118, 134 and 138) were administered to fistulated cows indoors on a hay and concentrate diet. All boluses were removed and replaced at regular intervals for weighing and examination.

RESULTS AND DISCUSSION

The results of testing the large boluses are

plotted on Figure 7. The common pattern of a large initial weight loss followed subsequently by little change was shown by boluses 61 and 63 which contained sugar and sodium bicarbonate respectively. Bolus 63 lost 80% of its weight within four days and bolus 61 lost 50% of its weight within the same period. Bolus 62 which contained 2 g zinc sulphate and had a 14 mm diameter opening, by contrast, eroded slowly and steadily over the eighty-two day test period, losing on average 0.67 g/d.

The removal of partial amounts of ZnO from the formulation as in boluses 81, 87, 100 and 102 had a marked effect on erosion. These results are plotted on Figure 8. Boluses 81, 87 and 102 had lost most, if not all, of their weight within eight days despite having varying percentages of zinc oxide. Bolus 100 was a little slower, losing most of its weight within eleven days.

There was a marked difference in results when 16.67% zinc sulphate heptahydrate was added to the mineral mix (mix A, Table 7). Figure 9 shows results of indoor testing of boluses 90, 118, 134 and 138. Bolus 90 containing 21% of mineral mix as zinc sulphate with one exposed face was similar in erosion to boluses containing 16.67% with two open faces. The active life of these boluses was 32-40 days which

was close to the original target of thirty days.

Similar boluses, numbers 142-150, tested outdoors have their results recorded in Table 8. In general boluses eroded within 35 ± 5 days at an average daily erosion of 0.667 ± 0.186 g over twenty-nine days. The coefficient of variation was 28%.

TABLE 8. Average daily erosion rates of mineral boluses tested outdoors

Cow Number	Bolus Number	Initial Bolus Weight (g)	Weight After 29 Days (g)	Average Release Rate Per Day (g)
34	142	33.640	18.968	0.506
	143	33.780	13.199	0.710
35	146	33.643	12.999	0.712
	147	33.666	22.090	0.399
36	148	33.612	6.968	0.918
	150	33.287	11.333	0.757

It was proved that zinc sulphate heptahydrate in fact did perform a regulating function when tested in different types of bolus. The large sized bolus

number 62 was particularly impressive when compared with similar sized boluses with different additives, showing that there was a possibility of developing a constantly eroding longer term release pellet. The glass fibre resin coat remained intact almost throughout the test period which was the first prolonged trial of this material.

Smaller 17 mm diameter boluses with two open ends eroded outdoors and indoors within the region of the time interval being sought. Although erosion rate was not strictly predictable on a daily basis, the general disappearance of two boluses per cow within 35 ± 5 days when manufactured entirely by hand with the inconsistencies which this might incur, was considered to be promising. For a mineral supplement, this was considered sufficiently accurate to justify developing this line of investigation further.

FIGURE 7. Erosion pattern of boluses 61, 62 and 63 which were 25 mm diameter. Each contained 30 g mineral and 30 g copper oxide needles plus the additives shown in the figure. The coating material was polyester resin and bolus testing was carried out in fistulated cows.

FIGURE 8. Erosion pattern of 17 mm diameter boluses tested in fistulated cows, containing 50% of the novel formulation mineral mixes B and C and 50% copper oxide needles. Mixes B and C contained varying amounts of zinc oxide and zinc sulphate as detailed in Table 7.

FIG.7 Sugar, sodium bicarbonate or zinc heptahydrate additives

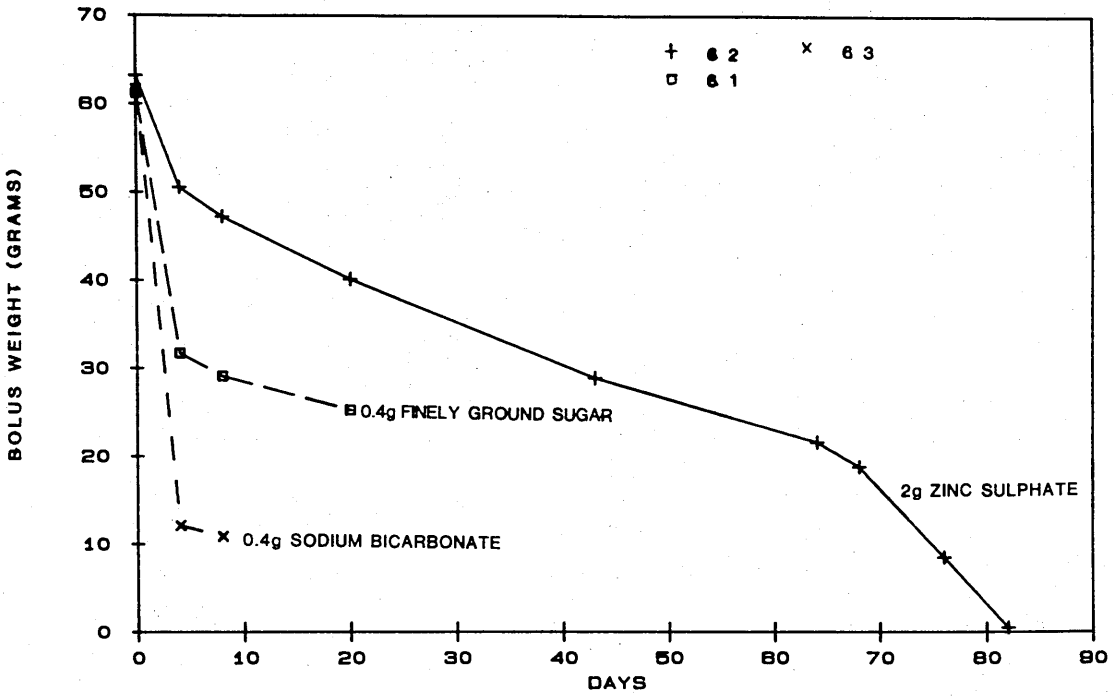


FIG.8 Mineral mixes B and C

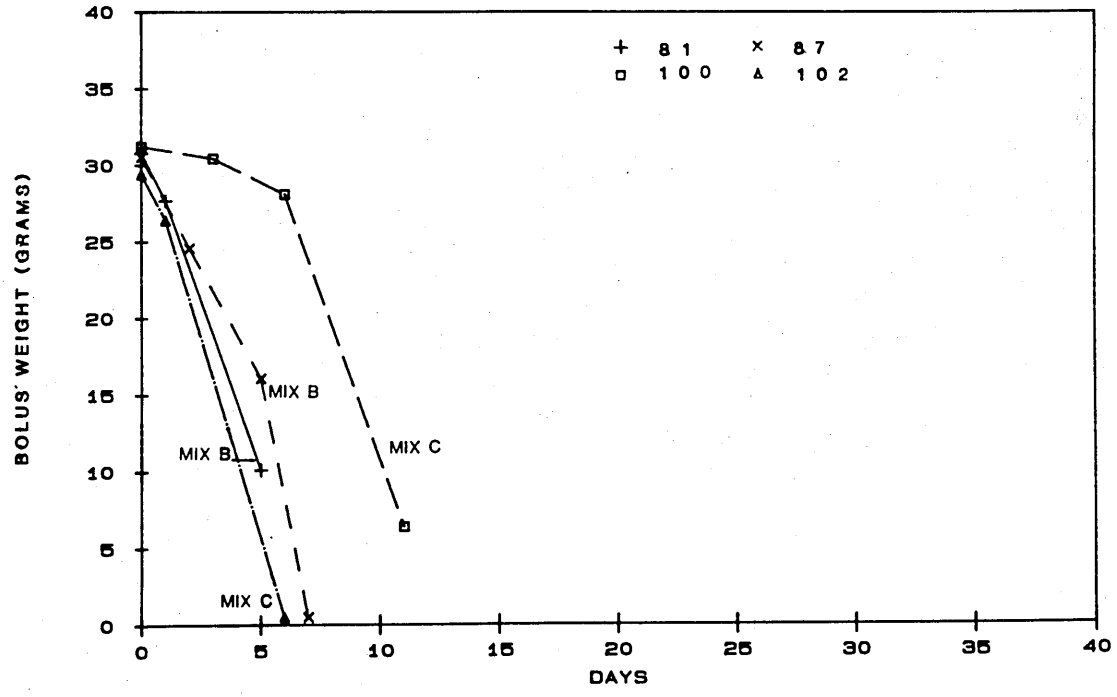
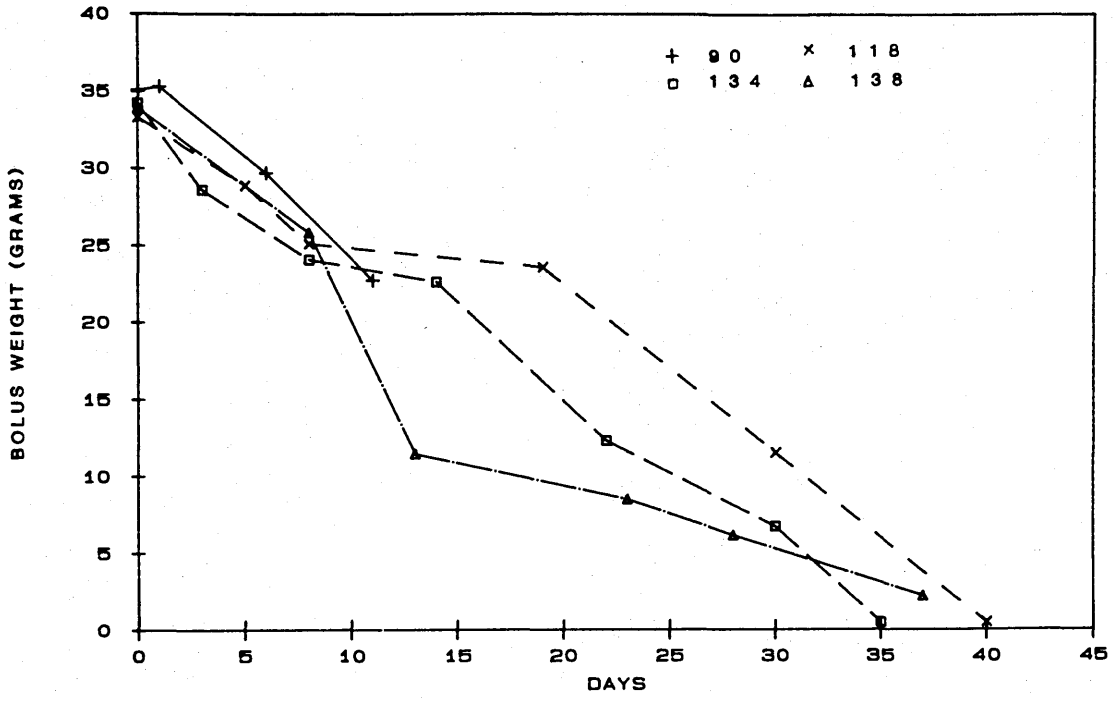


FIGURE 9. Erosion pattern of 17 mm diameter boluses containing 55% of mineral mix A (Table 7) and 15 g of copper oxide needles with two exposed faces and three coats of polyester resin. Testing was carried out in fistulated cows.

FIG.9 Boluses with zinc sulphate (mix A)



6. Alteration of Weighting Medium

INTRODUCTION

In parallel with development work on the mineral salts composition of the bolus, it was decided to experiment with alterations in the weighting agent since it was the largest single component of the bolus. Dense metallic particles were still required in order to impart sufficient specific gravity to the bolus for it to remain lodged in the reticulum, but an alternative to copper oxide needles (2-4 mm) was sought.

The alternatives available were iron filings, iron shot or copper oxide powder (either 50 mesh or 200 mesh size). Iron filings were dismissed because of their tendency to oxidise in the rumen environment. Rusty deposits were found on the exposed surface of previous boluses in which they were included (Section 2:1).

It could be commercially advantageous to produce a bolus for sheep weighted primarily with iron shot. Sheep have the facility for accumulating large amounts of copper which in some cases can lead to fatal copper toxicosis.

In the first instance it was decided to determine the difference between 50 mesh which is a relatively coarse grade of copper oxide powder, and 200 mesh copper oxide powder which is fine and soot-like. A hydrophobic lubricant magnesium stearate was also included in these boluses.

(i) Boluses

A1 and A2 contained 33.5 g of mineral mix, 29.5 g copper oxide powder (50 mesh) magnesium stearate 1.28 g. They were 25 mm in diameter, had one exposed face and were given four coats of glass fibre resin (Plastic Padding Ltd.). Boluses B1 and B2 were identical to the above, except that they contained 200 mesh grade copper oxide powder.

A variety of other boluses of different sizes with different combinations of weighting agents were manufactured as follows:

Bolus 14 was composed of 15 g mineral mix (as Section 2:2) and 15 g of iron shot coated once with two exposed faces.

Bolus 16 contained 15 g of mineral mix (Section 2:2) and 15 g copper oxide powder (50 mesh).

Bolus 29C of 25 mm diameter, contained 30 g mineral mix (Section 2:2) and 30 g copper oxide powder (50 mesh), coated with three coats of glass fibre resin (David's Isopon).

Both copper oxide powder (50 mesh) and iron shot together were used as a weighting medium in bolus 40B. It contained 7.5 g of each weighting agent, and 15 g of mineral (Section 2:2) and was coated three times with glass fibre resin leaving one exposed face.

These and further formulations of boluses 153-161 which were tested are listed in Table 9.

TABLE 9. /

TABLE 9. Component mixes of various boluses

Bolus Number	Diameter of Cylinder (mm)	Number of Exposed Faces	Constitutents of Formulation (g)			
			Mineral Mix	Copper Oxide Needles	Copper Oxide Powder (50 mesh)	Iron Shot
14	17	2	15	-	-	15
16	17	2	15	-	15	-
29C	25	1	30	-	30	
40B	17	1	15	-	7.5	7.5
43B	17	1	15	15	2.5	-
47	17	1	15	15	0.5	-
153	17	2	18*	4	-	10
154	17	1	18*	7	-	7
155	17	2	18*	7	-	7
157	25	1	35*	14	-	15
158	25	1	35	28.5	-	-
161	25	1	32	13	-	14

* containing zinc sulphate

(ii) Experimental

All boluses apart from 29C which was administered alone, were administered in combination with one other bolus to fistulated cows indoors. They were removed, weighed and replaced at intervals during and up to twenty-eight days.

RESULTS AND DISCUSSION

Figure 10 shows the erosion pattern of boluses A1, A2 and B1 and B2 over an eighteen day period. These results indicate that the A boluses containing 200 mesh copper oxide powder had negligible weight losses over the whole test period whereas the B boluses with 50 mesh powder displayed an acceptable erosion pattern over the eighteen days and the predicted life span of these two 70 g boluses would have been twenty and twenty-seven days respectively. In general, it was accepted that the 50 mesh powder would be a suitable alternative to the needle form.

The results of testing the remainder of the bolus formulations are shown on Table 10. In general, the results were fairly erratic and all the boluses had very rapid initial release rates which were unsuitable for further development. The lifespan of the boluses was very short, the longest lasting for twenty-eight days although several boluses were removed prematurely

when it was thought that initial release rates were too rapid to justify further testing. The best result was obtained with the large size bolus 157, containing both copper needles and iron shot weighting so this formulation was borne in mind for future testing. In the latter part of its erosion, bolus 157 settled to a release rate of approximately 1 g per day.

The alteration of the copper oxide needles component obviously has a large effect on the erosion characteristics of the bolus. It is postulated that needles act partly as strengthening rods in the bolus and partly as aids to disintegration. The random protection of the needles, and the air space around them probably acts as an aid to erosion since when they become exposed they will tend to project roughly and randomly from the surface. Copper oxide powder (50 mesh) most probably also has this roughening erosion encouraging effect without the rod strengthening feature, thus boluses containing powder erode more quickly, even when it is present in relatively small amounts. Fine powder does not encourage erosion in any way because of its smoothness, thus boluses tend to remain static. Presumably iron shot has a similar function to needles in that it is particulate material without a strengthening function, which also then, tends to encourage erosion.

The discrepancy in lifespan of boluses 16 and 29C as compared with A1 and A2 was most probably due to variations in the formulation.

Boluses A1 and A2 contained a hydrophobic agent magnesium stearate which would be likely to slow erosion, while 16 and 29C which did not contain this ingredient eroded much more swiftly. An additional factor which may also have been relevant was that numbers 16 and 29C were relatively early bolus examples which, in the opinion of the researcher were not quite so robust and well manufactured as later boluses when techniques for manufacture became more refined.

FIGURE 10. 25 mm diameter boluses tested in fistulated cows of identical composition except that A1 and A2 contained 46% 50 mesh copper oxide powder and B1 and B2 contained 45% 200 mesh copper oxide powder.

FIG. 10 Comparison of 50 mesh and 200 mesh copper oxide powder

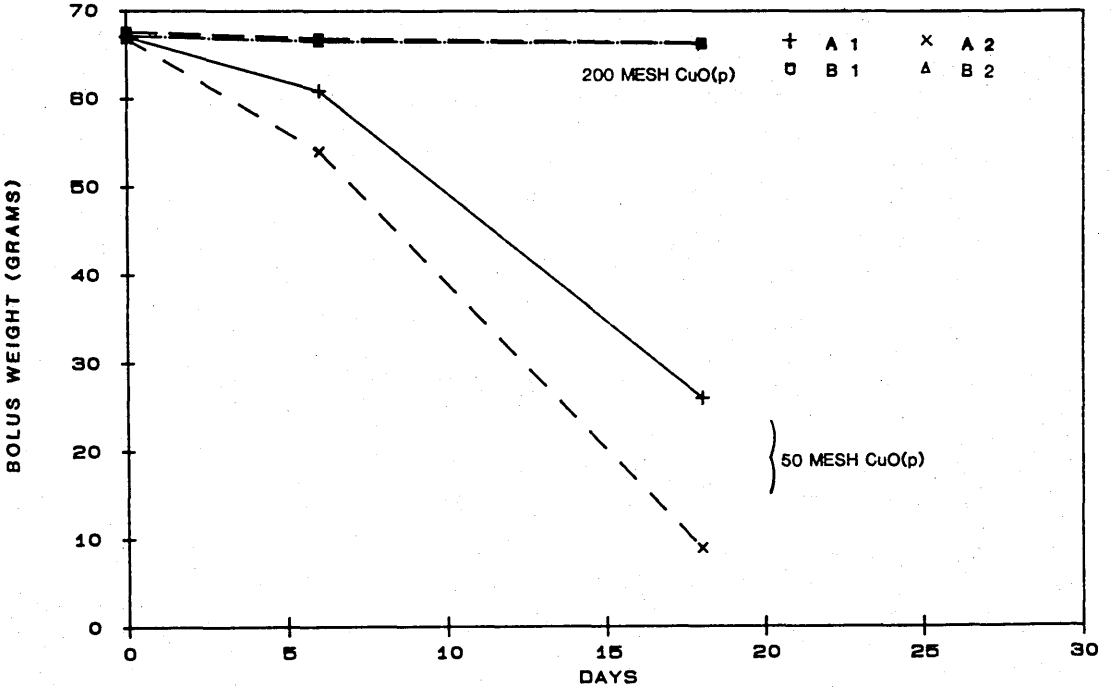


TABLE 10. Erosion data on boluses with different weighting agents

Bolus Number	Average Weight Lost Per Day (g)				Overall Erosion Rate Per Day	Total Life of Bolus (Days)
	0-3	3-10	10-17	17-25		
14	0.676				0.676	NK
16	5.513				5.513	NK
29C	20.10				20.10	3
40B	5.57				6.232	5
43B	3.345				3.345	NK
47	8.01				8.01	NK
153					3.68	9
154	2.59	2.50			2.54	13
155	3.88	0.379			1.725	NK
157	3.99	0.93	0.97	1.033	1.16	28
158	4.96					13
161					3.96	15

NK - not known. Boluses were removed after short test period.

7. Uncoated and Thinly Coated Boluses

INTRODUCTION

On a parallel with research on a coated bolus, uncoated boluses were again retested. There were basically two types, those with no coating at all and those with a thin layer of resin applied which was intended to flake off rapidly within a few days. It was known that tempering of the bolus occurred during exposure to the reticulum environment, causing erosion to gradually lessen when the exposed surface area became less active. It was hoped that if a thin resin coating could be applied which withstood the initial two or three days and then flaked off, the mineral matrix would have hardened sufficiently such that the increased surface area would enable a slow constant release to take place closer to the 1 g per day being sought. Seventeen millimeter diameter and 25 mm diameter boluses were tested, with and without additives intended to improve their erosion.

MATERIALS AND METHODS

(i) Boluses

(a) Uncoated

Boluses numbers 72 and 78 of 17 mm diameter were composed of 15 g of mineral mix (as

Section 2:2) and 15 g copper oxide needles with 1.5 g zinc sulphate heptahydrate. After compaction the boluses were prehardened by first fully immersing them in water and leaving them to dry then repeating the procedure.

(b) Thinly coated boluses

These were made in two sizes. Numbers 68 and 84 were 17 mm diameter and composed of the same mix as above. Bolus 84 was compacted at 1 ton psi instead of the normal 3 tons psi. These were prehardened by dipping once briefly in water, leaving to dry, and applying one thin coat of glass fibre resin (Plastic Padding Ltd.).

Two 25 mm boluses were composed as follows: Numbers 74 and 76 contained 30 g mineral mix (as Section 2:2), 30 g copper oxide needles and 3 g zinc sulphate heptahydrate. They were briefly immersed in water, left to dry and then were coated once thinly with glass fibre resin (Plastic Padding Ltd.). The whole of one face was left exposed in bolus 74 whereas bolus 76 had an 18 mm diameter opening exposed at one end, and an 8 mm diameter opening exposed at the

other end.

(ii) Experimental

Boluses were administered in pairs to fistulated cows indoors on a hay and concentrate diet. They were removed for weighing at regular intervals of up to thirty-five days.

RESULTS AND DISCUSSION

Results of testing the small uncoated and thinly coated boluses is shown on Figure 11. Although the uncoated boluses 72 and 78 were identical and were each administered with at least one other bolus, their release rates were quite different. Bolus 78 in fact broke into two pieces after six days, highlighting the vulnerability of the unsupported mineral core. Bolus 72 having hardened in the rumen environment, became almost totally inert after seven days and lost virtually nothing over the following nine days. The single coat boluses (numbers 68 and 84 - Figure 11) were composed of exactly the same mineral mix as the uncoated examples. Unfortunately the pattern of release rate was very similar despite the initial coating protection.

Number 68 bolus became three quarters uncoated within twenty-four hours but it rapidly lost almost half its weight within six days, and in the following

fortnight the rate of erosion had declined considerably. Another bolus was present with number 68 throughout its trial. Bolus 84 repeated the same formulation, coating, and conditions but the pressure of compaction was reduced to 1 ton in the hope that it might speed the erosion and prevent such a marked plateau. As time passed it became apparent that the same pattern was being followed, perhaps even more obviously than previously.

Bolus 74 on Figure 12 demonstrated that the large sized bolus was also unsuitable as a thinly coated device. When the coat broke off within twenty-four hours, more than 50% of the total weight was lost within ten days. Bolus 76 did not lose its coat as planned, and in fact showed a reasonable steady erosion rate of 0.82 g per day on average over the fourteen days of test.

In view of these results it was thought that much better erosion control was possible with the permanently coated bolus. A high degree of resistance to erosion did build up in the uncoated and thinly coated boluses after a period of up to ten days during which erosion was rapid and erratic. Once the bolus had "hardened" overall through exposure, it became less resistant to weight loss than coated boluses which were constantly having fresh material exposed as the resin coat flaked off during normal abrasion.

FIGURE 11. Erosion rate of boluses in the reticulum of fistulated cows. Boluses 68, 72, 78 and 84 were composed of copper oxide needles, mineral mix and 1.5 g of zinc sulphate heptahydrate and were 17 mm in diameter. Numbers 72 and 78 were uncoated while 68 and 84 had one thin layer of polymer resin applied. Testing was carried out in fistulated cows.

FIGURE 12. Erosion rate of boluses in the reticulum of fistulated cows. Boluses 74 and 76 were tested in the same way as above. They were 25 mm in diameter and contained 30 g mineral mix, 30 g copper oxide needles and 3 g zinc sulphate heptahydrate. One layer of polymer resin was applied.

FIG. 11 17mm diameter uncoated and thinly coated boluses

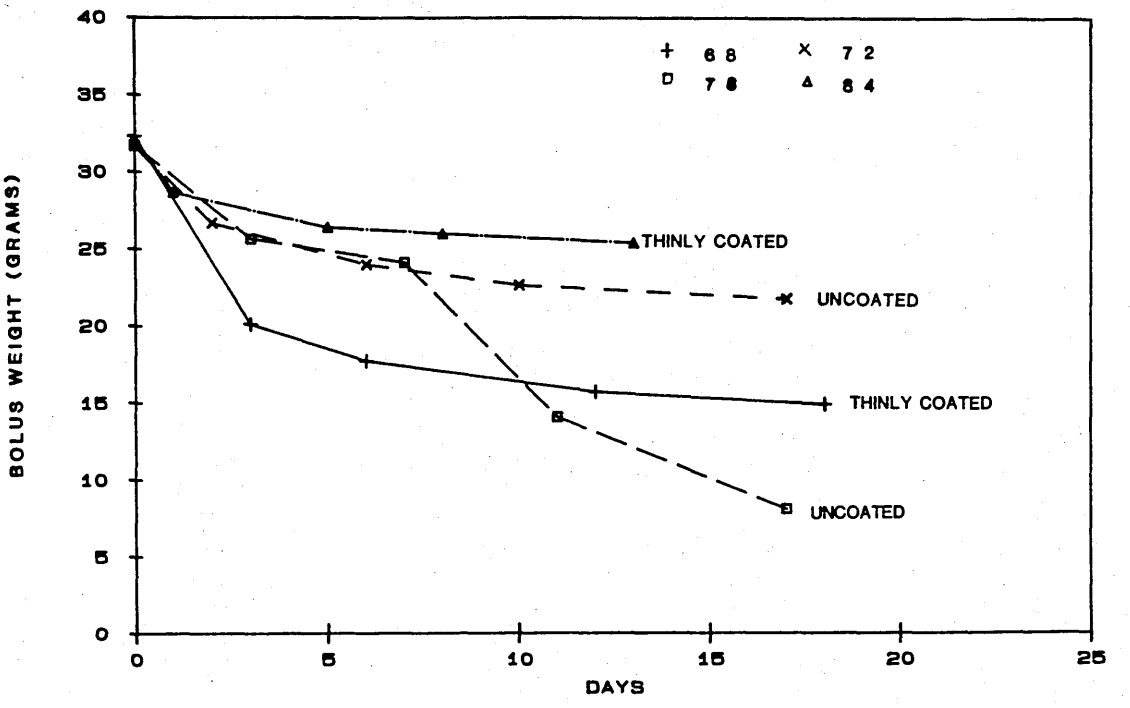
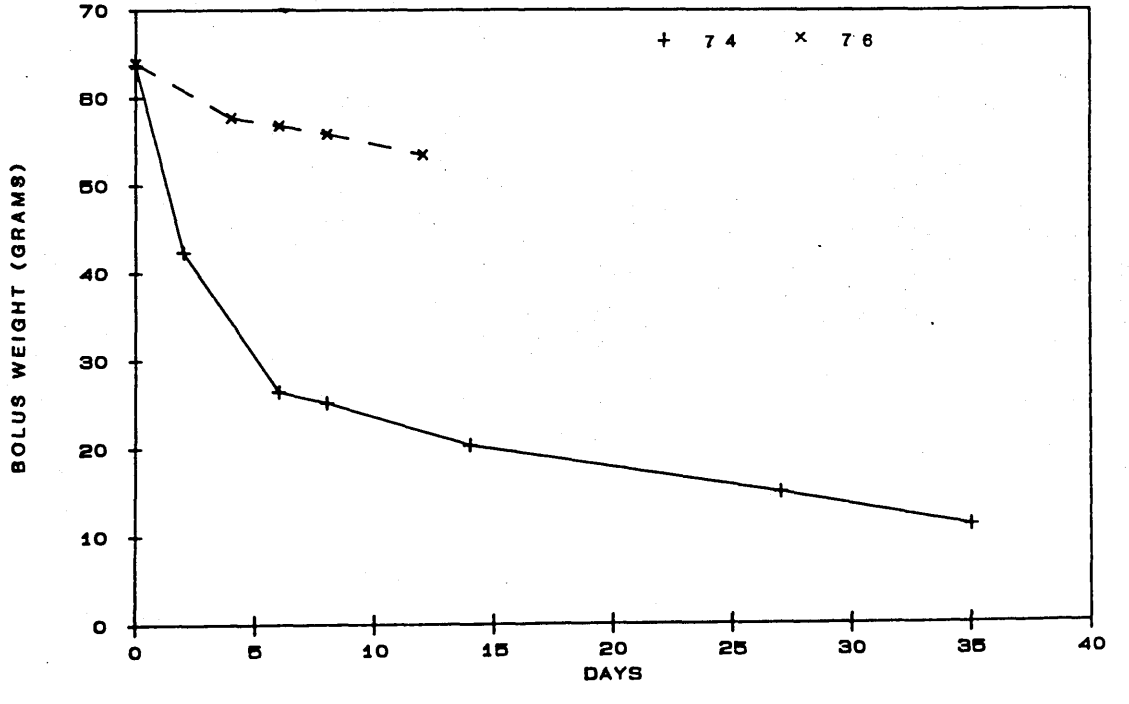


FIG. 12 25mm diameter thinly coated boluses



8. Large Bolus Formula Manipulations

INTRODUCTION

In order to supply the same daily quantity of mineral over a longer time span that the 17 mm or 19 mm diameter boluses, 1" (25 mm) diameter boluses were manufactured. When the 25 mm die was completely filled with loose mineral powder and weighting, then compacted, the total mass was between 60 and 70 g - double the quantity of the 17 mm diameter bolus. The 25 mm bolus had the additional advantages of being more robust and less prone to damage from handling and would fit an existing cattle balling gun for 1" diameter boluses.

The main problem associated with the large bolus was due to the initially large surface area exposed (19.6 cm^2). During the first batch of testing 25 mm diameter boluses, the most successful sustained release over a long period was by bolus 62 (see Figure 7) which eroded gradually over sixty-six days at 0.67 g/d, but theoretically should have lasted for ninety-five days in total. However the surface area initially exposed was only 14 mm in diameter. Although masking part of the exposed face with a thin coat of resin had been fairly successful in large boluses, it was not the sort of process which would be desirable in a

commercial operation and may also be unpredictable in effect. It was intended that the regulation of the erosion rate should proceed entirely via alteration of formulation.

MATERIALS AND METHODS

(i) Boluses

Table 11 shows the mineral constituents of boluses 124, 125, 126, 129, 130, 157, 160 and 167 tested in this section. All boluses apart from number 167 were composed from mineral mix as in Section 2:2 with no zinc sulphate heptahydrate included. Each bolus was coated three times with glass fibre resin leaving one face completely exposed.

(ii) Experimental

Boluses were administered to fistulated cows indoors on a hay and concentrate diet. Where possible they were administered in pairs, but the premature erosion of a partner meant that bolus 125 was present alone during days 4-42 and number 129 was present alone from days 0-10.

RESULTS AND DISCUSSION

Figure 13 shows the results of erosion of boluses 124, 125, 126, 129 and 130. In general a large percentage of the initial weight (about 50%) was lost quickly within seven days. The fact that boluses 125 and 129 were present alone for part of this time has obscured their erosion pattern. Increasing amounts of zinc sulphate appeared to increase the erosion rate, with bolus 126 containing 8 g of zinc sulphate being not recovered after twelve days, compared to number 130 with 6 g of zinc sulphate which eroded in twenty-eight days. Bolus 124 of the same formula as 130 was still eroding at 47 days. The factor which appeared to govern erosion was whether the entire bolus eroded before it "hardened" (bolus 130) or whether it might become tempered after half the bolus was lost in the initial surge (124).

Zinc sulphate was then entirely omitted from the formulation as with bolus 157 and 160. The results of this are shown in Figure 14. Bolus 160 also had additional manganese sulphate, a relatively water insoluble material, added in an effort to restrain erosion. Part of the normal copper oxide needles component in both was replaced with iron shot. However bolus 160 eroded very rapidly and was not recovered after thirteen days' testing. Bolus 157 however eroded in a promising manner losing an average

of 1.12 g/d until its coat began to peel off at around twenty-four days.

Another successful combination of ingredients was subsequently found in bolus 167. It contained zinc oxide, zinc sulphate and manganese sulphate in addition to iron shot and copper oxide needles. This bolus eroded steadily over sixty-one days, releasing approximately 0.4 g/d with a total estimated life of one hundred and sixty-two days. This was longer than the period aimed for with a mineral only bolus, but it could usefully become a carrier for other materials such as anthelmintics or growth promoters, which may be required to be administered over long periods.

Further experimentation with 25 mm diameter boluses was performed as described in a later section on commercially pressed cylinders (Thomson and Capper).

TABLE 11. Formulation used in large bolus studies

Bolus No.	Mineral Mix (No $ZnSO_4 7H_2O$	Cu(n)	Fe shot	Additional $ZnSO_4 7H_2O$	Additional $MnSO_4$	Additional ZnO
124	30 g	30 g		6 g		
125*	30 g	30 g		4 g		
126	30 g	30 g		8 g		
129≠	30 g	30 g		6 g		
130	30 g	30 g		6 g		
157	35 g	14 g	15 g			
160	30 g		28 g		3 g	
167	30 g (incl. $ZnSO_4$)	15 g	14 g	2 g	1 g	3 g

* 125 - present alone days 4-42

≠ 129 - present alone days 0-10

FIGURE 13. The release rate of 25 mm diameter boluses containing varying quantities of zinc sulphate and 30 g of mineral mix and 30 g of copper oxide needles tested in fistulated cows.

FIGURE 14. The release rate of bolus 157 and 167 containing approximately 50% of copper oxide needles and 50% iron shot as weighting plus mineral mix. Bolus 160 contained iron shot alone as weighting (details given on Table 11). Boluses were 25 mm in diameter and were tested in fistulated cows.

FIG.13 25mm diameter boluses of varying formulations

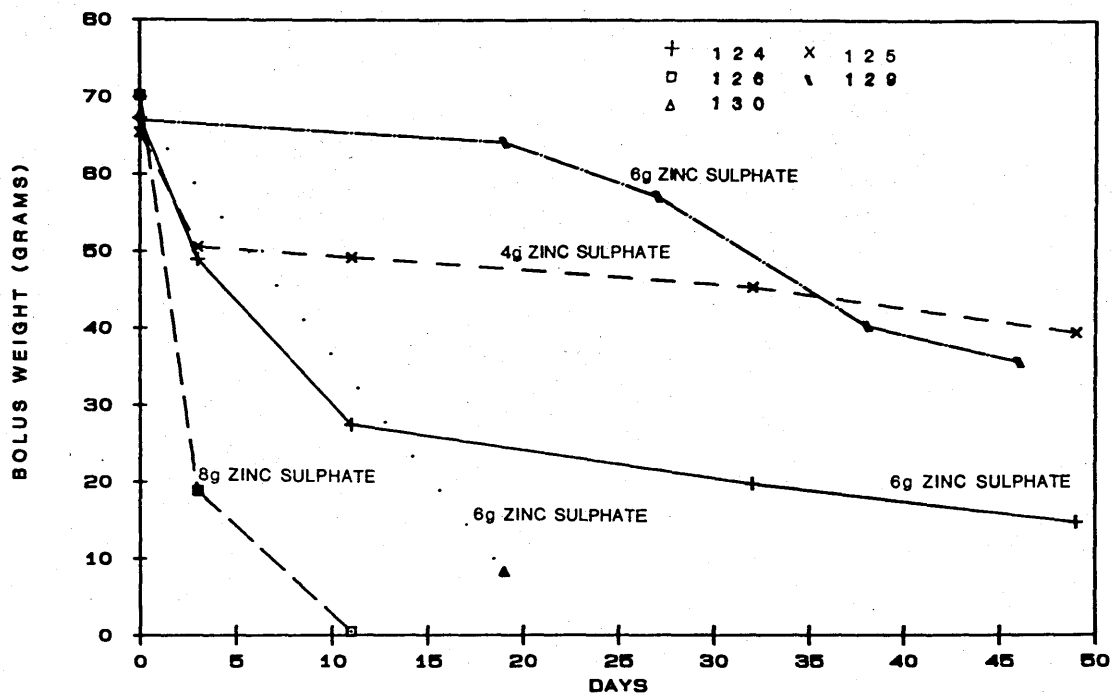
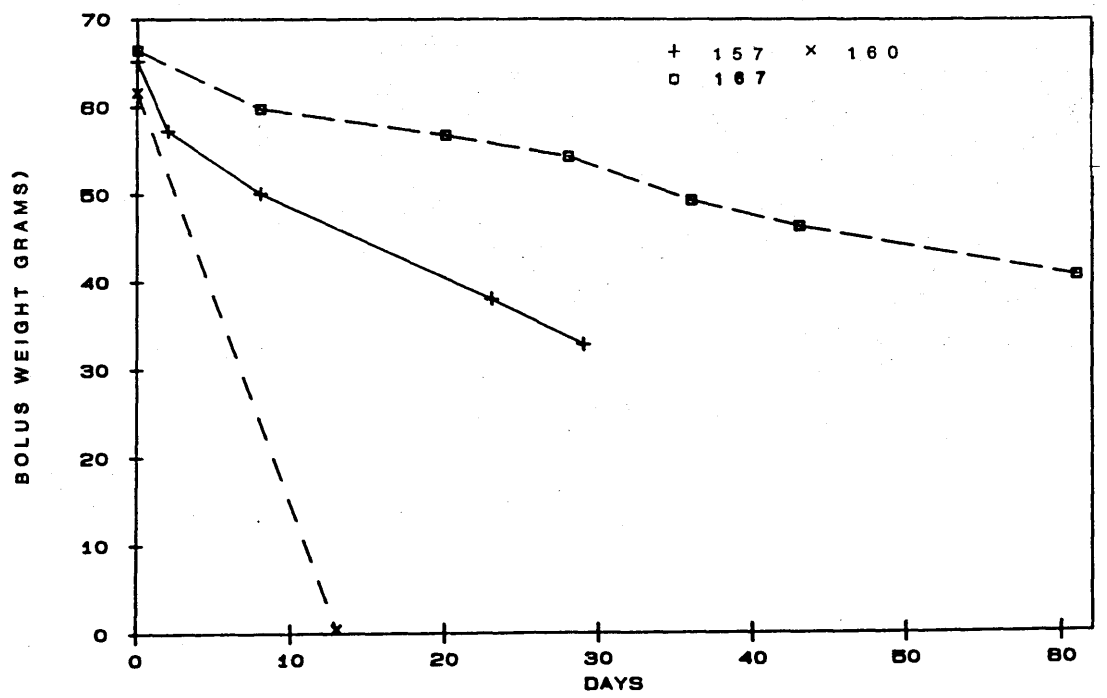


FIG.14 Erosion pattern of 157, 160 & 167



9. Studies in Surface Area Alterations

INTRODUCTION

When it was later proposed that the mineral bolus be developed as a carrier of medicated ingredients, such as anthelmintics, it became desirable to extend the life of the bolus to a target of 90-120 days. The daily release rate of the coated mineral bolus will vary in proportion, though not necessarily directly with the area exposed for dissolution and/or abrasion. The two main variables affecting the exposed surface area are firstly the diameter of the cylinder, which in this study has been varied from 17 mm to 25 mm, and secondly it is affected by whether one end or two ends of the cylinder are left uncoated. This, for any one size of pellet, would double the exposed area. Most studies of small (17 mm) boluses had been carried out with a two open ended pellet, but it was proposed that in this trial one end should be closed off, in order to extend the life of the bolus. Tests were also carried out to determine what degree the exposed surface area of the bolus influenced the release rate.

MATERIALS AND METHODS

(i) Boluses

All boluses tested in this trial were composed

of 55% mineral mix containing 16.67% zinc sulphate (as Section 2:5) and 45% copper oxide needles.

(a) 17 mm

Numbers 183, 185 and D19 were 17 mm in diameter with one open face. Boluses 151 and 152 were of the same size but with two exposed ends.

(b) 19 mm

Boluses 189 and 190 had one exposed face 19 mm in diameter.

(c) 25 mm

Boluses 191 and 192 had a 25 mm diameter exposed surface area.

(ii) Coating

All boluses were coated in identical fashion with Plastic Padding resin (Plastic Padding Ltd.) as described in Section 2:3.

(iii) Experimental

Boluses were administered in pairs to fistulated cows indoors by placing in the reticulum by hand and were removed, examined, washed, weighed and replaced at regular intervals.

RESULTS AND DISCUSSION

(a) Cylinder diameter influence

Boluses of the same basic mineral formulation, but with varying diameters were administered to fistulated cows on the same diet indoors. The results are shown on Table 12. All the boluses in this study had one exposed surface.

In the first three weeks of bolus testing, the release rate of boluses of the same diameter tended to be variable. In general this was associated with the "initial surge" of erosion within the first seven days. Although this was broadly related to bolus diameter, there was some overlap between the various cylinder sizes. Between days 7 and 20 the erosion rate of the different bolus types became more predictable, and this further steadied between days 20 and 35.

The average release rate (mg cm^{-2}) of exposed area was then calculated, using the average daily rates between days 20 and 35, since they were the most steady. Values for 17 mm and 19 mm diameter boluses were similar, but when results from each size were meaned and compared, there was a 7% increase in rate per cm^2 in changing from a 17 mm to a 19 mm diameter bolus, and a 34% increase in rate per cm^2 when a 25 mm diameter bolus was

compared to one of 19 mm diameter.

(b) Number of exposed faces

Table 13 shows the difference in erosion rate per cm^2 of exposed surface when one or two faces of a bolus of identical composition is compared. The typical erosion pattern of a one and two ended bolus is also shown on Figure 15. Bolus 152 had completely eroded within fifty days, and had shown a tendency to accelerate in rate towards the end, whereas bolus 185 eroded at a slow steady pace and had a projected lifespan of approximately two hundred days. This fact is partially represented in the figure for the average release rate per cm^2 of exposed surface area between days 25 and 40 shown on Table 13. The spurt of acceleration between approximately forty and fifty days in two open ended boluses is not represented in the table. In actual lifespan, two open faced boluses therefore erode in approximately one quarter of the time of those with one open face. The actual rate per cm^2 over an average period is up to twice as fast as that of the one ended bolus therefore quadrupling the erosion rate of individual two ended boluses. This observation may be explained if one assumes that each exposed surface is aided partly in erosion by contact with another exposed surface, other than a smooth base.

During normal rumen movement, it is thought that boluses jostle randomly. When two ends of each bolus are exposed, there is a 1:1 chance that contact between ends will be that of two roughened eroding surfaces. If only one end of each bolus is exposed, this chance is 1:4. The presumption is that the base when smooth and coated is less capable of causing abrasion, if it does so at all. This is diagrammatically represented by Diagram 2(a) and 2(b).

The rapid erosion of two ended boluses towards the end of their lifespan is explained by the fact that the coating, which chips off gradually from both ends, may dislodge entirely from a small fragment, causing it to lose its protection and erode more quickly.

The relationship between exposed surface area and erosion rate is not directly proportional, and is thought to be influenced by such factors as degree of contact between abraiding surfaces, and coating integrity. As a rough estimation, opening two ends of the bolus quarters its lifespan, and doubling the initially exposed area on a single ended bolus increases the rate of erosion by 60%.

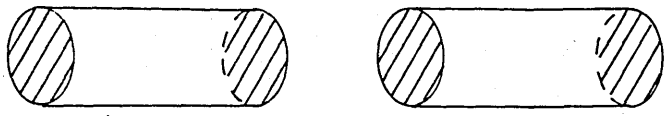
TABLE 12. Release rate of different bolus diameters calculated by mg per day (between d20-35) per cm^2

Bolus Number	Bolus Diameter (mm)	Exposed Surface Area cm^2	Average Release Rate (g/day) d0-7	d7-20	d20-35	d0-40	Average Release Rate ($mg\ cm^{-2}$) Exposed Area Betw 20-35d
183	17	2.27	0.192	0.131	0.155	0.191	68.28
185	17	2.27	0.411	0.112	0.154	0.190	67.84
189	19	2.83	1.344	0.344	0.197	0.549	69.61
190	19	2.83	0.580	0.206	0.219	0.329	77.38
191	25	4.91	1.91	0.746	0.630	0.938	128.31
192	25	4.91	0.718	0.347	0.456	0.469	92.87

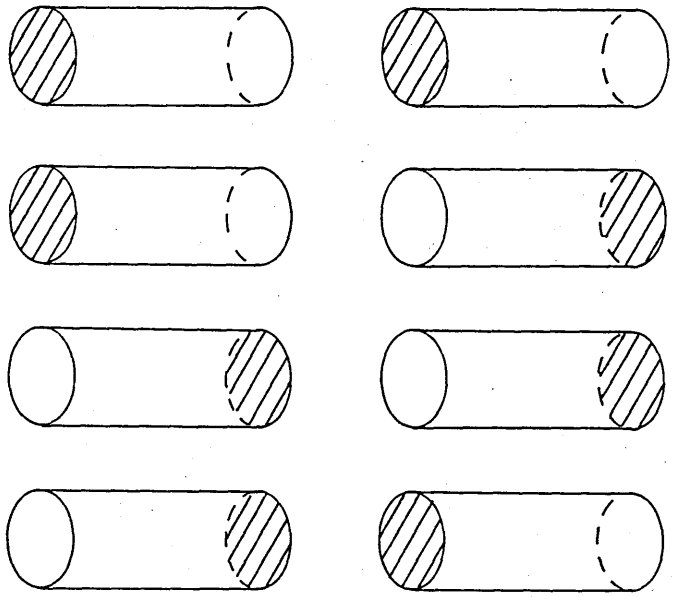
TABLE 13. Comparative release rate of one and two ended boluses ($mg\ cm^{-2}$) of identical size and formula

Bolus Number	Diameter (mm)	No. of Exposed Faces	Average Release Rate (g/d) d0-7	d7-20	d20-35	d25-40	Average Release Rate ($mg\ cm^{-2}$) Exposed Area Betw 25-40d
185	17	1	0.411	0.112	0.154	0.193	85
151	17	2	1.369	0.220	0.300	0.536	118
152	17	2	1.545	0.200	0.362	0.499	109
D19	17	1	0.496	0.199	0.097	0.119	52

DIAGRAM 2(a) and 2(b)



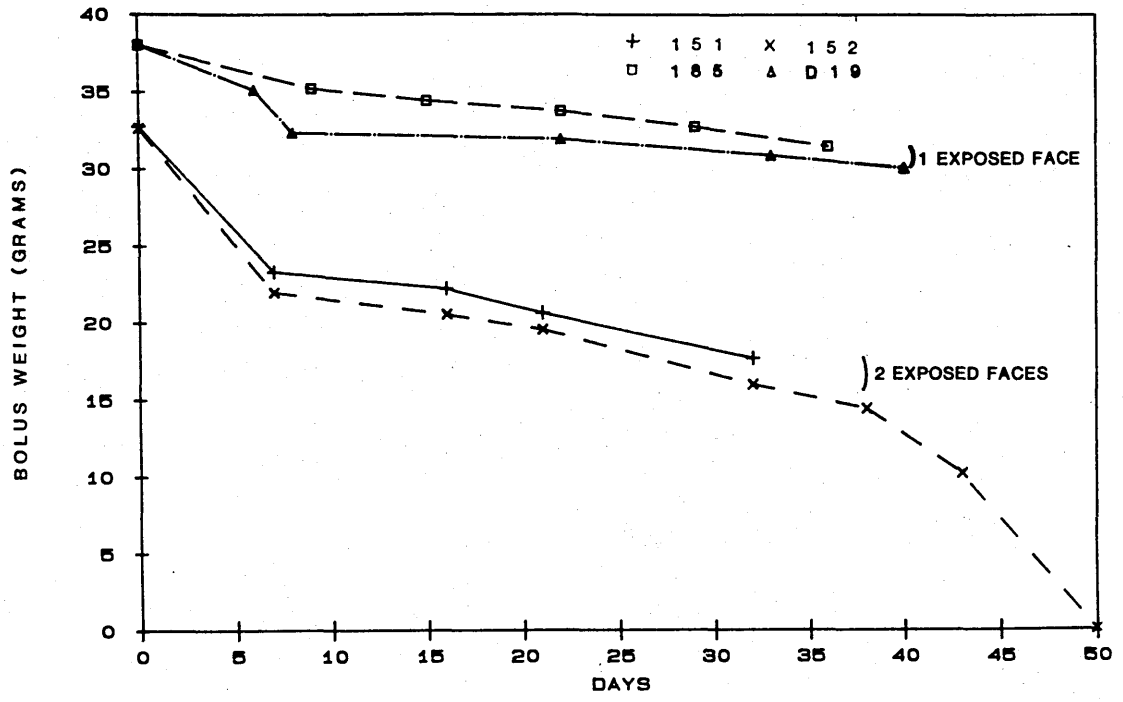
2(a) 2 x 2 ended bolus



2(b) 2 x 1 ended bolus

FIGURE 15. The erosion pattern of boluses 185, D19, 151 and 152 which were composed of 55% mineral mix containing 16.67% zinc sulphate heptahydrate and 45% copper oxide needles. All boluses were 17 mm in diameter. Numbers 151 and 152 had two exposed faces while 185 and D19 had one exposed face. Boluses were tested in fistulated cows.

FIG. 15 One or two open-ended boluses



10. Discussion of Mineral Bolus Development

The development of the trace mineral bolus along the lines described is obviously an imprecise art which relies to a large extent on trying to apply essentially empirical observation. Although attempts have been made to reduce considerable quantities of data to a single mathematical expression, the true number of variables cannot easily be quantified. For example, a bolus may start by eroding well and then gradually change to a slower less suitable erosion pattern, although apparently no conditions have altered. This would be noted as an observation but the precise cause and effect relationship could not be expressed. In a superficial study, examples were chosen of boluses in which the coating had remained intact throughout trials and which had been tested in the company of one other similar bolus. The constituents of such boluses were divided into columns, and statistical tests run by computer to try to evaluate whether any column had a close correlation with release rate. Superficially the closest correlation was between initial surface area exposed and subsequent release rate. Experimental testing has verified this fact and it has been proved that the number of exposed faces, and size of open face have a proportionately large effect on release rate.

Coating integrity is therefore also important as

it obviously controls the area of material exposed. It is shown to exert a more prominent influence on a bolus with good erosion properties, rather than those which are relatively less soluble.

There is some degree of variability in results which cannot always be strictly accounted for. Part of the reason for this has been thought to be due to handmanufacture of the boluses and small individual differences arising from handpressing and coating. Perhaps occasionally there may have been some settlement of ingredients of the mixes. On large scale commercial production, it is assumed that a lot of this variability could be removed through machine processing, granulation of ingredients, machine mixing and pressing.

Individual cow differences may possibly play some part in influencing results. A pairing effect has been noted from time to time where two boluses in an animal dissolve at a rate similar to each other but different from an identical pair in another cow. Yet it has not been possible to quantify this effect or trace any pattern where certain cows cause faster erosion than others. A table was drawn up after three and a half years bolus' testing to try to decide if any one cow appeared to exert an independent influence on erosion rate. It was not possible to draw any clear

conclusions. All fistulated cows have been kept under the same conditions and fed the same diet at any one time, but they are a varied group with respect to age, breed and size. In a field situation, it is likely that a commercial product would be administered to a more uniform group of animals. The degree of fit of the bung and cannula in the fistulated cows may, for example, cause some cows where it is looser, to have a more aerobic rumen environment, and consequently change the pattern of rumination slightly which in turn could differ from animal to animal. It has been observed that when the rumination rate is low or apparently absent, as in occasional isolated cases when a fistulated cow has failed to eat for several days, boluses in the reticulum, no matter what the formulation, tend to gain weight through deposits, rather than lose material. Obviously erosion is therefore dependent on normal rumen function. Small differences in individual animals may in turn alter erosion slightly.

Boluses which contained heptahydrated zinc sulphate in the mineral mix at a rate of 16.67% (Section 2:5) showed a reasonable degree of repeatability and the erosion rate could be manipulated by allowing one or both faces of the bolus to be exposed. Alteration of the diameter of the compressed cylinder also altered the erosion rate in a repeatable fashion. It was anticipated that yet better

uniformity would ensue from commercially manufactured boluses of this formula.

The bolus had now reached a stage where it became necessary to extend its testing into intact cattle and sheep in field studies. At this point an approach was also made to a commercial tabletting firm, Thomson and Capper Ltd., Runcorn, Cheshire, to enquire of the likelihood of producing this bolus on a commercial scale.

SECTION III

1. A Study of Three Different Methods of Mineral Supplementation in Sheep

GENERAL INTRODUCTION

The various methods used to provide supplements of trace minerals in deficiency situations were discussed fully in Section 1. The two most popular methods presently practised in the U.K. for provision of additional minerals outdoors are supplementation via the feed concentrates, and in cases where supplementary feed is not being provided, supplementation through the provision of free access minerals.

Supplementation of minerals via the concentrate ration involves incorporating a balanced mineral formulation into the food at the appropriate concentration, then mixing and pelletting the ration to ensure a well mixed even dispersion. This would then be trough fed on a daily basis. Obviously this is a fairly labour intensive operation which would only be carried out when grazing is sparse over autumn/ winter or when the food requirement is high. Such a method is mainly limited to lowland farms with ready access to grazing areas, and may be impractical for other enterprises. Supplementation via concentrates

is, in reality, a fairly short term remedy for mineral deficiency, and it is not a true solution to the problem of administering minerals.

In recent years, evidence has come to light that trough feeding behaviour in any one apparently similar group of animals, is highly variable. Some individuals consume far in excess of their share, while others eat virtually nothing (Kendall, 1977; McEleney, 1985). Therefore despite apparent provision of adequate levels of supplementation, some particular animals may actually be deficient.

Provision of free access mineral on a self-help basis is a common method of supplementing the diet of animals on hill farms. A bulk quantity of nutritionally balanced material with perhaps salt added to increase palatibility is provided and each animal is expected to consume a sufficient quantity to supplement its diet. The material would normally be placed in a prominent part of the grazing area, perhaps near a water source, so that animals would notice it and take some.

The most common problem associated with this method of supplementation is that some animals fail to consume any mineral, or take very low ineffective levels (Kendall, 1977). It is impossible to ensure

that every animal consumes a sufficient quantity of mineral, and the farmer is therefore left with a significant number of his stock just as susceptible to mineral deficiency as though no supplement had been provided.

For a mineral supplement bolus to be of any great value in practice, it should be shown that it is superior to these more traditional methods. The advantage should be that since each animal is individually treated, there should be no case of deficiency arising in any animal within a certain defined period which ought to correspond to the active life of the bolus.

A trial was therefore set up to assess the level of variation of mineral uptake in a flock of ewes provided with minerals mixed in with the daily trough fed concentrate ration, and then a similar assessment of mineral uptake was planned when ad lib free access powdered mineral supplement was provided. It was anticipated that the variation in these methods of supplementation might be found to be unacceptably wide. An experiment where a similar group of animals were administered with a prototype intraruminal mineral bolus was then carried out to compare the results of this trial with those of the two more traditional methods. It was appreciated that further development

of the bolus was required to prolong its life from the expected six to eight weeks if it was to be of practical application.

The following experiments comprised a study of three methods of mineral supplementation in sheep. Experiments 1 and 2 dealt with two common methods of administering minerals, compared with the third which was concerned with intraruminal supplementation. Comparisons have been drawn in the general discussion between these three methods with regard to efficacy and labour involved. Information on the role of faecal copper output as a measure of intake was also investigated in Experiments 1 and 2 and the results applied to the intraruminal supplementation trial.

(a) Free Access Mineral

INTRODUCTION

This experiment studied free access supplementation. Free access supplementation is commonly practised in sheep farming. Normally sheep would be provided with a bulk quantity of mineral mix, a mineral block, or a mineral fortified feedblock in some strategic area of their grazing, and be expected each to consume a sufficient quantity to satisfy their daily requirements. It has been shown in the past by several workers (Kendall, 1977; Ducker et al, 1981) that although excessive quantities of feed supplement may be provided, up to 19% of sheep will not partake of their share at all irrespective of the presence of a physiological necessity. Individual sheep may also consume vastly differing quantities which could vary from day to day. Daily group intake may be erratic and be related to extrinsic factors such as temperature and rainfall (Ducker and Fraser, 1975).

MATERIALS AND METHODS

(a) Sheep.

Thirty-two Dorset Horn ewes of varying age were maintained at grass with a Dorset Horn Tup on approximately 3.4 ha pasture in late autumn/early winter in Garscube Estate, Bearsden. The ewes

were blood sampled by the Vacutainer method from the jugular vein prior to the provision of supplement, and at seven and fourteen days after the supplement was first available. Faecal grab samples were collected at the same time. Blood was analysed for copper by the zinc displacement method, and faeces dried and analysed for copper and chromium. As a supplement to grazing, sheep were provided with 0.5 kg per head of ground barley daily, trough fed.

(b) Mineral supplement

"Sheepmin", a powdered mineral supplement, was supplied by Agrimin Ltd., Grimsby. As with all sheep foodstuffs, the copper content is normally nil. However copper was deliberately added to the mix for the purposes of this experiment, at the rate of 1500 mg/kg.

Although it is relatively easy to measure the total intake of a feed supplementation on a free access basis, this gives no indication of the individual uptake per animal. One effective way is to use a dietary marker or "tracer". The one most commonly used is chromium, added as chromic oxide, which is known to be completely undigested and passes through 100% in the faeces (Stevenson and De Langen, 1960; Curran,

Leaver and Weston, 1967). If chromic oxide is then added to a free access supplement, and faecal samples are taken from animals eating the supplement, faecal chromium gives a direct measure of the relative quantity of supplement consumed in the period prior to sampling.

Chromic oxide is indigestible and 100% is excreted in the faeces. Therefore the relative consumption of "Sheepmin" by each ewe could be estimated when chromic oxide was added in a known quantity.

"Sheepmin" was composed of the following ingredients:

6.25% Mg	14.3% Ca	vit. A = 300,000iu/kg
1500 mg/kg Cu	3.9% P	vit. D = 60,000iu/kg
10 mg/kg Se	25% NaCl	Chromic oxide = 48 g/kg
200 mg/kg Co	200 mg/kg I	5000 mg/kg Fe
1000 mg/kg Mn	1600 mg/kg Zn	375 iu/kg vit. E.

An appropriate daily amount of the mineral for sheep was recommended by the manufacturers to be between 5-10 g/day. A fresh 5 kg batch of the mineral and chromic oxide mix was provided daily in a zinc tub under a shelter in a central part of the grazing area. Residual quantities were removed each day, dried in an 80^{o}C oven, and the

amount of supplement collectively consumed on the previous day was calculated. Weather conditions were noted daily in order to investigate whether any change in conditions related to variations in the mineral intake of the ewes.

RESULTS AND DISCUSSION

The daily variation over fourteen days in group consumption of "Sheepmin" is plotted on Figure 16. The mean daily consumption of mineral over that period was 0.998 $\pm$ 0.365 kg per 33 sheep, and the coefficient of variation was 37%. During the first week, intake was slightly lower than during the second, that is 0.935 kg per day as compared with 1.12 kg. However, the coefficient of variation in the second week was 39% as compared to 36% on the first week. Some correlation apparently existed between weather conditions and supplement consumption. In general, when the weather was mild, and rainfall light to average, more supplement was eaten. Very cold or very wet weather was apparently related to lesser intake.

The figures for faecal chromium output, faecal copper and blood copper levels are shown in Table 14. The mean faecal chromium output over the first week was 1.33 $\pm$ 1.074 mg Cr/kg faeces DM, which has a coefficient of variation of 81%. For faecal copper

levels over the same period the mean was 0.098 $\pm$ 0.052 g Cu/kg faeces DM. This has a 54% coefficient of variation. Blood copper levels were within the normal range during weeks 1 and 2. The mean values were 0.863 $\pm$ 0.0971 mg Cu/kg blood and 0.781 $\pm$ 0.095 mg Cu/kg blood for weeks 1 and 2 respectively. During the second week, the mean faecal chromium output had increased to 1.715 $\pm$ 1.063 mg Cr/kg which had a 62% coefficient of variation. For the same period, the faecal copper output was 0.112 $\pm$ 0.052 g Cu/kg, with a 47% coefficient of variation. There was a statistical correlation significant at $p < 0.001$ for a positive relationship between faecal chromium output and faecal copper output from day 0-14 which is plotted graphically on Figures 19 and 20 for day 0-7 and 7-14 respectively.

The daily mean consumption of mineral by all the ewes is plotted on Figure 16, and the faecal chromium output by individual animals at the end of the one and two week periods is plotted in Figures 17 and 18. The mean individual mineral consumption for the first week was 28.37 g which implies that the amount of supplement consumed by individual sheep varied from 1.96 g to 101 g. During the second week the variation in consumption was between 1.4 g and 96.4 g. The coefficient of variation for collective consumption over the test period was 36.6%.

It appeared from studying individual faecal chromium values that all sheep ate at least a little supplement over the two week period, and different sheep each week consumed the lowest amount. The tendency to overconsume, however, was more restricted, tending to be associated with individual sheep. For example, sheep No. 5 consumed an average of 48 g per day the first week and 59.5 g the second week. An appropriate amount of mineral was considered to be between 5 and 10 g per day.

The close correlation between faecal chromium output and faecal copper output proves that there was a directly proportional relationship between copper intake and output. Therefore faecal copper analysis gives as accurate a guide to the individual intake of high copper supplement as does faecal chromium. This can be used later in assessment of the performance of the intraruminal bolus. A comparison of the amount of copper excreted can therefore give an indication of the quantity of copper released by the bolus, which in turn provides some indication of the relative degree of erosion of boluses in different animals.

Further discussion of results will be made in conjunction with the other two experiments in this section.

TABLE 14. Faecal chromium and copper levels and blood copper values of ewes given ad lib free access to mineral supplement

Ewe No.	Faecal Chromium (mg/kg) Wk. 1	Faecal Chromium (mg/kg) Wk. 2	Faecal Copper (g/kg) Wk. 1	Faecal Copper (g/kg) Wk. 2	Blood Copper (mg/l) Wk. 1	Blood Copper (mg/l) Wk. 2
1	0.765	0.579	0.075	0.049	0.69	0.73
2	1.196	0.451	0.095	0.050	0.81	0.80
3	0.800	0.075	0.068	0.033	0.06	0.95
4	NS	0.796	NS	0.065	0.85	0.75
5	2.244	3.175	0.160	0.178	0.72	0.63
6	1.562	NS	0.126	NS	1.12	0.76
7	NS	0.907	NS	0.057	1.03	0.88
8	0.867	2.223	0.074	0.141	0.86	0.80
9	0.240	2.333	0.040	0.144	0.93	0.67
10	2.385	1.628	0.162	0.094	0.93	0.80
11	3.748	2.004	0.182	0.136	0.80	0.76
12	1.931	0.636	0.121	0.047	0.83	0.76
13	NS	5.150	NS	0.235	0.91	0.81
14	2.122	1.742	0.131	0.113	0.73	0.63
15	NS	1.848	NS	0.216	0.87	0.64
16	0.598	NS	0.059	NS	0.85	0.80
17	0.998	2.379	0.081	0.146	0.76	0.73
18	0.145	2.103	0.035	0.119	0.86	0.86
19	1.568	1.682	0.109	0.110	0.86	0.75
20	NS	0.465	NS	0.053	0.86	0.83
21	0.092	0.877	0.029	0.062	0.98	0.87
22	0.800	NS	0.070	NS	0.83	0.80
23	NS	NS	NS	NS	0.86	0.74
24	0.505	1.587	0.060	0.110	0.83	0.76
25	1.121	2.446	0.086	0.151	0.83	0.81
26	0.876	0.639	0.074	0.068	1.00	0.95
27	1.432	2.723	0.105	0.161	0.80	0.71
28	4.732	2.639	0.264	0.155	NS	0.67
29	0.352	1.864	0.039	0.119	0.83	0.81
30	1.579	1.705	0.119	0.105	0.78	0.68
31	1.286	1.662	0.104	0.107	0.80	1.06
32	0.623	NS	0.073	NS	0.87	0.78
Mean	1.33	1.715	0.098	0.112	0.863	0.781
S.D.	±1.074	1.063	0.052	0.052	0.0971	0.095
Coeff of Variation	81%	62%	54%	47%		

FIGURE 16. The amount of mineral supplement collectively consumed (kg fresh weight) by 33 sheep per day when offered on an ad lib basis over a period of 14 days.

FIG. 16 Mineral supplement consumption per day

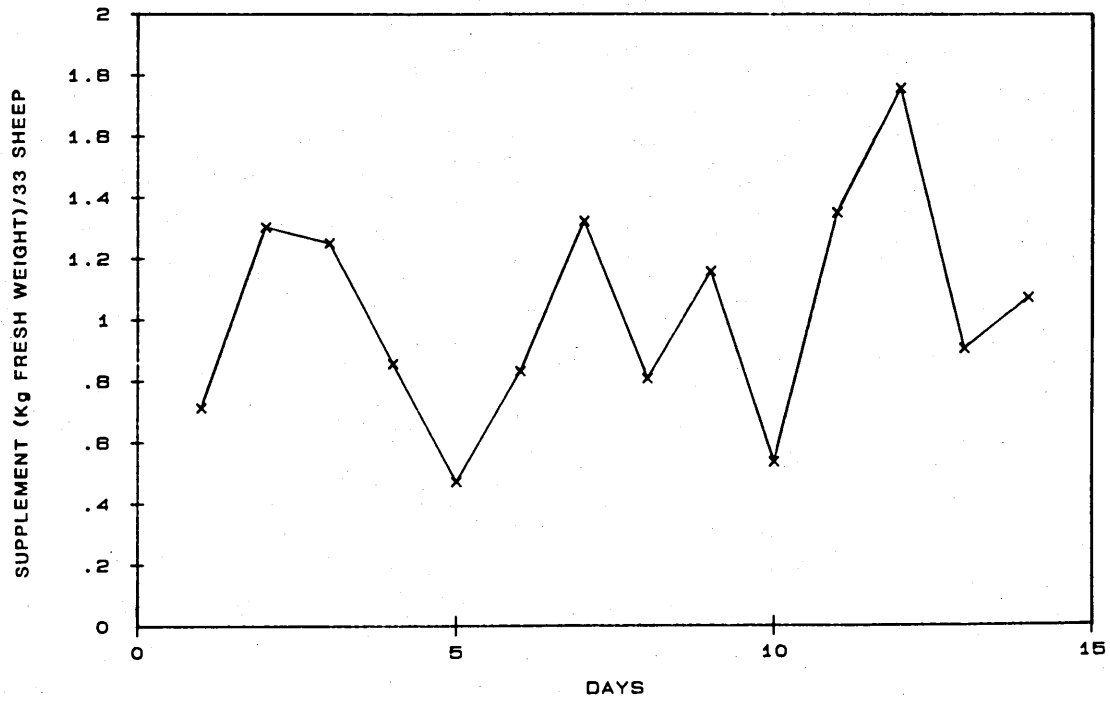


FIGURE 17. Chromium excretion (g)/kg faeces DM per sheep on day 7 after ad lib mineral supplement was provided for 1 week.

FIGURE 18. Chromium excretion (g)/kg faeces DM per sheep on day 14 after ad lib mineral supplement was provided for 2 weeks.

FIG.17 Chromium excretion on provision of ad-lib mineral supplement (Wk. 1)

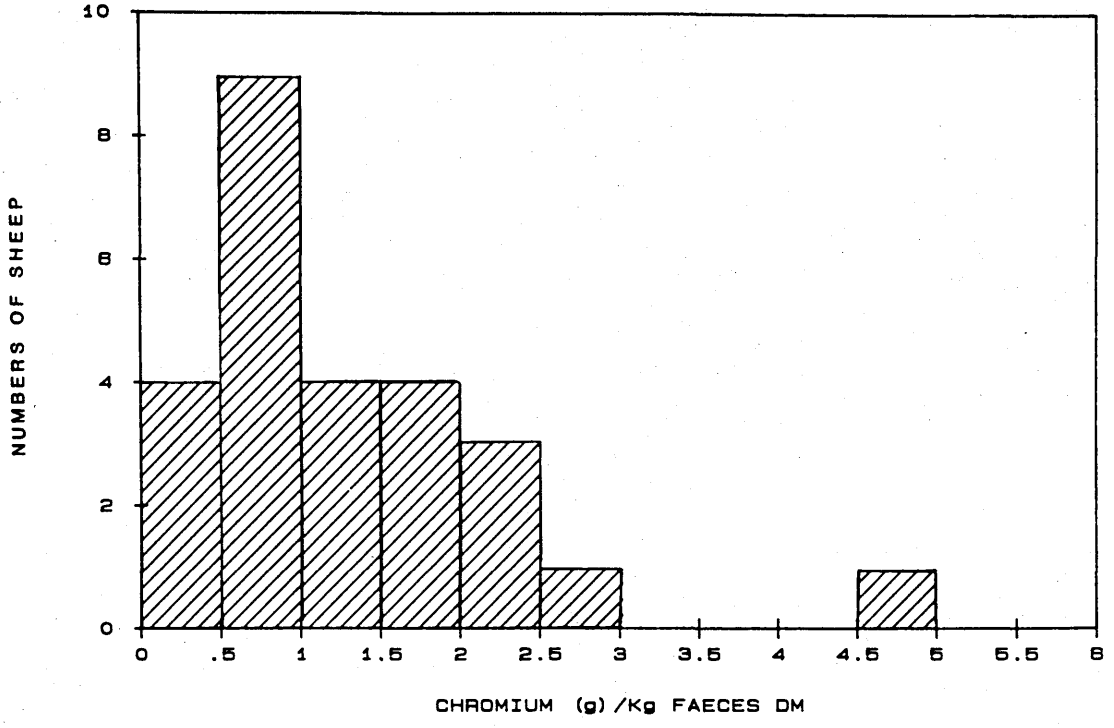


FIG. 18 (Wk. 2)

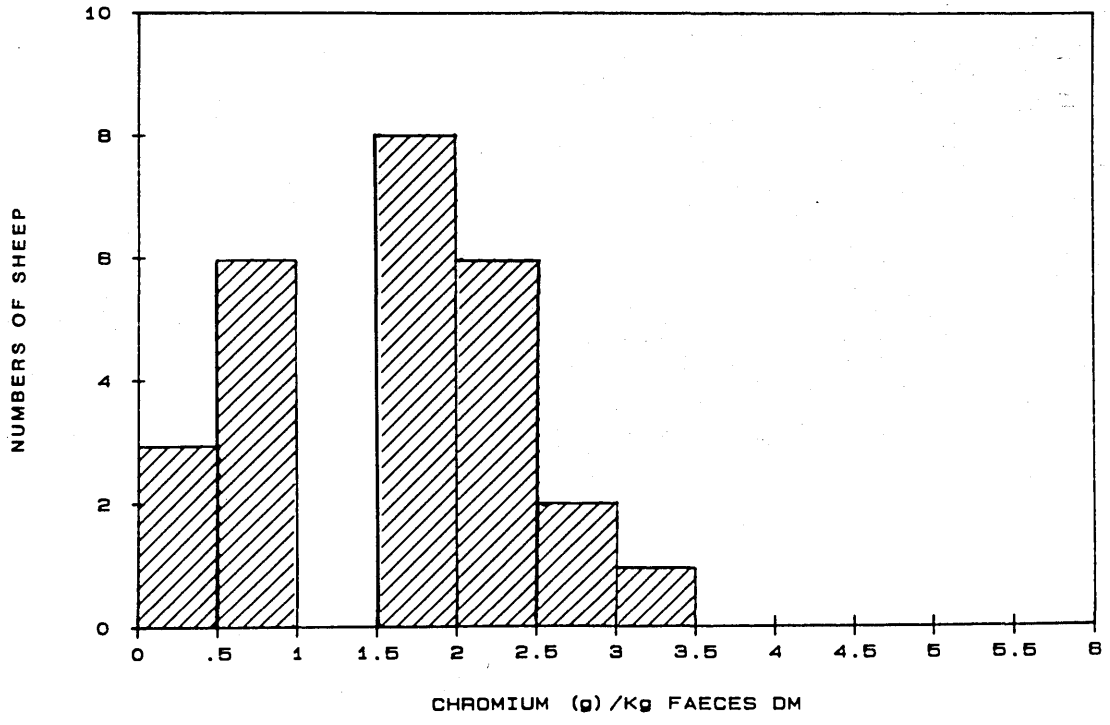


FIGURE 19. Copper content of faeces vs chromium content of faeces on day 7 after 1 weeks provision of ad lib mineral supplement.

$$y = 0.034 + 0.04787x$$

Correlation between x and y was significant at $p < 0.001$.

FIGURE 20. Copper content of faeces vs chromium content of faeces on day 14 after 2 weeks provision of ad lib mineral supplement.

$$y = 0.0349 + 0.0449x$$

Correlation between x and y was significant at $p < 0.001$.

FIG. 19 Copper in faeces vs chromium in faeces (Wk. 1)

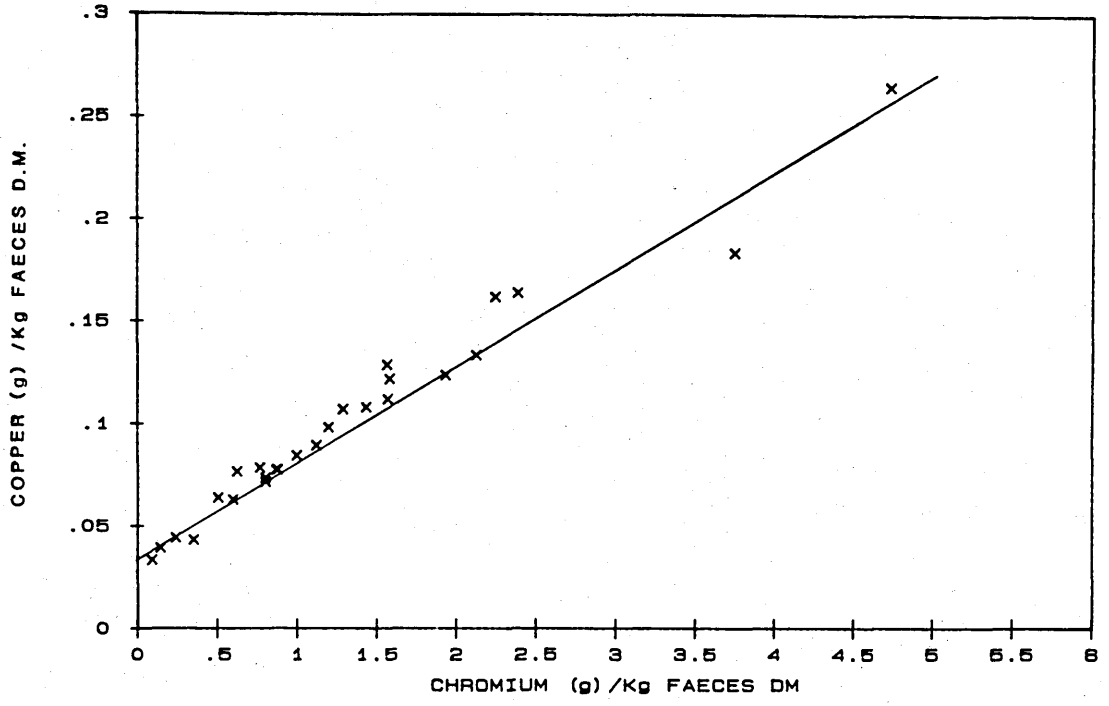
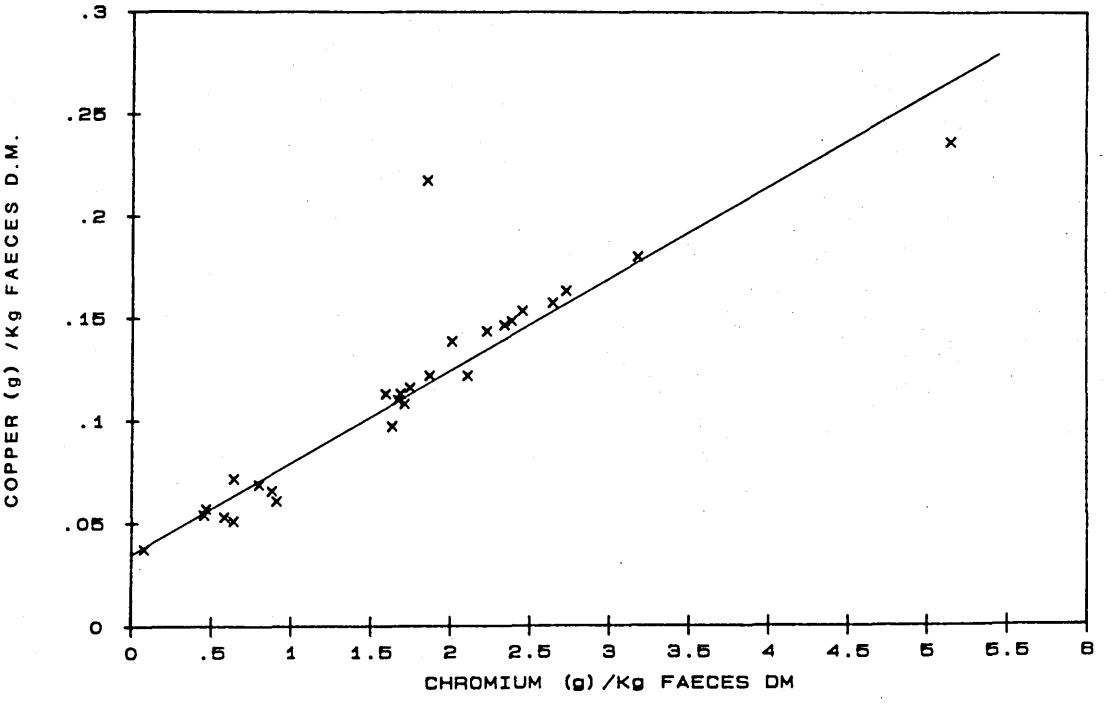


FIG. 20 (Wk. 2)



(b) Mineral Supplement Added to Concentrate Ration

INTRODUCTION

The second experiment was designed to test the variation in intake involved in another common method of mineral administration where supplementary feeding is practised. This method involves mixing the minerals into the normal feed and trough feeding the bulk mix at grass on a daily basis. The same quantity of food is supplied for each sheep and the assumption is that each consumes its share and in so doing satisfies its daily mineral requirement. Minerals and vitamins are mixed into the food in a measured dose. Observers have noted (for example Kendall, 1977) however that individual sheep behave differently while feeding and some ewes may consistently eat more or less than their share.

MATERIALS AND METHODS

(a) Sheep

Thirty-two Dorset Horn ewes of variable age were at grass on approximately 2.4 ha in late autumn with a Dorset Horn tup in Garscube Estate, Bearsden. All apart from the tup were blood sampled by the Vacutainer method from the jugular vein prior to the commencement of the

experiment and thereafter at seven days and fourteen days. Faecal grab samples were collected at the same time. Blood was analysed for copper by the zinc displacement method, and faeces dried and analysed for copper and chromium.

(b) Feeding

Mineral supplement of "Sheepmin" brand (Agrimin Ltd., Grimsby) was mixed in at a rate of 2.5% to 184 kg barley, 10 kg molasses and 240 g Cr_2O_3 and then cubed. The mineral content of "Sheepmin" was identical to that of the previous experiment including the high copper levels. The amount of copper and chromium supplied in the feed was as follows:

Chromium = 1.02 g Cr/kg food mix DM

Copper = 465 mg Cu/kg food mix DM

At the same time each day, the sheep were trough fed with 8.25 kg of barley mixture, which is equivalent to 0.25 kg each, containing 6.25 g per head of mineral supplement which was the recommended dose level.

RESULTS AND DISCUSSION

Table 15 lists the results of faecal chromium

analyses of the samples collected on day 7 and day 14. Also shown are the results of copper analyses of blood and faeces which were collected at the same time. By calculation from the faecal chromium outputs, it was possible to estimate how much food was consumed per animal on average, and these estimated feed consumption figures are listed in Table 16. Figures 21 and 22 show individual feed estimates in histogram form for days 7 and 14 respectively. The mean amount of concentrate consumed in the first week was 0.265 $\pm$ 0.1662 kg. This has a coefficient variation of 63%. The spread in values ranged from two animals which consumed less than 50 g on average, to one individual which consumed an average 815 g. During the second week the coefficient of variation had reduced to 35%, the mean consumption being 0.2502 $\pm$ 0.0885 kg. However the range in intake was still relatively broad at between 76 and 427 g on average. Grass consumption during the first week was estimated at 1.25 kg DM grass daily and during the second week this figure was estimated to be 0.98 kg DM grass daily. Reference to the individual feed intake figures for the two measuring periods (Table 16) indicates that there was a tendency for the same individuals to consume the greatest and least amounts of food in the two different periods. For example, ewe numbers 5 and 27 were amongst the highest consumers both weeks and numbers 23 and 9 had less than average consumption over the

two week period.

Blood copper analysis results shown on Table 15 gave a mean level of 0.848 $\pm$ 0.089 mg/l after week 1 and 0.837 $\pm$ 0.113 after week 2. These did not vary significantly from the mean of 0.844 $\pm$ 0.0879 mg/l calculated from samples prior to experimentation.

Faecal copper levels were an average 0.491 $\pm$ 0.0126 g/kg after one week which was significantly different ($p < 0.001$) from control values of 0.0305 $\pm$ 0.0038 g/kg. There was no significant difference for values between day 7 and 14. The mean value for day 14 was 0.0522 $\pm$ 0.0076 g/kg.

Faecal copper output versus faecal chromium output at day 7 is plotted on Figure 23. The correlation between these two measurements was significant at $p < 0.005$. During the second week this correlation was significant at $p < 0.001$, and this is plotted on Figure 24.

Further discussion of results is carried out in conjunction with the two other experiments in this section and is given at the end of the chapter.

TABLE 15. Faecal chromium, faecal copper and blood copper levels over a 2 week period in a group of ewes trough fed concentrates a mineral supplement

Ewe No.	Chromium (mg/kg) DM Wk. 1	Chromium (mg/kg) DM Wk. 2	Faecal Copper (g/kg) Wk. 1	Faecal Copper (g/kg) Wk. 2	Blood Copper (mg/l) Wk. 1	Blood Copper (mg/l) Wk. 2
1	0.211	0.302	0.043	0.062	0.86	0.77
2	0.250	0.200	0.041	0.042	0.82	0.84
3	0.197	NS	0.040	NS	1.03	1.18
4	0.197	0.438	0.036	0.050	0.84	0.84
5	0.548	0.676	0.057	0.071	0.73	0.70
6	0.200	0.444	0.037	0.057	0.93	0.86
7	0.278	0.435	NS	0.057	0.91	0.93
8	0.159	0.238	0.048	0.045	0.96	0.85
9	0.165	0.111	0.066	0.035	0.75	0.65
10	0.286	0.459	0.049	0.060	0.92	0.89
11	0.182	0.287	0.041	0.050	0.95	0.99
12	0.251	0.351	0.048	0.053	0.75	0.83
13	0.248	0.328	0.050	0.045	0.86	0.86
14	0.037	0.337	0.034	0.053	0.71	0.71
15	0.231	0.350	0.097	0.058	0.66	0.62
16	0.170	NS	0.049	NS	0.92	0.85
17	0.100	0.300	0.041	0.053	0.82	0.63
18	0.260	0.380	0.044	0.048	0.85	0.90
19	0.619	NS	0.052	NS	0.80	0.86
20	0.252	0.346	0.044	0.048	0.99	0.97
21	0.166	NS	0.048	0.039	0.94	0.95
22	0.261	0.267	0.046	0.050	0.84	0.89
23	0.033	0.253	0.048	0.045	0.84	0.86
24	0.630	0.423	0.073	0.060	0.82	0.81
25	0.271	0.318	0.053	0.054	0.84	0.84
26	0.270	0.278	0.043	0.047	0.97	0.90
27	0.867	0.623	0.061	0.057	0.85	0.86
28	0.225	0.360	0.037	0.052	0.68	0.70
29	0.532	0.577	0.057	0.058	0.82	0.82
30	0.281	0.456	0.044	0.057	0.80	0.73
31	0.319	0.499	0.042	0.056	0.82	0.84
32	0.325	NS	0.053	NS	0.85	0.85

TABLE 16. Food intake of individual ewes over experimental period

Ewe No.	Food Intake Week 1 kg	Food Intake Week 2 kg
1	0.198	0.207
2	0.233	0.137
3	0.185	NS
4	0.185	0.300
5	0.515	0.463
6	0.188	0.304
7	0.261	0.298
8	0.150	0.163
9	0.155	0.076
10	0.269	0.314
11	0.178	0.196
12	0.236	0.241
13	0.233	0.225
14	0.035	0.231
15	0.217	0.240
16	0.159	NS
17	0.094	0.205
18	0.244	0.260
19	0.582	NS
20	0.237	0.237
21	0.156	0.132
22	0.245	0.183
23	0.031	0.173
24	0.592	0.290
25	0.254	0.218
26	0.254	0.190
27	0.815	0.427
28	0.212	0.247
29	0.500	0.395
30	0.264	0.312
31	0.300	0.342
32	0.306	NS
	$x = 0.265$	$x = 0.250$

FIGURE 21. Barley cube plus mineral supplement (FW) consumption average per sheep per day after week 1.

FIGURE 22. Barley cube plus mineral supplement (FW) consumption average per sheep per day after week 2.

FIG.21 Barley cube consumption per sheep/day (Wk. 1)

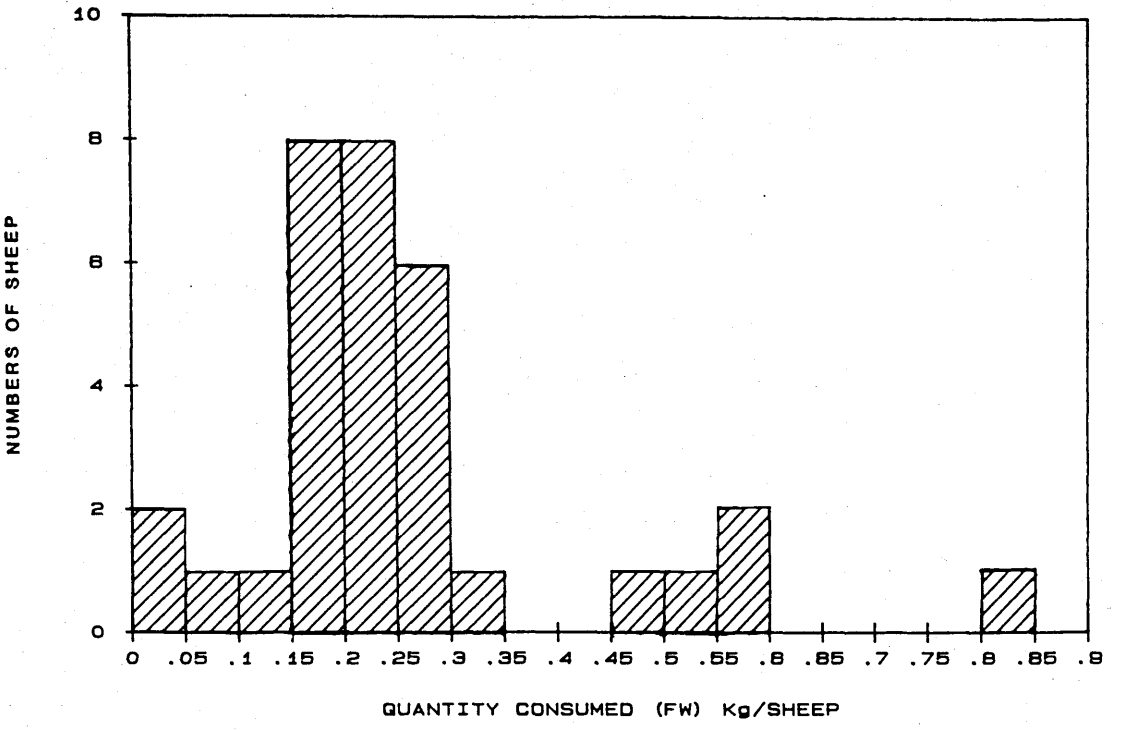


FIG.22 (Wk. 2)

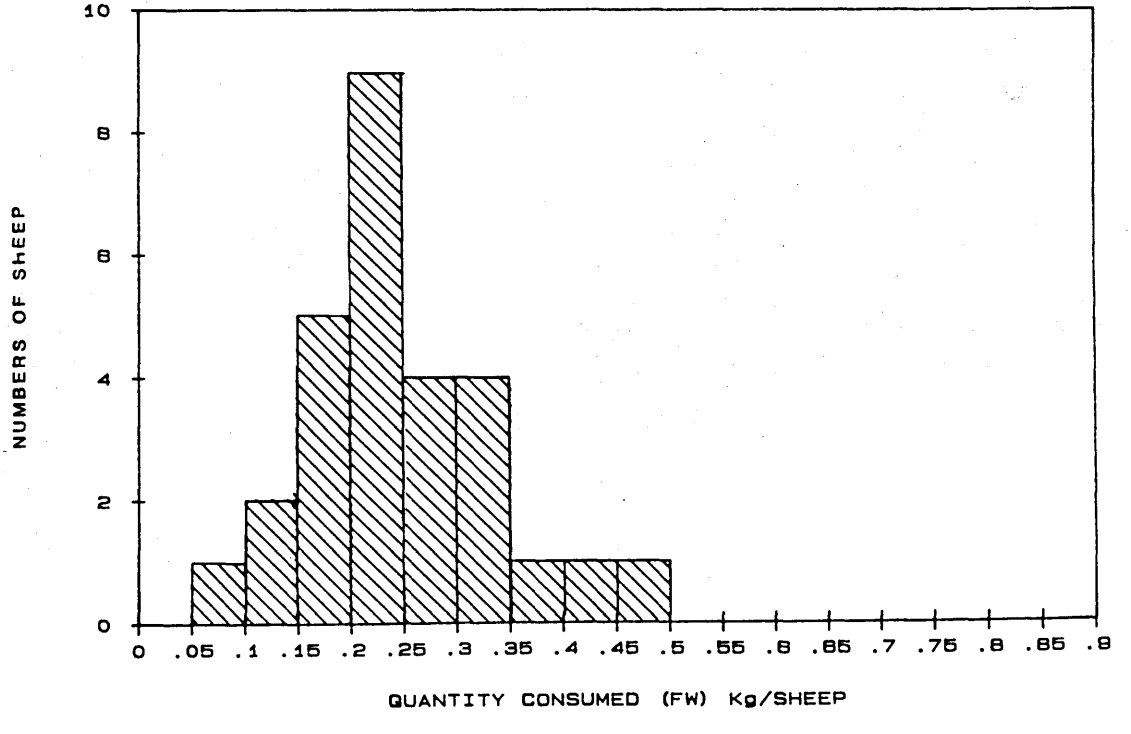


FIGURE 23. Copper content of faeces versus the chromium content of faeces at day 7 on trough feeding of barley cube plus mineral supplement for week 1.
Correlation was significant at $p < 0.005$.

$$y = 0.04123 + 0.02783x$$

FIGURE 24. Copper content of faeces versus the chromium content of faeces at day 14 on trough feeding of barley cube plus mineral supplement for 2 weeks.
Correlation was significant at $p < 0.001$.

$$y = 0.03499 + 0.0471x$$

FIG.23 Copper in faeces vs chromium in faeces (Wk. 1)

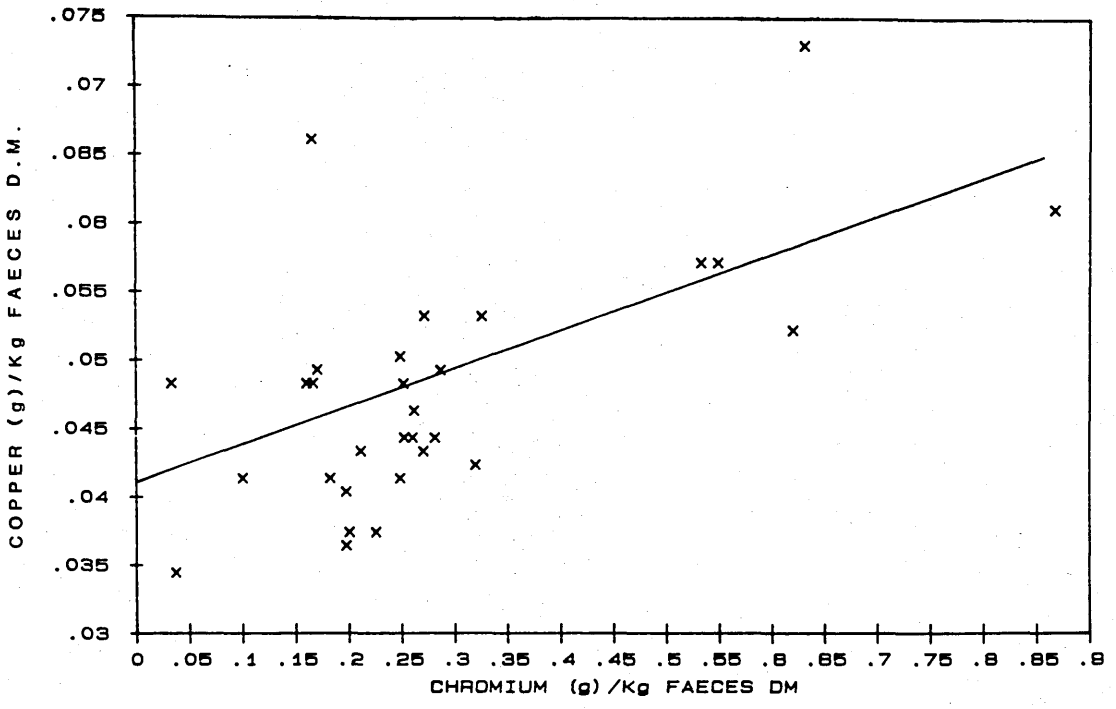
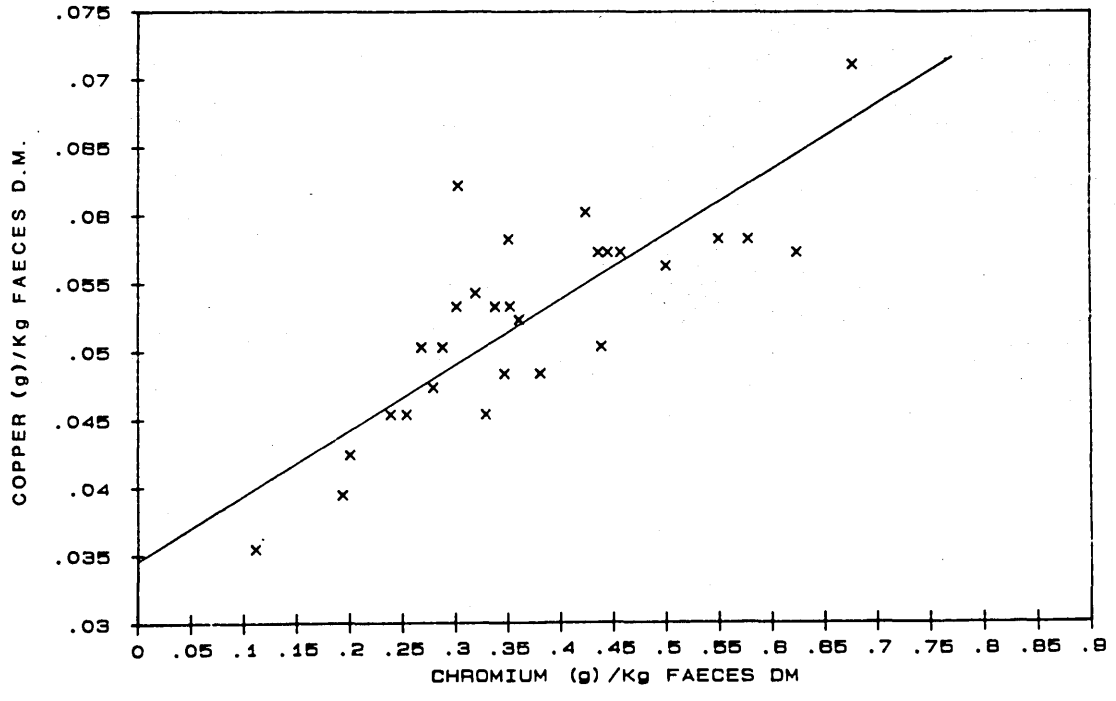


FIG.24 (Wk. 2)



(c) Oral Dosing With An Intraruminal Bolus to Sheep at Grass on Autumn Pasture

INTRODUCTION

This trial was regarded as an ideal opportunity to test the recently developed mineral bolus in intact sheep at grass.

Individual oral dosing with a successful slow release intraruminal device would be a solution to the problem of varied voluntary intake of minerals.

Only one handling session is required to administer the bolus and protection with this particular bolus should last about two months $\pm$ two weeks. Therefore fattening lambs at grass in autumn, some of which had been dosed with a bolus, were studied and observations were compared to those from the previous two experiments.

MATERIALS AND METHODS

(a) Sheep

The experimental animals used were 16 greyface x Suffolk cross fattening lambs of mixed sex close to finishing weight on autumn pasture with access to a field of rape at Cochno Farm, Duntocher.

Lambs were randomly assigned into two groups. One group of eight received two boluses per head intraruminally via a balling gun, the other group remained untreated as controls. All sheep were blood sampled by Vacutainer for copper estimation from the jugular vein on day 0 and day 21 of the experiment. Lambs were slaughtered on day 27. At slaughter, complete livers were recovered from all lambs, weighed and analysed for copper content. The rumen and reticulum were searched for bolus residues.

(b) Boluses

The mineral bolus was composed of 9 g of the standard mineral mix (already discussed in Section 2:2) and 7 g CuO(n). It was coated three times with Plastic Padding resin. The dimensions were approximately ½" x ⅞" and the density 2.8 g cm^{-3}. Two boluses contained sufficient to supply completely the ARC mineral requirements of sheep for six to eight weeks. Copper however was present in excess. Boluses were numbered and weighed before administration. After slaughter bolus residues were recovered so that their weight loss over the period could be calculated.

RESULTS AND DISCUSSION

Individual blood and liver copper levels are detailed in Table 17(i) and 17(ii). Bolused lambs on day 21 had a significantly higher ($p < 0.02$) blood copper level (0.885 mg/kg blood) than control lambs (0.604 mg/kg blood). There was no significant difference between the blood copper levels of control animals at day 0 and at day 21. The difference between blood copper levels in treated animals before bulletting and after 21 days was significant at $p < 0.001$. The mean liver copper level of the bolused lambs was 572 mg Cu/kg and that of the control lambs was 34.175 mg/kg. This was statistically significant at $p < 0.001$.

After twenty-seven days only 50% of boluses were recovered. Two lambs had neither bolus, four sheep had one bolus and two sheep had both. Table 18 shows the weights of copper lost from those boluses which were recovered and data on liver copper content. From this, the percentage accumulation of copper in the liver due to the bolus has been estimated. It is not certain whether, when one bolus was not recovered in a pair, the bolus (a) eroded more quickly than its partner, (b) was regurgitated and lost, or (c) was regurgitated, chewed and some or all of the particles swallowed. Minerals would be released into the alimentary tract in one dose if the bolus was chewed. Two possibilities (a) and (b) have been covered in the table. It is

likely from calculations given in the table that explanation (a) or (c) is correct as the percentage liver accumulation of copper by the postulated dissolution of one bolus alone exceeds by far the sort of result obtained where two boluses were recovered, poorly worn, where 6.3-6.8% of the released copper was stored in the liver. The four boluses which were recovered in pairs showed a fairly small variation in weight. The average release rate was 10.816 $\pm$ 1.636 g, the coefficient of variation being only 15%. This argues for explanation (c) being correct. Since all the boluses were identical it would be uncharacteristic for one of a pair to behave completely differently unless it was somehow altered - perhaps by chewing. Where two boluses have been recovered as an intact pair, they have behaved exactly as expected and had a projected life of about seven weeks.

TABLE 17(i) and 17(ii). Copper levels mg/kg blood in bolused and control lambs

Treated Lamb No.	Day 0	Day 21	Liver Copper mg/kg
21	0.66	1.24	652.00
23	0.55	0.86	71.79
25	0.47	0.85	919.26
27	0.34	0.82	643.55
29	0.56	0.82	834.82
31	0.48	0.85	695.65
33	0.54	0.84	159.60
35	0.70	0.78	600.90
Mean	0.537	0.885	572.2
S.D.	±.113	±.146	±301

Control Lamb No.	Day 0	Day 21	Liver Copper mg/kg
22	0.33	0.39	11.97
24	0.69	0.62	39.99
26	0.41	0.40	15.98
28	0.62	0.40	37.86
30	0.62	0.89	59.80
32	0.64	0.68	35.99
34	0.59	0.65	41.84
36	0.57	0.80	29.97
Mean	0.559	0.604	34.17
S.D.	±.124	±.192	±15.16

TABLE 18. Percentage accumulation of copper in treated animal's liver

Lamb No.	No. Bullets Recovered	Cu Dose if Both Bullets Dissol.	% Accum. of Cu in Liver	Cu Dose if One Bullet Dissol. (g)	% Accum. of Cu in Liver
21	2	8.399	6.80%	NA	
23	1	6.609	0.876%	0.527	10.98%
25	1	10.363	6.40%	3.587	20.54%
27	2	7.358	6.30%	NA	
29	0	12.187	4.78%	NA	
31	0	12.175	4.89%	NA	
33	1	6.380	1.86%	0.099	100%
35	1	9.018	6.37%	2.99	19.15%

GENERAL DISCUSSION

Each method of mineral supplementation studied has shown some extent of disadvantages.

Ad lib provision of a balanced mineral mix is cheap and labour saving, and although individual sheep intake varied widely, the majority of ewes had consumed a suitable amount to supplement their diet.

Over the two week period however there were three or four sheep each week which had a very low chromium output, implying low mineral intakes. This represents as many as 12% of the total flock. It has been shown by Cunningham (1949) and Kendall (1977) that sheep which do not consume the supplement have no less physiological need than others which eat large quantities and those sheep eating large quantities are not necessarily deficient. In certain situations where cobalt deficiency for example may be a problem, this low intake percentage of the flock is unacceptably large. Supplementary cobalt is required for vitamin B_{12} manufacture in the rumen and therefore must be consumed regularly and frequently if it is to be of benefit. There is also some evidence for weather conditions affecting group intake of the supplement. During a prolonged physiologically stressful period of poor weather, supplement consumption may be insufficient to sustain the sheep.

Mixing a balanced quota of minerals into the supplementary feeding would have been assumed to reduce some of the variation arising in ad lib supplement situations. Daily group feeding of supplementary feeding is the most labour intensive of these three methods.

As expected, trough feeding yielded a smaller coefficient of variation and a greater number of observations were clustered around the mean. Individual feeding behaviour however affected consumption and again there were three or four animals with a low intake each week, and about the same number with a high intake. It is well known that some sheep are "shy feeders" and do not assert themselves at the trough to eat their share. Such animals are often the younger members of the flock. In any case, in this experiment each individual animal tended to consume similar amounts from week to week so that in general the same animals were consistently under or over consuming. About 10% of sheep in the flock practised this low intake behaviour, which could leave them vulnerable to deficiency disease conditions.

The administration of an intraruminal bolus as a method of supplementation has many advantages compared to the previous two methods. Each animal is treated individually so that it is known that 100% of the

flock are protected from deficiency for a period of two months. The labour involved is only moderate and the method is particularly suitable for treating hill sheep which are infrequently handled. Steady provision of mineral as from the bolus is also of more physiological benefit than sporadic intake.

The main problem which arose with relation to intraruminal supplementation was the fact that only 50% of the boluses were recovered after twenty-seven days due probably to regurgitation and chewing. An improved formulation or density increase may partly improve retention. A relationship between increasing density and improved retention has been noted previously by researchers (Ritchie, 1966, and Dewey et al, 1958) Despite the 50% non-recovery of boluses the results showed very decisively that copper levels were much elevated in both blood and liver.

Copper is the major ingredient of the bolus, therefore it is the most obvious choice for tracing blood and liver levels throughout treatment. However, if the bolus were chewed and reswallowed, the copper oxide needles particles would be released and would settle in the abomasum and intestinal tract and slowly break down to release copper over a prolonged period (Judson et al, 1982). Copper is stored in the liver during periods of excess and mobilised as required to

maintain a state of copper sufficiency (Suttle, 1981b) as already discussed (see Introduction). Therefore blood copper levels can give no parallel for the fate of the rest of the mineral elements of the bolus.

If the bolus was broken by chewing, perhaps after 2-3 weeks, then swallowed, the net effect would be the same as that of administering an oral drench containing two or four weeks' supply of minerals depending on whether one or both boluses break. In the case of cobalt, live weight gains have been shown to be substantially reduced in deficient lambs supplemented by one monthly dose compared to those given the same quantity divided and administered daily (Stewart, Mitchell and Young, 1955). Similarly, zinc must be supplemented on a regular, preferably daily, basis. It has been demonstrated that plasma zinc levels fell rapidly one week after supplement was withheld from lambs (Mills et al, 1967).

Selenium, manganese and iodine can be stored in the tissues and therefore can be administered less frequently. Underwood (1981) recommends monthly dosing in the case of selenium, and notes that two oral doses of potassium iodide or potassium iodate during pregnancy are sufficient to prevent neonatal mortality and associated goitre in lambs when the dams are wintered on goitrogenic kale. Lambs on a

low manganese diet only showed signs of clinical deficiency after three months (Lassiter and Morton, 1968).

Thus, if the bolus is to be an effective vehicle for the treatment of trace mineral deficiencies, it is imperative that it should erode over the recommended period, otherwise deficiencies in zinc and cobalt could arise. If one bolus was present alone, previous results (see Section 2) indicate that its release rate would be inadequate to meet the dietary requirements of the trace minerals.

Another point which must be borne in mind with regard to bolus treatment is the possibility of toxic amounts of material being released after being fragmented due to chewing of the bolus, either during administration or regurgitation, chewing and swallowing.

It has been shown during development that small uncoated sections of bolus although eroding initially rapidly as the coat comes off, do settle to a steady release, albeit faster than a coated section. Thus it would be very unlikely that the entire bolus contents would be released at once. If the contents were released within, for example, one week, the quantities present in the bolus of all elements but copper would be most unlikely to lead to toxicity.

The copper content of the bolus is in excess of normal requirements since it is also present as a weighting medium. However, the presence of the copper in the form of needles prevents instantaneous copper release because in the ruminant gastrointestinal tract, needles are slowly dissolving and break down over a prolonged period of time. When administered in relatively large doses (10 g) to sheep in gelatin capsules, there have been few toxicity problems because of their delayed release. Ideally such problems of regurgitation and chewing would not arise if retention was improved. If improved to the stage of retention approaching 100%, there would be no better method for supplementing minerals. The mineral is present where it can be easily assimilated and all members of a flock, without exception, can be protected from the risks of deficiency. The amount of labour involved in dosing all the animals is cost effective in view of the benefits, particularly if a bolus with a longer life was developed.

2. Mineral Boluses Tested in Beef Suckler Calves

INTRODUCTION

A beef farm in Perthshire had a proven history of subclinical mineral deficiency in grazing stock. Low to marginal soil levels of copper cobalt and selenium were known to be present on the farm which in turn caused inadequate levels in the grazing. Metabolic profiles of animals from the same herd as the experimental group were charted on three occasions between August and December 1982. In general in 90% of the samples, blood levels of cobalt and selenium varied between deficient and marginal while copper levels were normal in 53% of the samples and low to marginal in the remainder.

Adequate copper levels are defined as being in the range of 0.65-1.00 mg/l of whole blood. Deficiency is expected to be manifest at blood values of less than 0.5 mg/l although 0.3 mg/litre is thought to be a more realistic figure by some workers (Smith and Coup, 1973). Marginal levels are considered to be between 0.5-0.65 mg/litre. Cobalt levels of less than 200 ng/litre are thought to be clinically deficient, and between 250 and 300 ng/l marginal. Selenium levels are not so clearly defined but a range of 10 to 20 units of

GSH-Px/ml blood is generally accepted to be marginal, less than this deficient, and greater than 20 units GSH-Px, adequate.

Over the previous winter on this farm a specially formulated supplement containing high levels of trace minerals has been included in the beef cow concentrate ration. However, when the growing calves and their dams were turned out to graze in Spring, supplementation of the diet was more difficult. It was thought that there would be physiological benefits from administering boluses to individual animals compared to non-treatment of the calves. Since the deficiency was only marginal the untreated control group should not suffer any ill-effects over the short experimental period.

MATERIALS AND METHODS

(a) Animals

The animals were a group of twenty-eight beef cross single suckling calves of 100-150 kg LW which were at grass with their dams having been turned out fourteen days previously from winter housing. For treatment application, the calves were penned in a group then individually held in a crush where they were blood sampled and given an oral drench of anthelmintic. Alternate animals were bolused and subjectively judged to be "large" or

"small" corresponding mainly to being an autumn or early spring born. All animals were blood sampled, and blood samples from three of each "large" and "small" treated and control animals were submitted for blood profile analyses performed by the East of Scotland Agricultural College to measure copper, cobalt and selenium levels. The remaining sixteen blood samples were analysed for copper by the atomic absorption method at the Animal Husbandry Department, Glasgow University.

After six weeks, the farm was revisted and the calves were blood sampled as before. As an additional measure, the farm manager made a subjective assessment, without knowing which animals had been bolused, as to whether each animal was in a satisfactory condition or not.

(b) Boluses

The boluses used had originally been formulated for animals of 70-90 kg LW. However on arrival at the farm, the calves were found to be larger, in the range 100-150 kg. Each bolus weighed approximately 20 g and was composed of 45% CuO(n) and 55% mineral mix. The dimensions were $\frac{5}{8}$" x $1\frac{1}{8}$", the density 2.9 g cm^{-3}, and the bolus was coated 3-4 times with Plastic Padding resin with one end

exposed. The amounts of each mineral supplied by two boluses over six weeks fulfilled or exceeded the ARC (1980) requirements and were as follows:

343	mg Cu/hd/day	131	mg Zn/hd/day
1.4	mg Co/hd/day	81	mg Mn/hd/day
0.407	mg Se/hd/day	2.57	mg I /hd/day

Boluses were admistered with ease via a standard sheep sized balling gun. Two boluses from this same batch were placed in the reticulum of a fistulated cow in Glasgow for a period of three weeks to give an indication of erosion data which could be related to the calf trial.

RESULTS

Tables 19-22 show the results of blood copper levels of the whole group of calves and metabolic profiles of the twelve selected animals. Techniques used in blood copper analysis is carried out for the blood profile (East of Scotland Agricultural College) tended to yield values lower than those performed for copper only (Animal Husbandry Department, Glasgow Veterinary School). Thus in all t-test statistical evaluations, data has been dealt with as paired observations. Statistical analysis showed that there

was a significant increase in blood copper levels ($p < 0.001$) in the treated group after six weeks. The untreated group showed no significant change.

There was a significant decrease in blood selenium levels in the untreated group ($p < 0.02$) over the six week period, whilst there was no significant change in the bolused group. Cobalt levels in general increased a little over the period of the experiment. In the animals which were not treated, this increase was significant at $p < 0.05$, but not significant in the treated group.

A copper level of less than 0.3 mg/litre was noted in one untreated animal (A121) on the second sampling, but apart from this all other animals were in the marginal to adequate range. Signs of deficiency were manifest in calf A121. Its condition was described as being poor, it had a rough unhealthy coat and was smaller than its peers. In addition, its selenium level was also clinically deficient while all the other animals were in the marginal to adequate range. According to the ARC (1980) guidelines, four animals (two treated, two untreated) were clinically deficient in cobalt. However, obvious signs were not detectable in the condition of the four calves. Two of the treated animals with the lowest cobalt levels were described as highly satisfactory. Animals described

as "less satisfactory" did not have particularly low blood levels of copper, cobalt, or selenium. In fact, some of them had similar, if not higher, blood levels than some highly satisfactory calves. Their lack of condition could have been due to other factors such as parasitism or lower energy intake.

After three weeks testing in a fistulated cow indoors, the two boluses had lost 1.735 g and 0.861 g. This was an average of 12.5% of the rate required.

DISCUSSION

The situation on this farm was one where marginal deficiency had been experienced in the past and was believed to be a continuing problem. Theoretically slow release intraruminal supplementation would be the ideal solution to the problem outdoors where there was to be no supplementary feeding. The mineral bolus developed in the laboratory contained the correct ingredients for dealing with the levels of deficiency. Unfortunately, the information given before the boluses were manufactured for the trial was that the calves were spring born and no more than 90 kg. The size of the bolus formulated only just covered the allowances of the larger calves used in this trial. Since it was found from observations in sheep that pairs of boluses apparently dissolved more quickly in a smaller

reticulum, compared to the rate in fistulated cows, only one face of the bolus was exposed and the relatively small diameter of $\frac{5}{8}$" used for pressing. It was obvious that the bolus dissolved much too slowly in the fistulated animal, but it is difficult to speculate on the rate with which the bolus may have eroded in the calves. Since physiological levels of minerals were not influenced to a great degree over the six week period, it is highly likely that the bolus dissolved too slowly in this particular trial. Without performing post mortem or operative retrieval of the intraruminal device, it is impossible to speculate about what the erosion pattern of this combination of ingredients might have been.

Supplementing copper via CuO(n) has been shown to be effective in this trial from the significant elevation in blood levels. The bolus contained about seven times as much copper as was required so that even if the release rate was very slow, as it was in the fistulated cow, the daily copper requirement would have been met. The maintenance of the same selenium levels over the six week period in treated animals compared to the fall in control animals' level was most probably attributable to the slow rate of bolus dissolution. Cobalt levels tended to hover around the adequate level in both groups of animals after the second sampling, implying that herbage levels were perhaps just

sufficient.

An improved bolus of a larger size with a greater surface area would probably have yielded more definitive information in a similar trial. If handling facilities on this farm had permitted weighing of the cattle, it would have greatly improved the accuracy of comparison between the bolused and control groups.

TABLE 19. "Small" untreated calves' metabolic profile and condition score

Calf No.	Location of Analysis	Blood Levels 11.5.83 Copper mg/l	Blood Levels 11.5.83 Cobalt vit.B_{12} ng/l	Blood Levels 11.5.83 Selenium GSH-Px units/ml RBC	Blood Levels 26.6.83 Copper mg/l	Blood Levels 26.6.83 Cobalt vit.B_{12} ng/l	Blood Levels 26.6.83 Selenium GSH-Px units/ml RBC	Subjective Condition Score
A121	ESAC	0.39	330	12.6	0.28	260	3.3	VP
M512	ESAC	0.56	340	16.5	0.44	590	12.1	S
M617	ESAC	0.41	145	13.0	0.41	150	11.9	S
M602	GUVS	0.60	-	-	0.63	-	-	VP

HS = highly satisfactory
S = satisfactory
P = poor
VP = very poor

ESAC = East of Scotland Agricultural College
GUVS = Glasgow University Veterinary School

TABLE 20. "Small" bolused calves' metabolic profile and condition score

Calf No.	Location of Analysis	Blood Levels 11.5.83 Copper mg/l	Blood Levels 11.5.83 Cobalt vit.B_{12} ng/l	Blood Levels 11.5.83 Selenium GSH-Px units/ml RBC	Blood Levels 26.6.83 Copper mg/l	Blood Levels 26.6.83 Cobalt vit.B_{12} ng/l	Blood Levels 26.6.83 Selenium GSH-Px units/ml RBC	Subjective Condition Score
M511	ESAC	0.48	640	12.2	0.59	235	11.0	P
M513	ESAC	0.39	235	14.7	0.48	200	9.8	HS
M514	ESAC	0.65	400	24.3	0.49	255	19.5	S
M522	GUVS	0.73	-	-	0.98	-	-	P
M397	GUVS	0.62	-	-	0.89	-	-	HS

HS = highly satisfactory
S = satisfactory
P = poor
VP = very poor

ESAC = East of Scotland Agricultural College
GUVS = Glasgow University Veterinary School

TABLE 21. "Large" untreated calves' metabolic profile and condition score

Calf No.	Location of Analysis	Blood Levels 11.5.83 Copper mg/l	Blood Levels 11.5.83 Cobalt vit.B_{12} ng/l	Blood Levels 11.5.83 Selenium GSH-Px units/ml RBC	Blood Levels 26.6.83 Copper mg/l	Blood Levels 26.6.83 Cobalt vit.B_{12} ng/l	Blood Levels 26.6.83 Selenium GSH-Px units/ml RBC	Subjective Condition Score
M395	ESAC	0.56	< 100	26	0.46	215	20.4	S
M508	ESAC	0.51	135	30.5	0.39	180	15.1	S
M399	ESAC	0.59	190	25.7	0.51	1000	16.5	S
M520	GUVS	0.56	-	-	0.71	-	-	S
M503	GUVS	0.59	-	-	0.75	-	-	S
M502	GUVS	0.75	-	-	0.82	-	-	S
M518	GUVS	0.51	-	-	0.71	-	-	S
Y10	GUVS	-	-	-	0.74	-	-	S
M517	GUVS	0.52	-	-	0.65	-	-	S

HS = highly satisfactory

S = satisfactory

P = poor

VP = very poor

ESAC = East of Scotland Agricultural College

GUVS = Glasgow University Veterinary School

TABLE 22. "Large" bolused calves' metabolic profile and condition score

Calf No.	Location of Analysis	Blood Levels 11.5.83 Copper mg/l	Blood Levels 11.5.83 Cobalt vit.B_{12} ng/l	Blood Levels 11.5.83 Selenium GSH-Px units/ml RBC	Blood Levels 26.6.83 Copper mg/l	Blood Levels 26.6.83 Cobalt vit.B_{12} ng/l	Blood Levels 26.6.83 Selenium GSH-Px units/ml RBC	Subjective Condition Score
M510	ESAC	0.55	100	20.5	0.52	145	10.5	HS
M398	ESAC	0.63	180	13.5	0.71	235	17.0	P
M504	ESAC	0.41	130	26.5	0.45	180	24.3	HS
M505	GUVS	0.66	-	-	0.88	-	-	S
M516	GUVS	0.74	-	-	0.72	-	-	S
M506	GUVS	0.67	-	-	0.75	-	-	S
M521	GUVS	0.63	-	-	0.76	-	-	S
M507	GUVS	0.60	-	-	0.81	-	-	P *
M519	GUVS	0.67	-	-	0.73	-	-	HS
M400	GUVS	0.69	-	-	0.81	-	-	P

* animal suffering from bloat

HS = highly satisfactory
S = satisfactory
P = poor
VP = very poor

ESAC = East of Scotland Agricultural College
GUVS = Glasgow University Veterinary School

3(i). Commercial Attempts to Produce a Mineral Bolus - Thomson & Capper

INTRODUCTION

An approach was made to a company specialising in commercial tabletting (Thomson and Capper Ltd., Runcorn, Cheshire). They are involved almost exclusively in the manufacture of compressed salts compacts, mainly of a small size, compared to the dimensions of the bolus which had been developed. Enquiries had revealed that in Britain they had the widest experience in producing compressed tablets on a commercial scale. Indeed at the outset of the project, they were responsible for the manufacture of the mineral boluses weighted with iron filings, which, on testing proved that such a concept was potentially workable.

The main difference between that original type of bolus and the current model was that all boluses were subsequently produced in a cylindrical mould and pressed end to end instead of side on. Sideways pressing of tablets of the dimensions of the bolus generally produces an object which is square in cross section. A tablet which is perfectly circular in cross section would require a very precise amount of mould fill and would certainly have flashlines running longitudinally. These would need to be sanded off

which would introduce another undesirable step in the production process.

It was intended that Thomson and Capper exactly mimic on a large scale automated operation the bolus which was being produced in Glasgow on a handpress. Details of the present mineral bolus were given, as outlined in Table 23. In the factory there was a Bipel press, which, it was considered, could be modified to produce the Glasgow bolus. Two new moulds were ordered for manufacture, 25 mm and 18 mm diameter, which would fit the press. For economy purposes in commercial production, it was decided that one size, 18 mm, should replace the 17 and 19 mm moulds.

TABLE 23. Handpressed bolus specifications

Dia. Mould (mm)	Length Unpressed (mm)	Length Pressed (mm)	Weight (g)	Density (g cm^{-3})
17	102	47	33	3.08
19	112	61	50	2.87
25	102	51	70	2.80

All the mineral mixes which were to be incorporated into the boluses manufactured at Thomson and Capper were supplied to them directly by Agrimin Ltd., Grimsby,

according to instructions from Glasgow. Table 24 details the constitution of the basic mineral mix.

TABLE 24. Mineral constituents (g) of bolus mix

Cobalt sulphate ($CoSO_4 7H_2O$)	78.2
Sodium selenite ($NaSeO_3$)	36.8
Potassium iodide (Kl)	139.6
Zinc oxide (ZnO)	5211.7
Zinc sulphate ($ZnSO_4 7H_2O$)	3600.6
Manganese sulphate ($MnSO_4 H_2O$)	10299.5
Vitamin A (500,000 iu/g)	1116.5
Vitamin D (100,000 iu/g)	
Vitamin E (50% strength)	1116.5
Total	21599.4 g

It was recommended that bulk quantities of mineral and CuO(n) and iron shot weighting agent be commercially pressed as detailed in Table 25.

TABLE 25. Recommended formulae for commercially pressed boluses

Bolus Mix	Expected Bolus (mm) Dimension dia x lgth	Mineral (g)	CuO(n) (g)	Fe shot (g)	Approx. Wt. per Bolus (g)
1	18 x 45	20.5	17	0	38
2	18 x 23	10.3	8.5	0	19.2
3(a)	18 x 45	21.2	8.8	8.8	39.2
3(b)	25 x 50	38	16	16	70.5
4	25 x 50	36	30	0	66.5
5(a)	18 x 25	10	0	7	17.5
5(b)	18 x 45	20.5	0	14.3	39.3

MATERIALS AND METHODS

The press converted for the purposes of producing the bolus was a 35 ton Bipel machine. The dies, which it subsequently transpired Thomson and Capper had purchased, were 16 and 25 mm diameter. The method of compacting the bolus was essentially similar to laboratory production except that the whole operation was automated, performed horizontally and was capable of producing 2 x 25 mm boluses in one action or 4 x 16 mm. A hopper filled with mixed mineral and weighting was sited above the die and automatically

filled it. The rams then advanced, compacted the boluses, withdrew, and the boluses dropped out before the die filled again. The whole process was very rapid and was capable of a production rate of several hundred boluses per hour. Generally the first few boluses to come through were of inferior quality and were discarded.

(a) Mineral mix

Pregranulation of material is an essential step in commercially producing compressed objects. During operation of the press, finer ingredients would settle out in the hopper with normal machine vibration, which would alter bolus properties and make them non-uniform. The process of pregranulation involved dampening the complete mineral mix and adding an agent which encouraged particle aggregation - in this case, gum acacia, and gently machine mixing until the mineral dried into granules rather than powder. The process was also intended to increase the density. The weighting agent was thoroughly mixed with the granulated mineral before filling the hopper.

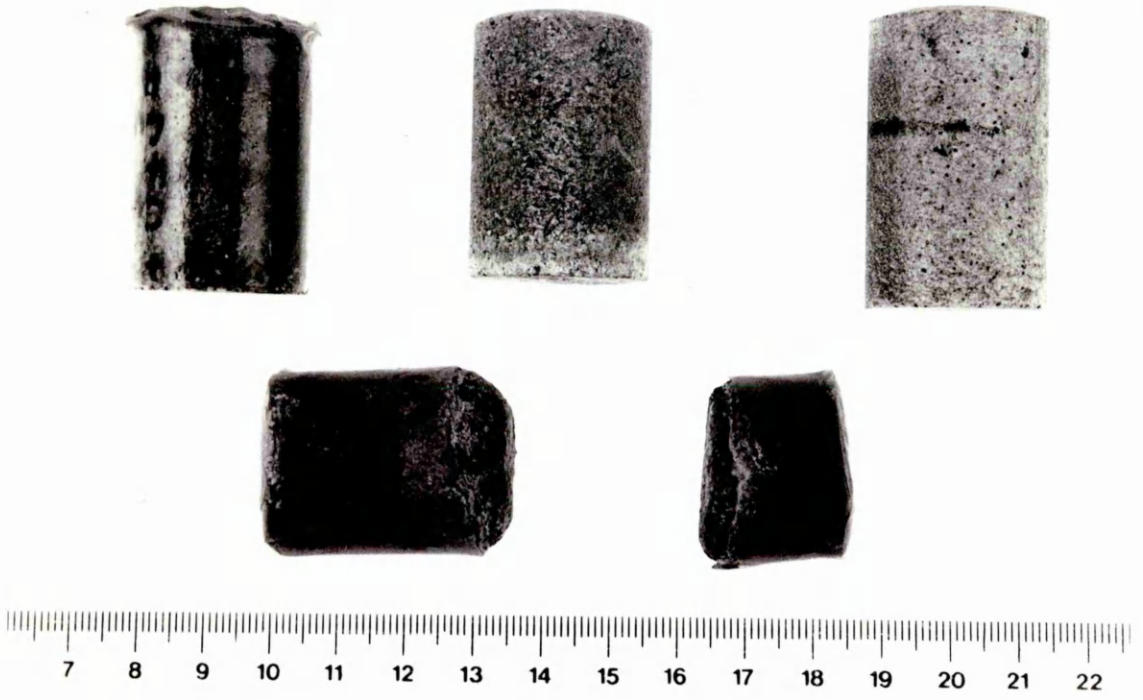
Boluses produced at Thomson and Capper were then sent to Glasgow for normal coating treatment and were tested in fistulated cows.

(b) Boluses

The first batch of boluses were received from Thomson and Capper in July 1983, manufactured from mixes 3, 4 and 5 (see Table 25). In general, the boluses were well compacted with a very smooth finished surface. One cylindrical face was flat, and the other slightly domed (see Plates 5 and 6). Table 26aoutlines the dimensions of the Thomson and Capper boluses as compared with those of the same formulation which had been hand manufactured.

TABLE 26a Specifications of different bolus sizes produced by hand and by Thomson and Capper

Mix No.	Method of Manufacture	Bolus Dia. mm	Bolus Length mm	Weight (g)	Density g cm^{-3}	No. of Exposed Faces
3(a)	Hand	17	45	38	2.7	1
3(a)	T & C	16	31	21	2.87	1
3(b)	Hand	25	50	70.5	2.8	1
3(b)	T & C	25	32.75	58.59	3.15	1
4	Hand	25	50	66.5	2.8	1
4	T & C	25	37	58	2.9	1
5(a)	Hand	17	20	17.5	2.8	2
5(b)	Hand	17	40	34.5	2.8	2
5	T & C	16	27.2	17.34	3.07	1


7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22


5 6 7 8 9 10 11 12 13 14 15 16

'LATE 5. From left to right top row: Coated 25 mm diameter bolus of mix 1; uncoated bolus of mix 4; uncoated bolus of mix 3b. Bottom row: Two partly worn boluses of mix 4.

LATE 6. Various uncoated, coated, and partly worn boluses of 16 mm diameter containing copper oxide needles. Bolus in middle of top row is an example of a partly worn mix 5 (see Table 25).

The most obvious difference between handpressed and Thomson and Capper boluses was that, on average, Thomson and Capper boluses were about two thirds of the desired length, although the large boluses contained about 85% of the material required. Although the boluses had the appearance of being well compacted, those containing copper oxide needles were dark in colour with a powdery coating on all surfaces. It was difficult to distinguish particles of copper oxide needles in cross section so it was assumed that fractionation had occurred during manufacture. About one third of bolus 3a type arrived broken into two pieces with another one third of those remaining having small chips on the exterior. Only slight force was required to break this bolus into pieces compared with handmade boluses. Type 3b were again darker in colour indicating fragmented copper oxide needles. Unlike the smaller Thomson and Capper boluses of the same mixture (3a), these boluses had a hard exterior which was difficult to chip or break experimentally, and all sixteen boluses arrived fully intact. The majority of the consignment of type 4 boluses were chipped or broken on arrival. The copper oxide needles weighting again had been crushed into powder and small particles so that the boluses were a dark brownish colour with a crumbly, soft constitution.

By contrast, type 5 boluses were hard with a shiny exterior. Few had chipped corners and there were no broken pieces in the batch. The iron shot appeared to be fairly well dispersed throughout, though occasionally there were slight concentrations at the base and on the wall of the bullet. In some cases such concentrations of shot had fallen out of the wall leaving a pitted exterior.

It transpired that copper oxide needles had in fact been intentionally broken up during the production process. The mineral mix, as outlined in the specifications (Table 23) had an approximately 2:1 reduction ratio from unpressed material to a compacted bolus. Thomson and Capper production department anticipated that should the material be first pregranulated, this ratio would diminish and be perhaps 3:2. However this was not found to be the case, and the ratio remained as before, with the air space between granules more than compensating for the aggregation within particles. Thus in order to reduce the compaction ratio Thomson and Capper pressed boluses, then broke them up by force in a process known as "slugging", then repressed the material until a more desirable size of bolus was produced. Physical constraints on the length of travel of the ram, imposed by the actual dimensions of the Bipel press, prevented

filling the mould to a greater degree and producing a larger bolus in this way.

However the "slugging" process was responsible for totally altering the physical properties of the copper oxide needles bolus. Iron shot boluses were produced at a very high pressure after one compaction but it was reported by an employee that the pressure used was "far in excess of safe working even for high compression machinery" and had caused extensive damage to the machine tooling.

(c) Experimental design

Each type of Thomson and Capper bolus was paired with its corresponding handpressed bolus. In the case of mix 5, handpressed boluses were produced in two lengths, but these were paired with two Thomson and Capper mix 5 boluses of one length. Thus there were five pairs of boluses. Each bolus was coated with four applications of polymer resin (Plastic Padding Ltd.) then weighed, and each pair placed in the reticulum of a fistulated cow at grass. All boluses apart from handpressed 5b, 5b1 and 5b2 had only one face exposed. Simultaneously, four cows which were due to be fistulated, had pairs of bullets administered by mouth via a cattle balling gun, with the intention of recovering them in several weeks following the fistulation

operation. The advantage is that the erosion rate would have been unaffected by the repeated drying and weighing involved in monitoring the boluses in fistulated animals and thus would provide a useful comparison. Only large bullets likely to last for at least one month were administered in this instance, i.e. types 3b and 4.

Table 26b summarises the bolus numbers tested in this trial and indicates those which were hand-pressed, and those which were produced by Thomson and Capper.

TABLE 26b Summary of numbers of boluses tested

Bolus No.	Method of Manufacture	
	Hand	Machine
3(a)5	*	
3(a)6		*
3(b)1	*	
3(b)4		*
3(b)		*
3(b)2	*	
3(b)3	*	
3(b)5		*
3(b)6		*
4(1)	*	
4(2)	*	
4(3)	*	
4(4)		*
4(5)		*
4(6)		*
5(a)2		*
5(a)6		*
5(a)13		*
5(a)14		*
5(a)8		*
5(b)	*	
5(b)1	*	
5(b)2	*	

RESULTS AND DISCUSSION

Results of testing type 3 boluses which contained iron shot and copper oxide needles are shown in Figure 25. Bolus 3(a)5 of 17 mm diameter was handpressed and had two open ends while 3(a)6 was 16mm diameter, produced by Thomson and Capper, and was coated so that only one end was exposed. After two weeks, erosion had virtually ceased in both cases. The larger 25 mm diameter bolus of the same mixture produced by Thomson and Capper, 3(b)4 and 3(b)8, dissolved very quickly, 3(b)4 in fact was gone within two days and replaced by 3(b)8 which also dissolved quickly. The equivalent handpressed bolus 3(b)1 lost almost half of its weight within seven days but thereafter settled to a reasonable release rate of 0.42 g/d. Weights of boluses 3(b)2, 3(b)3, 3(b)5 and 3(b)6 of type 3 design which were recovered after fifteen days from cows fistulated during the test period are shown on Figure 26. Handpressed boluses 3(b)3 and 3(b)2 have eroded at an apparently more reasonable rate than the equivalent 3(b)1 which was removed and weighed several times. Thomson and Capper boluses 3(b)5 and 3(b)6 were not found after fifteen days in the reticulum after oral administration.

The erosion pattern of boluses 4(1)-4(6) which contained 55% mineral mix and 45% copper oxide needles is shown on Figure 27. The handpressed boluses 4(1) and 4(3) had a fairly constant rate but the commercially

manufactured type (4(4)-4(6) seemed to be variable and outwith the ideal range. When boluses 4(1), 4(3), 4(4) and 4(6) were recovered from cows to which they had been orally administered pre-fistulation, a similar pattern emerged. Bolus 4(3) had been slightly chewed on administration but bolus 4(1) was seen to be eroding at a similar rate to bolus 4(2) (0.57 g/d) as compared with 0.46 g/d approximately of 4(1). It is highly likely that the general disruption in copper oxide needles integrity was responsible, at least in part, for this difference. Bolus 4(5) manufactured by Thomson and Capper, lost approximately half its weight within two days but there was then no change in its weight within the next four days. Boluses 4(4) and 4(6) were recovered by fistula after oral administration, and were found to vary in weight lost by 15 g.

Results for the small sized 16 mm diameter iron shot boluses type 5 made by Thomson and Capper are shown on Figure 28 and were fairly erratic and inexplicable. One particular Thomson and Capper bolus (No. 5(a)6) eroded extremely slowly in the presence of another similar bolus over a period of thirty days. However, on two separate occasions a similar pair of boluses were administered to different fistulated cows, only to discover that there was no residue after several days (for example 5(a)2 and 5(a)8). Evidence from the erosion of boluses 5(a)13 and 5(a)14 appeared to

indicate that erosion was indeed very rapid in those cases since two small residues were found after only six days. Handpressed boluses of this size, numbers 5(b), 5(b)1 and 5(b)2 containing 45% iron shot eroded less rapidly than manufactured examples, taking into account the fact that 16 mm diameter Thomson and Capper boluses had a smaller surface area, and hand-pressed also had two open faces, not one. Handpressed boluses of a larger size although with the same diameter and two open faces are shown on Figure 29. The average release rate in these examples is 0.38 $\pm$ 0.03 g/day at eighteen days. Unfortunately similar problems were encountered in the laboratory with the iron shot component damaging the die and ram.

Thomson and Capper subsequently manufactured, at safe working pressures, further boluses containing iron shot. Although the boluses were of enhanced density, they lacked "bond" and were crumbly and variable.

FIGURE 25. The erosion pattern of boluses of type 3 mixture which contained 22.5% copper oxide needles, 22.5% iron shot and 55% mineral mix. Bolus 3(a)5 was 17 mm in diameter, and 3(a)6 was 16 mm in diameter. Numbers 3(b)1, 3(b)4 and 3(b)8 were 25 mm in diameter. All boluses were administered to fistulated cows.

FIGURE 26. The erosion pattern of boluses 3(b)2 and 3(b)3 (handmade) and 3(b)5 and 3(b)6 (machine pressed) which were orally administered to cows pre-fistulation and then recovered via the fistula after 15 days' testing.

FIG.25 Handmade and Thomson&Capper boluses (type 3)

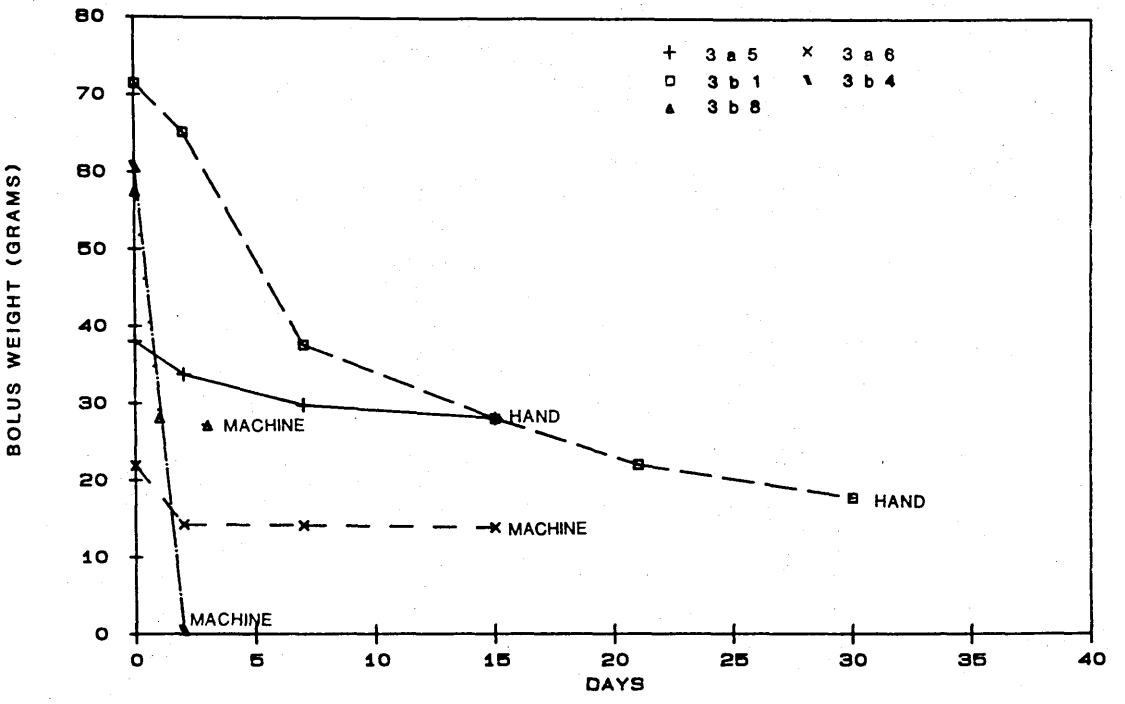


FIG.26

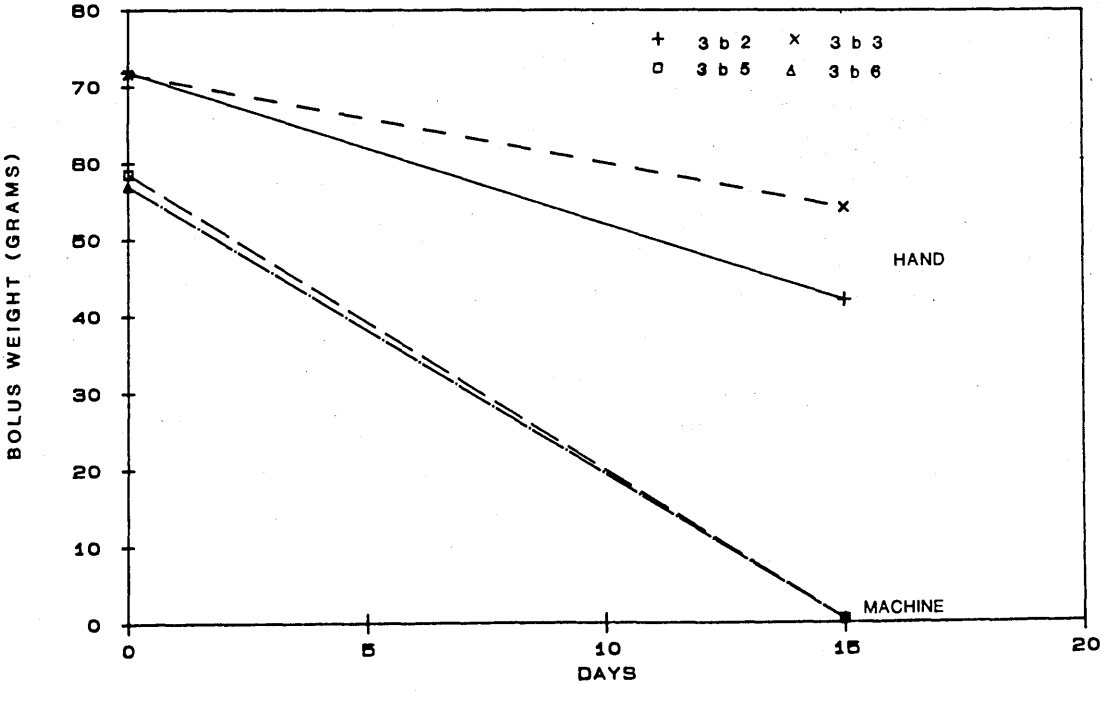


FIGURE 27. The erosion pattern of boluses 4(1)-4(6) which contained 55% mineral mix and 45% copper oxide needles. Boluses 4(2) (hand made) and 4(5) (machine pressed) were removed and weighed at intervals, but the remaining boluses were orally administered in pairs pre-fistulation and were recovered via a fistula after 15 days' testing.

FIG.27 Handmade and Thomson & Capper boluses (type 4)

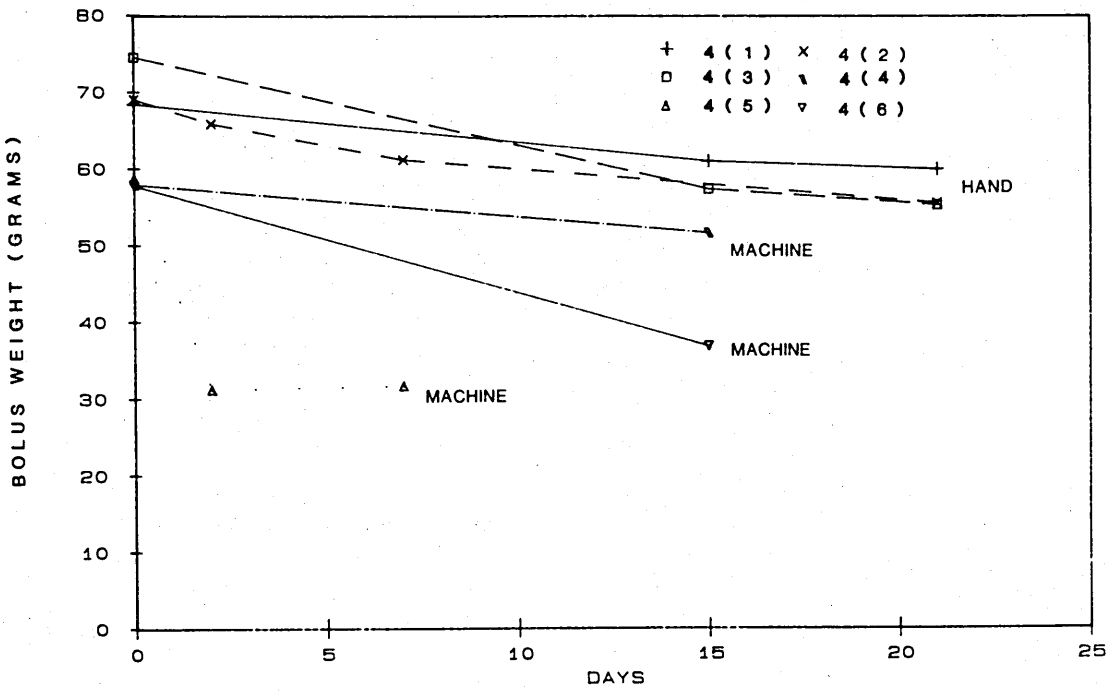


FIGURE 28. Boluses composed of 45% iron shot and 55% mineral mix of 16 mm diameter and one exposed face manufactured by Thomson and Capper, and administered in pairs to fistulated cows.

FIGURE 29. The erosion pattern of boluses of the same formulation as above which were 17 mm diameter with two open faces, administered in pairs to fistulated cows. These boluses were hand made.

FIG.28 Type 5 Thomson & Capper boluses

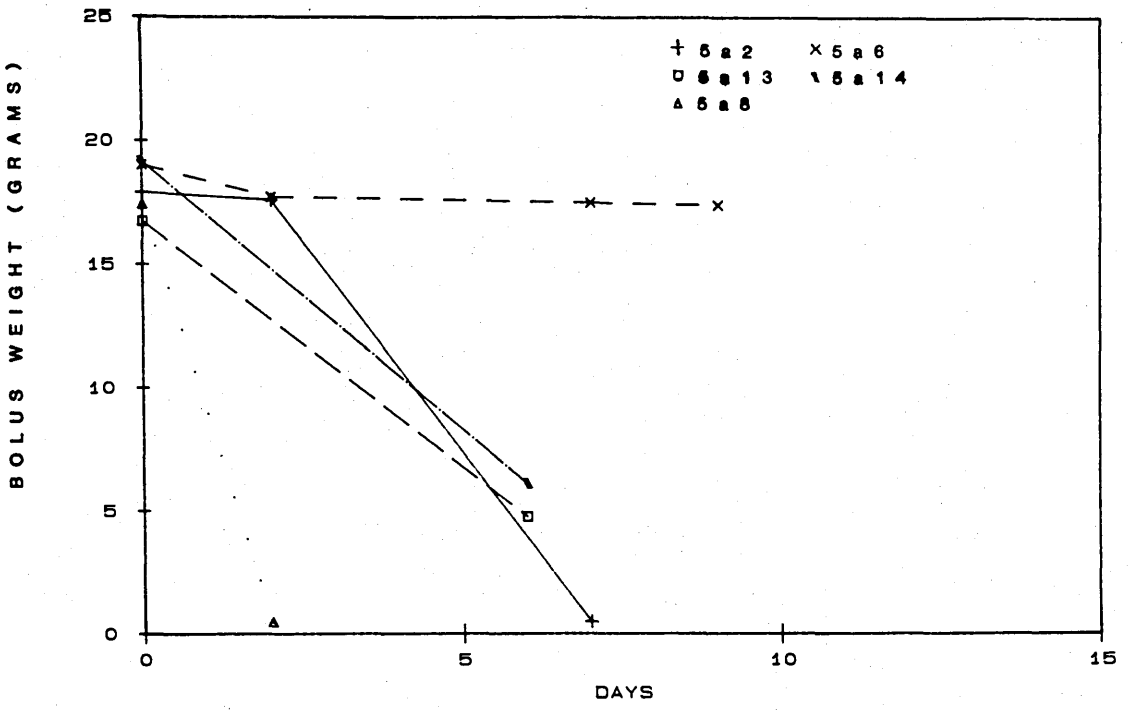
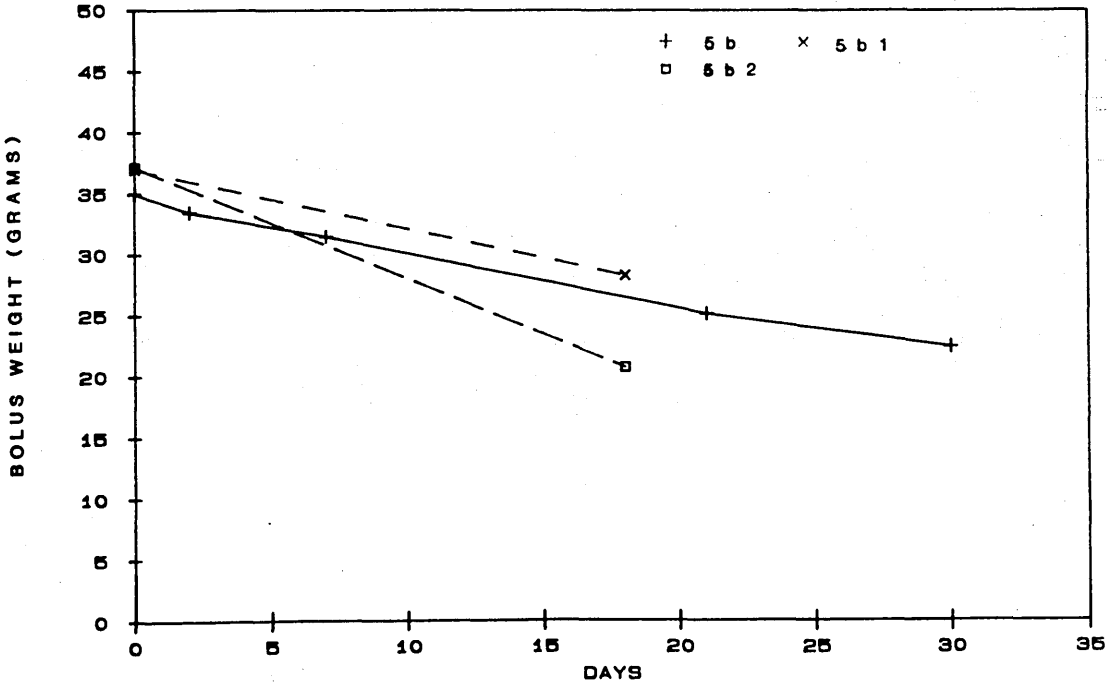


FIG.29 Type 5 handmade boluses



3(ii). Sheep Sized Thomson and Capper Boluses

INTRODUCTION

Despite extensive investigations it was found that it was not possible on an industrial scale (at Thomson and Capper Ltd.) to extend the length of the 25 mm diameter cattle sized bolus much more than to about 30-35 mm. It was considered that virtually no machine in Europe was capable of producing a larger length compressed object of the desired dimensions without expensive and extensive modifications. The smaller size of bolus, although the same finished length, could possibly have been used as a sheep supplement since sheep require a very much reduced quantity of mineral relative to cattle.

The boluses that were produced on an industrial scale had greater densities than handpressed mixtures of the equivalent dimensions. Reductions in the proportions of copper oxide needles were proposed and manufactured to test whether reasonable densities could still be maintained. For sheep, it would be highly desirable to have a bolus containing less than 50% CuO(n) because of legal restrictions on the administration of copper in sheep feedstuffs. The absolute daily requirement of adult sheep for copper is 3-20 mg per day. Table 27 shows the amount of

copper contained per two bolus administration at the varying percentages of copper oxide needles inclusion. Bolus 4D is closest to providing the actual copper requirements, yet its density still appeared to be within the adequate range.

TABLE 27. Amount of copper contained per two bolus administration in different formulae

Bolus Type	% CuO(n)	Cu (mg)	(Wks) Estimated Period of Erosion	Cu (mg) Daily Dose	g cm^{-3} Density
4A	50	16.758	6	399	3.3
4B	25	7.182	6	171	2.95
4C	12.5	3.192	6	76	2.82
4D	5	1.317	6	31.35	2.7

Tested in fistulated cows, the average release rates were as shown on Table 28. Bolus 4A6 had two open ends, 4A11 and 4A8 had only one, but none of this industrially manufactured type eroded particularly successfully. The B type had two open faces and lost weight at a steady rate over twenty-six days. Had they continued at this rate, the estimated life of B7 and B4 would have been approximately another 120 days. Boluses 4C1 and 4C4 were present in the rumen for part of the

test period so it is difficult to make judgements on erosion characteristics. The best results were obtained with the 25% and 5% copper oxide needles boluses open at both ends. Their estimated life, however, would be about 140 days in fistulated cows. Because of the relatively greater amount of mineral mix present in the 5% CuO(n) bolus it contained sufficient mineral and copper for about eight weeks full supplementation for sheep. A trial of retention and erosion in sheep themselves was then performed, because fistulated cows were not expected to make a good prediction for bolus behaviour in sheep, and although boluses appeared to be very slow, the smaller reticulum of sheep may cause an increase in erosion rate.

TABLE 28. Average release rates of different Thomson and Capper bolus formulations

Bolus No.	No. of Exposed Faces	% Mineral	% Needles Copper Oxide	Release Rate Average/d (g) d0-5	d6-25	d0-25
4A11	1	50	50	0.337	0	0.072
4A8	1	50	50	0.093	0.033	0.047
4A6	2	50	50	0	0.011	0
4B7	2	75	25	0.340	0.066	0.132
4B4	2	75	25	0.147	0.120	0.127
4C1	2	87.5	12.5	0.402	*0.003	0.12
4C4	2	87.5	12.5	0.383	*0	0.12
4D4	2	95	5	0.593	0.096	0.215
4D11	2	95	5	0.461	0.051	0.164

* located in the rumen for part of this time

MATERIALS AND METHODS

A group of twenty Suffolk ewes which were due for slaughter because of age or infertility were administered with 4A, B, C or D type Thomson and Capper boluses which either had one or two open faces. Two randomly paired boluses were administered per ewe via an intraruminal balling gun. Ewes were kept at grass on Cochno Farm Duntocher, then slaughtered forty-three days post bulletting. At the abattoir, the oesophagus was ligatured, and the reticulum and rumen searched for bolus residues.

Boluses

These were of types 4A-4D as detailed on Table 27 Ten boluses each were of types A, B, C and D, with 50% of these having one exposed end, and 50% having two exposed ends. Boluses were coated three times with glass fibre resin coating (Plastic Padding Ltd.).

RESULTS AND DISCUSSION

Results of recovered boluses are shown on Table 29 The remaining nine sheep were devoid of any boluses so that the overall recovery rate was 35%. The overall x release rate for boluses recovered was 0.168 $\pm$ 0.081 g which has a 48% coefficient of variation. If the result from A1 is excluded, this reduces to 0.15 $\pm$ 0.059 g with 38% variation.

TABLE 29. Results of bolus recovery after 43 days' testing

Ewe No.	Bolus Type	No. Open Faces	Original Weight	Weight on Recovery	Average Weight Loss Per Day (g)
R102	A1	1	22.487	6.615	0.37
	A9	1	22.586	19.691	0.07
R222	A3	2	21.378	16.026	0.13
	A4	2	21.552	14.002	0.18
R236	A2	1	23.535	18.063	0.13
	C2	1	18.24	0	-
Y55	A6	1	21.748	16.978	0.11
	B5	1	19.130	0	-
R56	A10	1	22.157	17.565	0.11
	D3	1	15.684	0	-
R181	B3	2	19.625	11.451	0.19
	B12	2	19.299	0	-
R12	B8	1	18.129	13.366	0.13
	B9	1	18.895	0	-
G73	C8	2	18.418	14.120	0.10
	C11	2	17.100	0	-
R1	C10	2	17.885	11.042	0.21
	C13	2	17.891	0	-
R177	C6	2	16.862	0	-
	D1	2	16.915	11.729	0.12
R90	C12	2	16.771	7.195	0.22
	D10	1	17.885	5.842	0.28

Seventy percent of A type boluses were recovered - all of those with a single open face, and 40% of the two ended samples. Only 23% of the three remaining types were found on slaughter. It seemed most likely that boluses not recovered were in fact regurgitated, because their density was insufficient to keep them lodged in the reticulum. Some of the bolus residues of the different types were fairly large, implying that boluses not recovered may not necessarily have eroded. It is difficult to draw any conclusions about the relative erosion rates of the different bolus types because of insufficient data, but it appeared that no matter the type or whether one or both faces were exposed, the rate of erosion was similar. If the A boluses had continued to erode at an average rate of 0.12 g/d they would have lasted for about 180 days, a period of more than six months. Results from equivalent testing in fistulated cows showed a very similar range of average daily erosion rates, except in the case of the A type boluses which were appreciably slower in cattle, although there is no obvious reason as an explanation. Bolus 4A8 was present in a different cow with a different partner from the other 4A boluses yet still had a low release rate. In sheep, no such difference came to light. Fistulated cows are therefore a most useful tool in screening possible sheep boluses. In practice fistulated cow erosion was a little faster but the general trend was correct. There is also the

advantage of minimal loss through regurgitation in fistulated cows, and the rumen can be searched if boluses are inexplicably not recovered from the reticulum.

4. Thomson and Capper Boluses in Fattening Lambs

INTRODUCTION

Following the testing of Thomson and Capper boluses containing variable percentages of copper oxide needles in cast ewes, it was decided to attempt to investigate more closely the release rate of these boluses in a more uniform group of animals.

Fattening lambs which were expected to be ready for slaughter within two months were selected for experimentation for several reasons. It is possible to recover the liver and any bolus residues when the sheep are slaughtered without affecting the carcase value. It was anticipated that the natural spread over time in attaining finishing weight would lead to fairly evenly spaced observations.

Subclinical cobalt deficiency in fattening lambs is of important economic consequence in Britain. Affected lambs fail to thrive and tend to fall short of the required slaughter weight in autumn (Lewis and Anderson, 1983). Economically damaging ill-thrift also occurs in young sheep in subclinical selenium and copper deficiency situations. Andrews et al (1974) have proved the weight gain advantage of lambs dosed with intraruminal selenium pellets compared to untreated

lambs. Copper deficiency is widespread throughout Britain and the most common effect, neonatal swayback, is well documented. However significant differences in weight gains between copper sufficient and subclinically copper deficient lambs have been noted by Whitelaw et al (1983).

In the field situation where there is a suspicion that trace element deficiency may be a hindrance to efficient production, it is often difficult to differentiate which particular element may be lacking in the absence of expensive testing procedures, particularly since copper and cobalt deficiencies occur frequently in combination. Therefore a combined trace element and vitamin supplement such as the bolus supplies would have a wide practical application in lamb production systems where, as in most cases, lambs are fattened at pasture.

MATERIALS AND METHODS

(a) Sheep

Forty-three Greyface (Border Leicester and Blackface) x Suffolk cross fattening lambs, both males and females, grazed on autumn pasture on an area of 8 ha of ryegrass with access to a 2 ha field of rape at Cochno Farm, Duntocher. All were blood sampled by Vacutainer from the jugular vein

would make other workers.

and twenty-five lambs were then randomly selected and administered with two boluses per animal via a standard sheep-sized intraruminal balling gun. As they attained the correct finishing weight for slaughter (greater than 40 kg), the lambs were again blood sampled. At slaughter the oesophagus was tied and the reticulum and rumen searched for any bolus residues in treated lambs. Complete fresh livers were taken from all sheep and weighed, then dried and analysed for copper content (see Appendix 1). In practice the lambs attained their appropriate weight at intervals over a ten week span. They were despatched in four groups during this period. Eleven lambs were slaughtered on day 12, eleven at day 24, twelve at day 32 and nine at day 69. Blood plasma samples were collected up to several days prior to slaughter and were analysed as one batch with a Precisnorm standard by atomic absorption.

(b) Boluses

The boluses used in this trial were manufactured by Thomson and Capper Ltd., Runcorn, Cheshire. The mineral mix was intended to be identical to that used for laboratory boluses but zinc sulphate monohydrate was used for the mix instead of the heptahydrated form. This mistake was not discovered until later, and is likely to have

influenced the erosion rate of the bolus.

There were two types of bolus. Type B was composed of 75% mineral mix and 25% copper oxide needles. In absolute quantities, this was approximately 4.5 g CuO(n) and 13.4 g mineral mix per bolus. Type C was composed of 87½% mineral and 12½% copper oxide needles, giving a bolus containing on average 2 g CuO needles and 14 g of mineral mix. The dimensions were as follows: the diameter for both boluses was 16 mm, and the length was 3 cm on average. Type B bolus density was 2.98 gcm^{-3}, and Type C density was 2.83 gcm^{-3}. One end of the cylinder was slightly domed, and the other end flat. Boluses were identified by numbering with an indelible marker pen, prior to coating three times with Plastic Padding glass fibre resin, keeping the flat face open and clear of resin. There were three different treatment groups plus one control group containing eighteen animals. Thirteen lambs received one B and one C type bolus, a copper oxide dose of 6.5 g; five animals received two B type boluses, a CuO dose of 9 g; and seven animals received a pair of C boluses, a dose of 4 g CuO(n). Sufficient quantities of minerals were supplied by the two boluses to completely fulfil the recommedned mineral requirements of the lambs for a period of

up to six months, although copper was present in excess.

(c) Calculations

In calculating the accumulation of copper by the liver from copper supplied in boluses, the average control liver copper value for that sampling date was deducted from each experimental liver, and the remaining value used to calculate the percentage copper accumulation.

T-tests were performed to determine the significance between data from treated and untreated lambs.

RESULTS

Tables 30 to 33 detail for the four slaughter groups respectively, the individual lamb data on blood plasma and liver copper levels, the weight loss of boluses recovered, and estimated liver copper accumulation. Column 6 shows the figure calculated had any bolus not recovered at slaughter completely eroded. Column 7 makes the assumption that boluses not recovered were regurgitated and lost.

The lambs had been experimentally subjected to four basic treatments and then slaughtered when their bodyweight was sufficiently high. In practice lambs

were judged to be ready for slaughter in smaller groups over a wider timespan than was anticipated during the planning of the experiment. In order to have sufficient numbers for statistical comparisons to be valid, it was therefore decided to analyse results in two groups as either treated or control, and discard between treatment analysis. Accordingly, Figure 30 shows the mean blood plasma copper levels of the treated and control lambs over the experimental period several days preslaughter. Post mortem mean liver copper levels are also shown on Figure 31.

(a) Plasma copper

Blood plasma copper levels in the treated group were higher than those of the control group at day 28 (Figure 31) but this difference was not statistically significant. Similarly, paired t-test comparison of blood copper levels from day 0 and day 28 in bolused and control groups failed to prove the existence of a statistical difference. Over the blood sampling period as shown on Figure 31, the mean copper level of the treated group was initially 0.65 mg/l rising to 0.71 mg Cu/l on day 28 and at sixty-eight days it was 0.60 mg Cu/l. The control group had a higher level initially of 0.68 mg Cu/l, falling to 0.59 mg Cu/l after sixty-eight days.

The precisnorm standard analysed alongside the plasma samples gave a result in the lower part of the expected range, 0.22 mg/l less than the recommended mean. Although the plasma results are apparently bordering on marginal status, it is likely that the analytical technique has pitched them a little low, and in fact they are probably well within the normal range.

(b) Liver copper

Liver copper levels were significantly higher at all sampling dates than control values ($p < 0.01$), despite a wide variation in values of both treated and control lamb livers. Overall (Fig. 30) the mean copper level of the treated animals' livers was 156 mg Cu/kg DM higher than that of the control. An alternative way of looking at the results is to examine the total copper content of the liver. Table 33a summarises the values for treated and control lambs and shows the degree of statistical significance. No correlation relationship existed between blood copper level and liver copper level.

TABLE 30. Results of lambs slaughtered on day 12

Lamb No.	Bullet Administered	Blood Cu Level at d.7 µg/ml	Total Liver Copper (mg)	Bolus Weight Loss (g)	% Copper Accum. assuming both bullets	% Copper Accum. assuming those not recovered were lost
424	B29	0.49	79.183	18.522*	1.69	18.48 ≠
	B25			1.867		
80	B38	0.64	75.074	16.702*	1.634	7.89
	B42			4.357		
76	C20	0.67	74.458	3.757	7.198	
	C28			5.164		
35	B39	0.68	56.275	2.782	3.71	
	B23			3.425		
457	B41	0.70	45.586	3.413	2.79	
	C36			2.917		
493	C26	0.65	20.749	17.549*	0.405	1.25
	B28			4.202		
72	C48	0.46	20.57	17.603*	0.531	5.597
	C34			1.845		
Y117	C17	0.64	24.273	16.961*	0.618	2.437
	B48			2.878		
99	-	0.56	6.858	-	-	-
	-			-	-	-
91	-	0.82	19.306	-	-	-
	-			-	-	-
Y127	-	0.69	4.546	-	-	-
	-					

≠ not included in statistical estimates

* boluses not recovered at slaughter

TABLE 31. Results of lambs slaughtered on day 24

Lamb No.	Bullet Administered	Blood Cu Level at d.17 µg/ml	Total Liver Copper (mg)	Bolus Weight Loss (g)	% Copper Accum. assuming both bullets dissol.	% Copper Accum. assuming those not recovered were lost
Y119	B46	-	78.14	13.176	2.056	
	C47				4.062	
428	C18	0.74	70.78	6.641	3.281	
	B19			5.085		
Y120	B32	0.65	39.59	4.237	0.868	2.829
	C42			19.134*		
71	B26	0.58	33.39	2.245	0.816	3.958
	C29			17.292*		
57	C27	0.67	74.38	5.500	4.353	
	B47			4.00		
Y116	B45	0.63	38.33	15.921*	0.456	
	C23			17.955*		
473	C34	0.60	30.65	18.921*	0.407	
	C43			18.008*		
Y126	-	0.81	5.012			
	-					
Y97	-	0.49	26.50			
Y113		0.72	15.35			

TABLE 32. Results of lambs slaughtered on day 32.

Lamb No.	Bullet Administered	Blood Cu Level at d.28 µg/ml	Total Liver Copper (mg)	Bolus Weight Loss (g)	% Copper Accum. assuming both bullets dissol.	% Copper Accum. assuming those not recovered were lost
Y130	C26	0.992	28.043	9.376	1.099	
	C19			5.217		
S83	B35	0.41	61.849	4.787	1.985	5.207
	C40			15.543*		
S41	C16	0.48	15.356	3.197	0.168	1.053
	C24			16.870*		
S58	B49	0.58	64.868	18.597*	1.177	6.837
	B43			3.867		
S97	C37	0.58	62.961	17.302*	2.058	6.833
	B30			3.730		
Y20	C45		51.861	17.666*	1.115	
	C32			17.022*		
Y118	-	0.72	15.373			
	-					
Y100	-	0.81	6.454	-		
	-					
S3	-	0.74	24.259	-		
	-					
S86	-	0.62	9.490	-		
	-					
Y58	-	1.08	9.109	-		
	-					
N.T.	-	0.58	14.500	-		
	-					
Y128	-	0.38	4.746	-		

TABLE 33. Results of lambs slaughtered on day 70

Lamb No.	Bullet Administered	Blood Cu Level at d.68 µg/ml	Total Liver Copper (mg)	Bolus Weight Loss (g)	% Copper Accum. assuming both bullets dissol.	% Copper Accum. assuming those not recovered were lost
Y37	C35	0.63	44.310	17.554*	1.149	
	C38			16.782*		
S96	B18	0.64	15.174	16.183*	0.174	0.652
	B37			5.893		
Y40	C22	0.49	43.802	18.048*	0.723	
	B17		16.048*			
S94	B27	0.65	52.746	16.887*	1.114	6.623
	C41			6.832		
Y129	-	0.57	4.732			
	-					
S95	-	0.63	14.954			
	-					
NT	-	0.67	3.735			
	-					
S421	-	0.504	2.377			
	-					
S85	-	0.67	11.681			
	-					

FIG 30 Mean liver copper at postmortem in bolused and control lambs

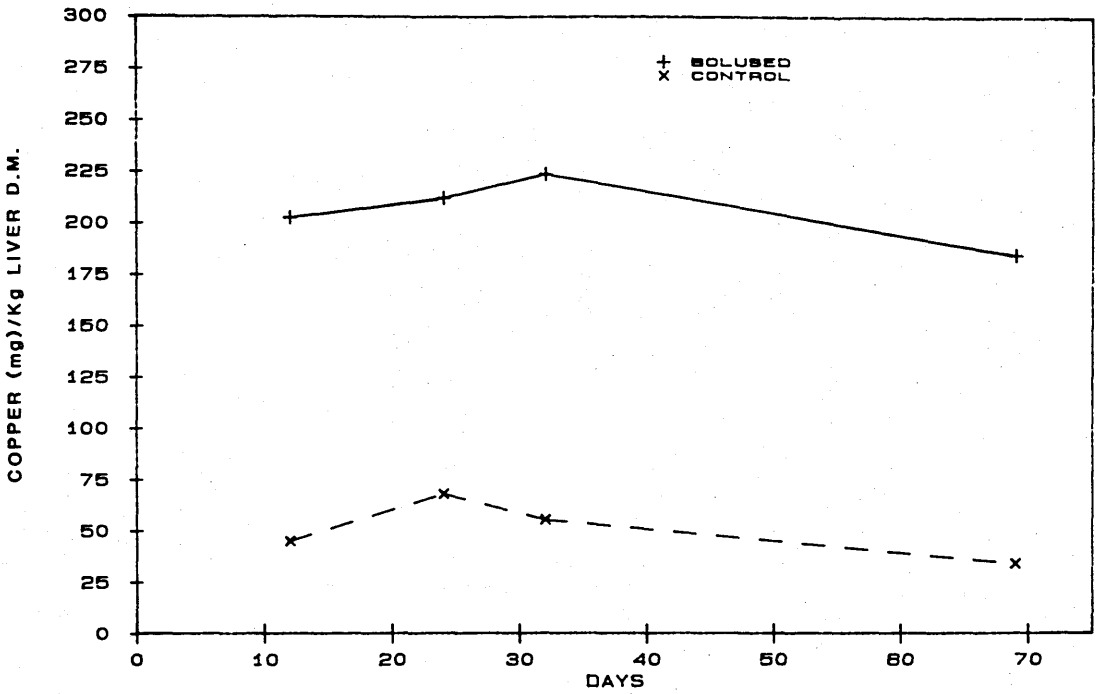


FIG.31 Average lamb copper levels in blood plasma

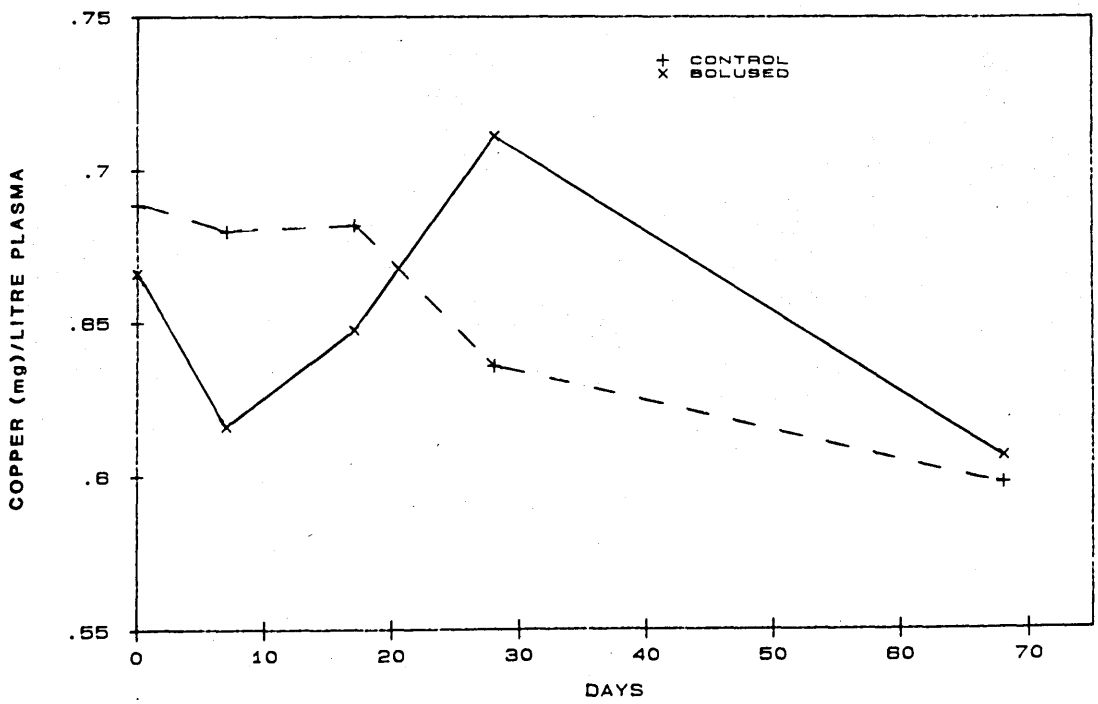


TABLE 33a Total liver content (mg) of lambs at different slaughter dates

	d12	d24	d32	d70
Bolused x	49.521±25.409	52.180±21.133	47.489±20.868	39.008±16.410
Control x	10.237± 7.939	15.621±10.747	11.990± 6.658	7.496± 5.503
Significance of difference	p 0.01	p 0.01	p 0.01	p 0.05

(c) Boluses

After twelve days, 69% of all the boluses administered were recovered from the reticulum at slaughter. After twenty-four days, 57% were found, after thirty-two days, 50% were found and after seventy days only 25% were recovered. Of those boluses retrieved from the reticulum at slaughter, the weight loss had gradually increased from week to week. After twelve days, it was 3.328 ± 1.019 g; at twenty-four days the average weight loss was 5.618 ± 3.312 g; at thirty-three days this was approximately the same at 5.029 ± 2.253 g, and at seventy days, it had increased to 6.363 ± 0.664 g. At seventy days this figure represents 33% of the original rate of weight loss, and an average daily erosion over seventy days of 0.091 g/d. However,

if the weight lost after twenty-four days was deducted, and the rate of erosion calculated between day 24 and day 70, it averaged only 0.016 g/d. Continuing to lose 0.016 g/d after approximately 5.5 g had been lost in an initial burst, it was estimated that this bolus would last in excess of two years. The actual weight loss of recovered boluses overall, averaged for B = 4.373 $\pm$ 2.566 g and for C = 4.939 $\pm$ 2.143 g which was not statistically significantly different. The recovery rate of bolus B was 61% and that of bolus C 41%.

No attempt was made to recover copper oxide needles from the gastrointestinal tract of the lambs. It is assumed that with this type of device, the needles dissolve within the framework of the bolus. If the boluses break up for any reason (such as chewing on administration), the needles may be released and thereafter lodge in the intestinal tract.

DISCUSSION

The results show that the administration of two slow release mineral boluses to a group of lambs outdoors is effective in significantly raising the copper status of the liver. The actual proportion of an oral copper dose which is retained in the liver of sheep outdoors is

similar for a range of copper compounds and has been investigated by several workers. MacPherson and Hemingway (1968) found that on average 1.79%, 1.80% and 1.61% of liver copper accumulated respectively when copper sulphate, copper glycine and copper EDTA were given by oral drench to groups of sheep outdoors. Edgar (1942) found figures varying between 1 to 3.9% (average 2.3%) when sixteen sheep were given 25 mg copper sulphate daily at grass. Hemingway et al (1962) found an average figure of 2.4% copper retention of orally administered copper sulphate in grazing sheep's liver.

The CuO wire supplied as the copper source within the bolus has been shown to have a wide range of accumulation in different animals in the experiment, varying from 1.099 to 7.198% where two bullets were recovered partly worn. A possible source of some of the variation is likely to have arisen from pre-treatment liver copper levels in bolused animals. Control animals were seen to have a wide spread in liver copper level. An estimate was made of initial pre-treatment copper levels of bolused animals, based on control animals at slaughter. In extreme cases, the initial liver copper was perhaps significantly higher or lower than the estimate. However, Suttle (1974a) has demonstrated that copper metabolism in sheep varies widely due to differing abilities to

absorb copper via oral supplementation. He has shown that intravenous copper infusion produced considerably more constant results during a copper repletion trial with deficient ewes.

While the bolus did demonstrate its ability to elevate liver copper levels, there was some question over the fate of boluses which were not recovered. Two estimates of percentage copper accumulation were therefore made, to encompass the explanation that what was not recovered had eroded completely, or alternatively, that it had been regurgitated and lost. Dealing with animals where both bolus residues were recovered, the average percentage liver copper accumulation of 3.5% was within the normally accepted range. Where one or both boluses was not recovered, and assumed completely dissolved, the average percentage copper retention was 0.949%, significantly lower than 3.5%. If the non-recovered bolus was assumed to have been completely lost, the average liver copper accumulation figure of 4.26% from the erosion of one bolus was not significantly different statistically from 3.5%. It would appear then that boluses which were not found at slaughter had been regurgitated. When neither bolus was present at slaughter, the average copper accumulation by the liver was relatively low ($0.77 \pm 0.35\%$), therefore it was unlikely to have arisen from the rapid erosion of both boluses. The increase

in liver copper levels of these lambs compared to untreated animals implied that perhaps the boluses dissolved for a period of time and then both were regurgitated.

There was no detectable difference in eventual liver copper content between animals given two B or two C type boluses despite their different copper oxide needles content. The margin between the copper oxide dose is likely to have been too narrow. Indeed Whitelaw et al (1980) found no difference in physiological response in two groups of sheep dosed with 4 or 8 CuO(n). When copper concentrations in the blood plasma were within the normal range as with this experiment, Judson et al (1982) showed that they were unaffected by liver copper load. This was found to be the case and blood levels did not significantly alter over the sixty-nine day period.

Since a greater percentage of the more dense bolus B was found at slaughter, there is likely to be a density-dependent retention effect. Ritchie (1966) has demonstrated that recovery rates of intraruminal devices varied directly with their densities over a period of twenty-six days. The fact in this case, that the percentage of boluses not recovered increased at each sampling date implied not that the boluses had completely dissolved, but that perhaps size also played a part in regurgitation. When boluses had

dissolved to a certain degree, and their size reduced accordingly, they may have been more vulnerable to regurgitation.

Both B and C boluses dissolved at approximately the same rate throughout the trial yet their recovery rates varied by 20% further promoting the theory that it was not rapid erosion which influenced the recovery rate, but density.

Had the bolus administered remained in the reticulum throughout the trial, it seems that they would have proved to be a reasonably effective mineral supplement, although perhaps dissolving up to four times more slowly than required.

A large number of boluses were known to have been rejected. Earlier investigators (Dewey et al, 1958) recommended that densities of heavy cobalt boluses should exceed 4.1 gcm^{-3} in order to improve on rejection rates which at 2.7 gcm^{-3} density varied between 7 and 23% after the first month. Even with selenium boluses with an average density of 5.0 gcm^{-3} (Andrews, Grant and Brunswick, 1974), a rejection rate of 16% over twelve months was recorded.

The problems of rejection of this type of bolus in sheep must be overcome before further progress can be

made. It is likely that an increase in density would partly solve the situation although how to accomplish this in practice in a compressed bolus with copper oxide needles weighting is not certain.

5. Copper Levels in Bolused and Control Ewes and their Lambs

INTRODUCTION

The previous experiment (number 3:4) has shown that the intraruminal bolus caused an elevation in blood and liver copper levels in treated lambs. One common manifestation of copper deficiency is immediate or delayed swayback in newborn lambs. Supplementation of ewes with copper in late pregnancy prevents the occurrence of this disease by indirectly maintaining adequate copper levels in the lambs. The experimental bolus could be an effective form of supplementation for this purpose, and could be preferred to the alternative methods of oral dosing or copper injection. The experiment described below was designed to test whether lambs born to dams bolused during pregnancy showed any advantage with respect to copper levels, compared to lambs of untreated ewes. Two different copper doses in the boluses administered were intended to determine whether there was any link between copper dosage of the ewes and the subsequent lamb blood and liver copper levels, and ewe copper levels in blood and faeces. Whitelaw (1983) showed that in a deficiency situation on improved hill pasture, the onset of hypocupraemia was marginally delayed in lambs of ewes dosed with 4 g copper oxide needles ten days

prepartum. The boluses tested in this trial contained the improved formulation with heptahydrated zinc sulphate (as Section 2:5) which was expected to erode more rapidly than the previously tested Thomson and Capper boluses containing monohydrated zinc sulphate.

MATERIALS AND METHODS

(a) Sheep

A flock of ninety-eight Greyface (Border Leicester and Blackface) ewes were brought indoors about six weeks prior to lambing and bolused about three weeks before the first ewe was due to lamb. Ewes were randomly assigned to three similarly sized groups. All were blood sampled from the jugular vein by Vacutainer prior to bolus treatment. Boluses were administered in pairs via an intraruminal balling gun to a total of sixty-three ewes, thirty-two receiving two of one type, and thirty-one receiving two of the other type. After exactly two weeks, all ewes were blood sampled as before and faecal grab samples were taken. Lambing commenced the following week and spanned a period of twenty-six days with blood and faecal samples being collected from the ewes shortly after they had lambed. Following sampling, except in rare cases when lambs were poorly, the

ewes and lambs were turned out to grass soon afterwards. Twelve weeks after the start of the experiment, all lambs and ewes were collected in a pen, blood sampled and faeces taken from all the ewes. Any ewe or lamb which died over the experimental period had its liver removed, dried and analysed for copper content. Blood and faecal samples throughout were also analysed for copper content. If it was possible, boluses were recovered from the reticulum of dead ewes.

(b) Boluses

There were two types of bolus which were composed of the mineral base outlined in Section 2:5, of the same dimensions (16 x 32 mm) as the previous experiment. Type R had 26% of its weight as copper oxide needles and type Y had 13% copper oxide needles. The density of a type R bolus was 2.96 gcm^{-3} and its weight was approximately 14.25 g so that 3.7 g consisted of copper oxide needles. The total dose to each ewe was therefore 7.4 g copper oxide needles. Each ewe receiving type Y boluses received only 3.2 g copper oxide needles in total since the average bolus weight was 12 g. The density of type Y was approximately 2.89 gcm^{-3}.

Types R and Y contained sufficient minerals for a

period of up to five months although copper was present in excess in R.

Boluses were manufactured by Thomson and Capper Ltd., Runcorn, Cheshire, and the comparison was of the preferred formulation containing zinc sulphate in its heptahydrated form.

RESULTS

Individual ewe blood copper levels for the entire experimental period are shown in Table 34. Prior to administration of the bolus, the mean ewe plasma copper level was 1.04 $\pm$ 0.15 µg/ml. The normal spread is 0.6 to 1.6 µg Cu/ml plasma, so these values were well within the adequate range. After two weeks the average values had increased slightly to control = 1.124 $\pm$ 0.133 µg/ml, R bolused = 1.154 $\pm$ 0.159 µg/ml and Y bolused = 1.214 $\pm$ 0.156 µg/ml. The differences between these are non-significant.

Faecal copper levels were significantly different in both bolused groups two weeks post treatment. For the Y group $p \leq 0.01$ and $R \leq 0.05$ compared to control values. R and Y did not vary significantly from each other. Thereafter samples were taken as and when sheep lambed. In the first two weeks of lambing (three to five weeks post bulletting) the average

blood levels were as follows: R bolused = 1.16 $\pm$ 0.187 µg Cu/ml, Y bolused = 1.264 $\pm$ 0.176 µg Cu/ml, control = 1.271 $\pm$ 0.261 µg Cu/ml. There was no significant difference between any of these values. For the same period, the faecal copper excretion for each group was similarly non-significant. In the second fortnight of lambing blood copper levels did not vary significantly between groups nor did faecal copper content. Tables 35 and 35a show lamb and ewe liver copper values from animals which died. The lamb liver copper levels did not vary statistically when those born of treated ewes were compared with those born of untreated ewes. However, the copper content of ewe livers from treated and untreated animals were statistically significantly different at $p < 0.02$.

At the later sampling date, twelve weeks post bulletting, no difference was found in ewe faecal copper levels. The untreated group had an average copper content of 38.039 mg/kg, the R group had an average level of 37.371 mg/kg, and the Y group had a level of 39.997 mg/kg. Plasma copper levels in lambs were as follows: lambs born of R group ewes = 0.833 $\pm$ 0.155 µg Cu/ml, those born of Y group ewes = 0.842 $\pm$ 0.187 µg Cu/ml, and those born of control ewes = 0.854 $\pm$ 0.169 µg Cu/ml. There was therefore no statistically significant difference among these three groups.

Boluses were recovered from three out of four treated ewes which died ten to twelve days post bulletting. Prolapsed uteri were found to be the cause in all three cases. Boluses residue weights are shown on Table 36. The average loss of the four residues was 6.280 ± 1.997 g.

It is not certain whether the two boluses not found at post mortem had completely eroded or else been regurgitated. Judging by the residue weights, the boluses were dissolving relatively quickly, and were, on average, 57% of their original weight,which, had they continued to dissolve at that rate, would imply an active life of about twenty-eight days.

TABLE 34. Individual ewe plasma copper levels over the experimental period

NS = no sample

Ewe No.	Bolus Treatment	Day 0 Plasma Copper Levels (µgCu/ml)	Day 14 Plasma Copper Levels (µgCu/Ml)	Lambing Day (in brackets) Plasma Copper Levels (µgCu/ml)		Day 82 Plasma Copper Levels (µgCu/ml)
R15	R	1.21	1.04	1.37	(34)	NS
R20	Y	0.80	1.26	1.16	(43)	1.01
R22	R	0.68	1.10	1.09	(27)	0.91
R23	Y	1.06	1.45	1.23	(48)	1.54
R30	R	1.01	1.35	NS		NS
R41	R	1.09	1.12	1.21	(43)	1.05
R45	O	1.07	1.41	1.23	(25)	NS
SR47	R	1.42	1.50	1.36	(22)	1.38
Y42	Y	1.32	1.32	1.10	(27)	1.33
Y50	R	1.01	1.40	1.35	(32)	1.05
R54	O	0.96	1.23	1.18	(27)	0.97
R59	R	0.95	1.18	1.02	(29)	1.05
R60	Y	0.88	1.11	1.55	(48)	0.95
R61	O	0.73	1.10	NS		NS
R67	O	0.99	1.24	NS		NS
R78	O	0.88	0.87	1.14	(34)	1.08
Y85	Y	0.99	0.99	1.16	(25)	0.90
Y86	O	0.84	1.10	1.00	(28)	1.04
R86	Y	1.37	NS	0.98	(28)	0.81
Y89	Y	1.08	NS	NS		NS
Y90	R	0.97	1.07	0.94	(28)	1.10
R99	Y	1.19	1.36	1.20	(22)	1.54
R129	O	1.00	1.14	1.05	(32)	1.25
R136	Y	1.04	0.94	1.11	(32)	1.04
R149	Y	0.76	1.25	NS		0.98
R150	O	1.04	0.95	0.95	(29)	1.13

Ewe No.	Bolus Treatment	Day 0 Plasma Copper Levels (μgCu/ml)	Day 14 Plasma Copper Levels (μgCu/ml)	Lambing Day (in brackets) Plasma Copper Levels (μgCu/ml)	Day 82 Plasma Copper Levels (μgCu/ml)
R151	Y	0.88	1.04	NS	NS
R164	O	1.06	1.26	1.25 (25)	NS
R166	R	0.93	1.38	1.18 (39)	1.10
R167	R	0.98	NS	NS	NS
R182	Y	0.88	1.46	1.35 (27)	1.09
R189	O	0.87	0.89	1.51 (34)	1.11
Y191	O	1.21	NS	NS	NS
Y192	O	1.11	1.16	NS	NS
R197	Y	1.06	1.41	1.37 (27)	1.36
Y200	Y	0.98	1.17	1.31 (32)	0.97
R211	O	0.85	1.08	NS	1.07
R215	R	1.52	1.06	1.09 (22)	0.99
R221	R	0.90	1.05	1.29 (28)	NS
R223	O	1.03	1.23	1.15 (32)	1.16
R227	O	1.06	1.04	1.46 (27)	0.97
R232	O	1.09	1.35	1.24 (28)	1.03
R237	O	0.98	1.16	1.48 (25)	1.18
R240	O	1.10	1.41	NS	NS
R241	Y	1.10	NS	NS	NS
R251	O	1.12	1.17	1.19 (39)	1.39
R252	Y	1.02	1.09	NS	1.11
R253	R	0.94	0.95	NS	NS
R256	R	0.81	NS	NS	0.91
R260	R	0.79	1.04	1.50 (25)	NS
R262	R	0.98	1.02	2.02 (36)	1.01
R263	R	0.96	1.14	1.39 (36)	NS
R264	O	0.95	1.25	NS	1.21
R268	O	0.85	1.12	0.86 (36)	0.88
R269	Y	0.96	1.59	1.52 (28)	1.65
R271	Y	1.23	1.22	NS	1.38
R272	O	1.16	0.88	1.11 (36)	1.06

Ewe No.	Bolus Treatment	Day 0 Plasma Copper Levels (µgCu/ml)	Day 14 Plasma Copper Levels (µgCu/ml)	Lambing Day (in brackets) Plasma Copper Levels (µgCu/ml)		Day 82 Plasma Copper Levels (µgCu/ml)
R273	O	0.99	1.03	1.13	(25)	1.35
R276	R	1.16	1.15	1.29	(22)	1.07
R288	O	0.85	1.08	NS		NS
R421	O	1.11	NS	1.64	(39)	NS
R422	O	1.03	1.13	1.10	(32)	0.80
R423	O	1.16	1.22	1.87	(27)	1.15
R424	R	1.04	NS	1.06		0.95
R426	Y	0.93	1.17	NS		0.83
R428	R	1.00	1.57	2.20	(32)	1.31
R429	O	1.16	0.98	0.98	(28)	0.87
R430	O	0.98	0.87	NS		NS
R431	Y	1.26	1.20	1.25	(36)	1.22
R432	Y	1.14	NS	1.47	(34)	NS
R433	R	1.31	1.25	NS		1.22
R434	O	1.03	1.20	1.33	(25)	1.01
R435	R	1.22	1.36	NS		NS
R436	O	1.28	1.12	1.85	(43)	NS
R437	R	1.18	1.10	0.98	(39)	1.25
R438	O	0.90	1.24	1.27	(25)	NS
R440	O	1.00	1.18	1.04	(25)	0.90
R441	Y	1.21	1.35	1.43	(32)	1.44
R442	R	1.24	1.34	1.30	(39)	NS
R443	Y	1.16	1.08	1.52	(29)	0.76
R444	Y	1.15	NS	1.13	(34)	1.09
R445	R	0.94	1.05	0.84	(27)	0.91
R446	Y	1.25	1.09	NS		0.90
R447	Y	1.15	1.10	1.26	(22)	0.93
R448	Y	1.00	1.30	1.18	(25)	0.87
R449	O	0.88	1.00	NS		1.15
R450	R	1.07	1.15	1.27	(39)	1.20
R451	R	1.03	1.08	NS		1.19

Ewe No.	Bolus Treatment	Day 0 Plasma Copper Levels (µgCu/ml)	Day 14 Plasma Copper Levels (µgCu/ml)	Lambing Day (in brackets) Plasma Copper Levels (µgCu/ml)		Day 82 Plasma Copper Levels (µgCu/ml)
R453	Y	1.13	1.20	1.20	(32)	0.84
R454	O	1.01	1.26	1.16	(32)	NS
R455	Y	1.00	1.14	NS		NS
R456	R	0.88	1.17	1.09	(29)	1.03
R457	R	0.93	1.17	1.00	(32)	0.99
R458	Y	1.07	NS	NS		NS
R459	R	1.04	1.07	1.05	(32)	0.85
R460	O	0.92	1.10	1.27	(34)	1.24
S481	R	1.06	1.04	1.17	(29)	NS
R452	R	1.19	1.05	1.89	(43)	0.83

TABLE 35. Lamb liver copper content at post mortem

Lamb No.	Week of Death	Treatment of Parent Ewe	Cu mg/kg DM Liver
458(i)	2	Y	68.24
458(ii)	2	Y	104.72
241(i)	2	Y	85.90
241(ii)	2	Y	61.89
Ewe No. Y60 No number	7	Y	6.70
x(Cu mg/kg) in liver = 65.49 $\pm$ 36.85			
428(i)	5	R	142.53
428(ii)	5	R	270.55
Ewe 164 No number	5	R	387.55
Ewe 221 No number	5	R	180.51
x Cu(mg/kg) in liver = 245.29 $\pm$ 108.98			
321	5	O	271.90
346	5	O	126.25
429	6	O	40.24
x Cu(mg/kg) in liver = 146.13 $\pm$ 117.10			

TABLE 35a Ewe liver copper content at post mortem

Ewe No.	Week of Death	Treatment	Cu mg/kg DM Liver
241	2	Y	249.77
458	2	Y	218.64
x Cu(mg/kg) in liver = 234.205 $\pm$ 22.01			
454	5	O	99.29
288	6	O	177.95
Y192	6	O	107.40
x Cu(mg/kg) in liver = 128.21 $\pm$ 43.26			
R164	7	R	247.74
R435	2	R	N.S.

TABLE 36. Bolus weights on recovery from treated ewes

Ewe No.	Bolus Administration	Bolus Weight on Recovery (g)	Approx. Weight loss (g)
R241	Y(i)	7.883	4.117
	Y(ii)	6.919	5.081
R458	Y(iii)	5.977	8.273
	Y(iv)	Not Recovered	Not Known
R435	R(i)	6.601	7.649
	R(ii)	Not Recovered	Not Known
R164	R(iii)	Not Recovered	Not Known
	R(iv)	Not Recovered	Not Known

DISCUSSION

Lamb copper levels did not appear to be influenced in a significant way by maternal supplementation of additional copper and minerals at 2-6 weeks prepartum. In a deficiency situation, the onset of hypocupraemia may be delayed in lambs of treated ewes but obviously there was no definite effect on lambs in circumstances of sufficiency.

The bolus was shown to be efficacious in that there was a significant advantage in the liver copper content in ewes which had been bolused and later died from other unrelated causes compared with similar untreated ewes. Indeed the last ewe which died (on week 7) had almost exactly the same liver copper in mg of dry matter as the first ewes which were sampled on week 2, proving the long term effectiveness of the bolus despite residue evidence which implied that the active life of the bolus was about one month. Copper is capable of being stored in the liver, and copper oxide needles administered in this way may be accumulated towards a liver copper store, irrespective of length of bolus life, and may be mobilised when dietary copper is low.

Sixty-seven percent recovery of boluses after twelve days post bulletting is in accordance approximately with results from the previous experiment

(Section 3:4) when lambs were serially slaughtered and bullet recoveries noted.

Retrospectively it would have been desirable to have made attempts to improve the bolus retention rates noted in the previous trial by experimental alteration of the shape or density of the boluses. Regrettably analysis of previous results could not be fully accomplished before this trial was underway. At the time it had been thought that more extensive information on the same types of bolus would be beneficial prior to the knowledge of the extent of regurgitation of these boluses in the previous experiment. In ewes, bolus retention rates may, in any case, have been superior, because their reticula are deep pouches compared with the more shallow pocket in the lamb.

The inclusion of heptahydrated zinc sulphate apparently improved the erosion rate as predicted, compared to Thomson and Capper sheep sized boluses tested in experiments which had the mistaken addition of zinc monohydrate. Therefore it may be technically possible to adjust erosion to the desired length by manipulation of the zinc sulphate mono- and hepta-hydrate components.

SECTION IV

1. Mineral Bolus as a Drug Carrier

Since the erodable bolus had been reasonably satisfactory as a supplier of minerals, attempts were made to use the matrix as a carrier for the provision of other materials on a slow release basis, such as anthelmintics and growth promoters. The effective dose of anthelmintic or growth promoter when administered on a slow release basis, compared to the normal single shot amount, is known to be much reduced. Anderson et al (1980) quote that one tenth of the single oral dose of oxfendazole administered continuously over four days was completely effective against adults of Ostertagia, Trichostrongylus and Nematodirus species.

The period of time over which a slow release drug treatment should be effective, ideally would be as long as possible, with the ultimate aim with an anthelmintic that it could be administered at turnout and prevent parasitism over the complete grazing season in a similar way to Paratect (Pfizer Ltd.).

When medicated ingredients were added to the existing bolus, it was anticipated that the release rate would be changed, and perhaps some of the

properties of the bolus altered. The limits of tolerance of variation in daily erosion would obviously be much narrower than that of a mineral only bolus because of the danger of toxicity from excess, or in the case of anthelmintics, from the possibility of clinical disease becoming established if the release rate was too low or if the bolus dissolved too rapidly leaving the animal susceptible to infection.

It was decided that the large 2.5 cm bolus should be mainly used in experiments on the development of levamisole, monensin and morantel boluses in order to lessen the percentage of material added to the basic mineral constituents.

2. Levamisole Boluses

INTRODUCTION

Levamisole hydrochloride is a white crystallic highly water soluble broad spectrum anthelmintic in common usage. Although it is available in drenches and injections, at present there is no slow release system of administering this anthelmintic. It was estimated (J. Armour personal communication) that an appropriate dose for cattle was 0.15-0.2 g/d when administered in a slow release intraruminal bolus. It was intended to develop a bolus with an active life of 90-120 days.

MATERIALS AND METHODS

(a) Boluses

The first bolus containing levamisole (L1) had 6 g of levamisole incorporated into the mineral (12 g) component and then copper oxide needles were carefully mixed in, and the bolus compacted in a 17 mm diameter mould, and coated with two ends left exposed as described in Section 2:3. This in combination with another identical bolus would then supply two months anthelmintic treatment.

After preliminary testing of L1, boluses were manufactured as detailed in Table 37. All were 25 mm in diameter apart from number L3 which was 19 mm, and all had one exposed face.

(b) Experimental

Boluses were administered in combination with another bolus of a similar size to fistulated cows indoors. They were examined and weighed at approximately weekly intervals.

RESULTS AND DISCUSSION

Bolus L1 began by eroding at an impressively steady pace, losing 0.5 g per day over forty days (Figure 32). The constancy and regularity of the erosion was actually an improvement on boluses which contained mineral alone. Two boluses of this type in combination would therefore supply all the anthelmintic necessary for a two month treatment period.

The larger boluses, L2, L3, L8, L7, L12 and L13 erosion pattern is shown on Table 37. There was some variability in the release rates over the first seven days, but thereafter the erosion was fairly steady when the bolus coating remained intact. One of the most impressive features of levamisole boluses was that they were flat topped and kept the same surface area

throughout testing. The glass fibre resin coating adhered well to the surface and was obviously chipped off very slowly as the bolus eroded. Plate 7 illustrates this point - bolus (d) has an equally flat top as bolus (a). Where compared with Plate 8 of growth promoter boluses (described in a later section) levamisole boluses have superior dissolution characteristics.

Although growth promoter boluses have a reasonably constant surface area, they do not have the uniformly flat top of the levamisole boluses.

When the weights of L2 and L7 were plotted graphically their release rate was remarkably similar (Figure 32) despite the fact that they contained different amounts of levamisole (L2 had 23%, L7 had 25%). Had the coating remained fully intact, and the boluses continued to erode at about 0.8 g/d, they would have lasted a total of 100 days and supplied the target amount of approximately 0.2 per day. A similar release rate was shown by bolus L13, although it was initially faster at days 0-7. The original objective of developing an anthelmintic treatment to cover the grazing season would then have been fulfilled.

Despite the very promising results with levamisole boluses, further improvements on the coating and release

rate were not purused. It was simply noted that levamisole was very well suited to this metbod of administration and could possibly be developed further in the future.

TABLE 37. Average weight lost/day (g) by levamisole boluses

Bolus No.	Constitutents of 25 mm dia. boluses	Average Weight Loss Per Day (g)						
		d0-d7	d7-d14	d14-d21	d21-d28	d28-d35	d35-d42	d42-56
L2	18 g levamisole 25 g mineral mix 35 g CuO(n)	0.19	0.53	0.76	0.87	0.84	2.03^{o}	2.45^{o}
L3^{x}	14 g levamisole 8 g mineral mix 20 g CuO(n)	3.13	0.81	0.86	0.79			
L8	18 g levamisole 25 g mineral mix 35 g CuO(n)	0.19	0.72	*0.41 over 14d				
L12	20 g levamisole 25 g mineral mix 35 g CuO(n)	0.87	1.34/	1.74≠				
L13	18 g levamisole 20 g mix A 35 g CuO(n)	1.99	1.08	0.91				
L7	20 g levamisole 25 g mix A 35 g CuO(n)	1.02	0.94	0.45	1.48/	1.73≠	1.56 average	

x 19 mm diameter bolus
* bolus present alone for part of this period

/ base beginning to break
≠ base off completely - two ended
o coating coming off sides

FIGURE 32. The erosion pattern of boluses L1, L2, L3, L7 and L14 which contained mineral mix, copper oxide needles and levamisole hydrochloride according to Table 37. L1 (not on table) contained 6 g levamisole and was 17 mm in diameter with two exposed faces. L3 was 19 mm in diameter with one exposed face, and the remainder had one exposed 25 mm diameter face.

FIG.32 Release pattern of boluses containing Levamisole hydrochloride

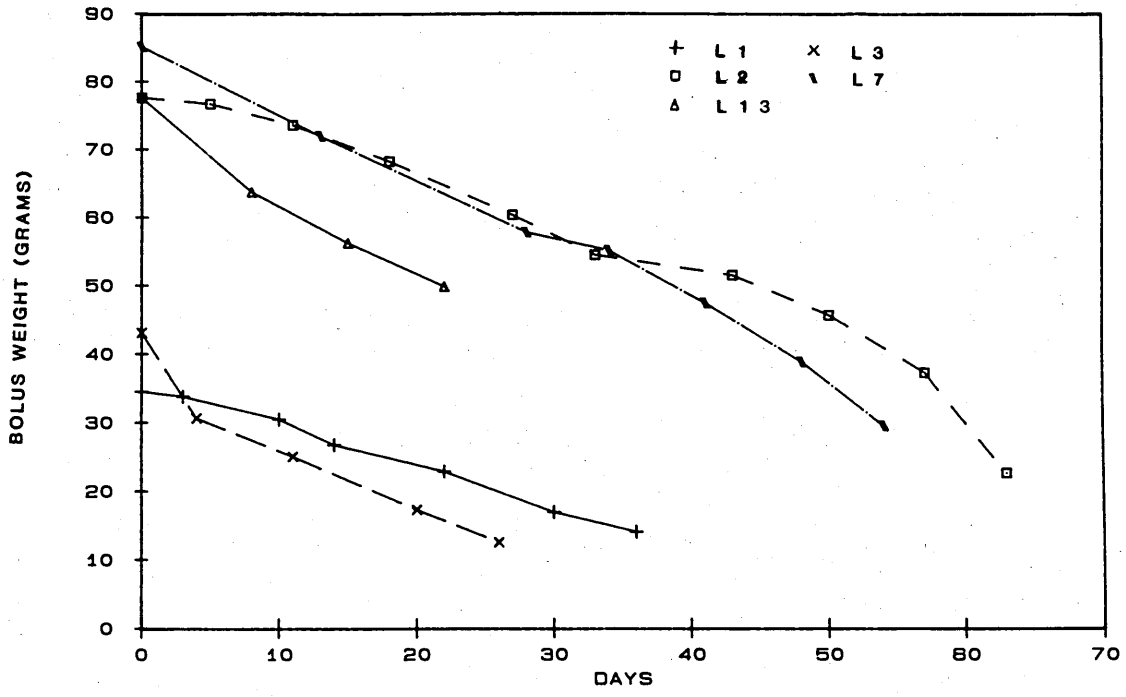
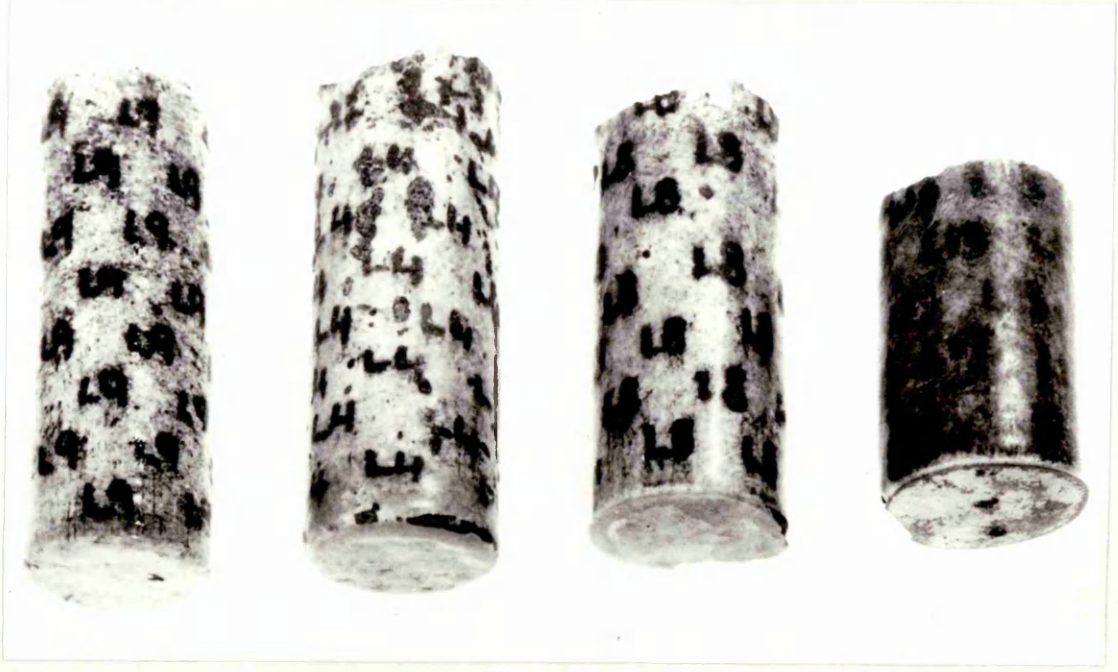


PLATE 7. Boluses containing levamisole hydrochloride showing progressive erosion from left to right.



3. Boluses Containing Monensin Sodium

INTRODUCTION

Monensin sodium has been recommended for improved food conversion and weight gain in growing and fattening cattle. This is achieved by improving the efficiency of rumen fermentation and altering the proportions in which the volatile fatty acids are produced. The ruminal propionate levels increase and acetate levels decrease but the total of volatile fatty acids and the level of butyrates in the rumen remain largely unchanged (Dinius et al, 1976).

Monensin has been used in the control of coccidiosis in lambs (Bergstrom and Maki, 1974), and a similar role in pigs has been suggested (Roberts and Walker, 1981). Other uses in cattle include the control and prevention of ketosis in dairy cows fed on poor quality silage (Rodgers and Hope-Cawdrey, 1980), and the prevention of acute pulmonary oedema and emphysema by reducing the ruminal conversion of L-tryptophan to 3-methylindole (Hammond et al, 1978).

Production of a slow release bolus containing the growth promoter monensin sodium was investigated with a view to commercial application.

Pure monensin sodium was obtained through the Calbiochem - Behring Corporation. A premix "Romensin" ccntaining 100 g monensin/kg for adding to animal feed is readily available at less cost but this was not suitable for inclusion in a bolus, because of the low concentration content of the active ingredient.

The target dose rate for a 250-450 kg beast is 0.2 g/hd/day of monensin. A bullet to last one month therefore would contain 6 g monensin.

It is recommended, particularly for heavy animals, that monensin be gradually introduced into the diet. To start with, half of the normal dose rate should be administered. Therefore it was a cause for concern that the normal pattern of mineral bolus erosion which has an "initial surge" of dissolution would lead to toxicity since the animals would receive a relatively large dose of monensin over a short period. As a precaution, the first two boluses manufactured were arranged in layers. The top 1 cm of bolus M1 at the exposed face contained no monensin; it was contained in the lower layer mixed with the remainder of the mineral. Since the effect of monensin on dissolution was completely unknown, only one gram of monensin was incorporated in the first two boluses.

MATERIALS AND METHODS

Monensin boluses M3, M7, M8 and M12 were manufactured according to the method detailed in Sections 2:2 and 2:3 using the mineral base of Section 2:2 unless otherwise specified. In all boluses but M1 and M2, the total amount of mineral in the formulation was added in small amounts for thorough handmixing with the monensin. Copper oxide needles were then added. In boluses M1 and M2, copper oxide needles were added to the mineral mixture, thoroughly mixed, and approximately one quarter of this decanted into another pot. Monensin was then thoroughly mixed with the remaining material. On pressing M1 and M2, half of the pure mineral and copper oxide needles mix was firstly poured into the mould followed by the mineral and monensin mix, and the remaining pure mineral mix was poured on top. Pressing then proceeded as normal. Boluses were coated four times with Plasting Padding glass fibre resin leaving the number of exposed faces as detailed in Table 38. Formulae and size are also detained in Table 38. Boluses were administered in pairs to fistulated cows on an indoor hay and concentrate diet.

TABLE 38. /

TABLE 38. Formulations of different monensin test boluses

Bolus	Mineral (g)	Copper Oxide Needles (g)	Additional Zinc Sulphate	Levamisole (g)	Dia. mm	No. Open Faces	Quantity Monensin (g)
M1	18	15.7	-	-	17	1	1
M2	18	15.7	-	-	17	2	1
M3	14	15	-	-	17	1	3
M7	12	15	2		19	1	3
M8	30	31.5	3		25	1	6
M12	25	31	2.22	2	25	1	6

RESULTS AND DISCUSSION

The erosion pattern of boluses M1, M2 and M3 are shown in Figure 33. The mean weight loss of M1 over thirty days was 0.46 g/d; that of M2 0.328 g over twenty-nine days; M3 was 0.205 g/d over twenty-three days. However in all cases the erosion rate plateaud out during certain periods, particularly during the mid point of the trial period.

Monensin sodium is a fairly dense water insoluble powder. When incorporated into boluses, it was simply mixed by hand, but particular care was taken to ensure an even distribution. The sandwich arrangement of M1 and M2 was easily accomplished in practice, but when it was attempted to make a 3 g monensin sandwich (almost 10% of the total bolus weight), the bolus crumbled in the centre.

Since the results of M1 and M2 (Figure 33) seemed to show that monensin inclusion basically slowed release rate, half of one month's requirement (3 g) was incorporated into M3. However difficulty was experienced in coating monensin boluses - the liquid glass fibre resin tended to run off in streaks soon after application, due possibly to monensin's hydrophobic qualities. Between subsequent applications of resin, the boluses were sanded and great care was taken to attempt to produce a reasonably resilient cover.

PLATE 8. Progressive erosion of boluses containing monensin sodium.

Once it became evident that M3, despite having two open ends was eroding very slowly, plans were made to incorporate zinc sulphate heptahydrate into the matrix. Additional zinc sulphate is known from earlier development work on the mineral bolus, to have an accelerating effect on release rate. M7 was 19 mm in diameter, had 2 g additional zinc sulphate heptahydrate but still dissolved very slowly (see Figure 34). It was decided at this point to try the large 2.5 cm diameter bolus with the full 6 g (one month's) monensin requirement. The intention was to supply two of these per animal and design them to erode overy sixty days. M8 did erode at approximately the desired rate, but this was due in part to large sections of the coating peeling off and being relatively insoluble, the whole mineral matrix continued to erode slowly once the coating was removed. Knowing that the anthelmintic, levamisole hydrochloride was an excellent regulator of this method of sustained release, 2 g were added to bolus M12 in addition to monensin and zinc sulphate. This produced the desire result (see Figure 34) and the coating also remained relatively intact until the end.

A batch of 12 x monensin containing boluses of the following formulation were manufactured: 2.5 g zinc sulphate heptahydrate, 3.0 g levamisole hydrochloride, 25 g mineral mix, 5 g monensin and 30 g

copper oxide needles and used by research workers in California in a fog fever prevention trial in intact calves, along with several other treatments. It was reported that these boluses were the most efficacious treatment, and performed well in the field (R. Breeze, personal communication).

It was decided not to continue the further development of this bolus at this particular time. The known relatively low tolerance of cattle to monensin excess has been well documented (Collins and McCrea, 1978; Wardrope et al, 1983) and casts some doubts on this sytem of administration as a marketable device. If the bolus were to be chewed on administration, there would be a risk of toxicity, or alternatively if the animals reticulum contained an abnormally large quantity of gravel, the release rate might increase and reach toxic levels.

FIGURE 33. The erosion pattern of boluses M1, M2 and M3 of 17 mm diameter and varying formulations containing monensin sodium as detailed on Table 38, tested in fistulated cows.

FIGURE 34. The erosion pattern of boluses M7, M8 and M12 which contained varying amounts of monensin sodium (see Table 38). Numbers M8 and M12 were 25 mm in diameter, and M7 was 19 mm. Boluses were tested in fistulated cows.

FIG.33 Release pattern of boluses containing Monensin sodium

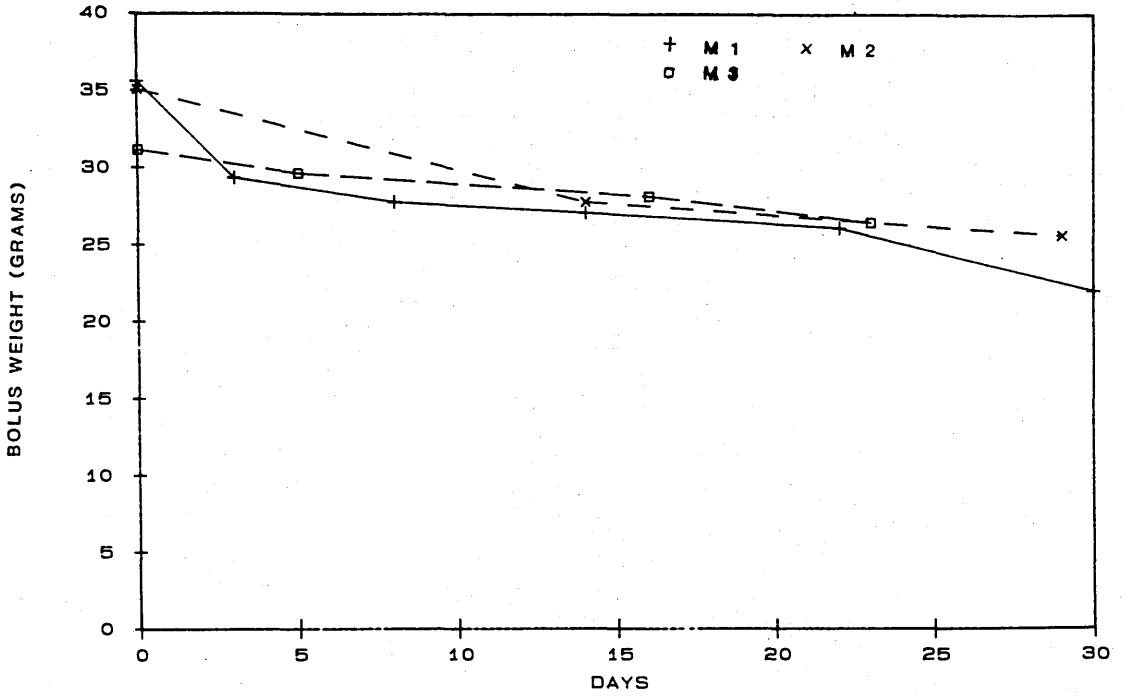
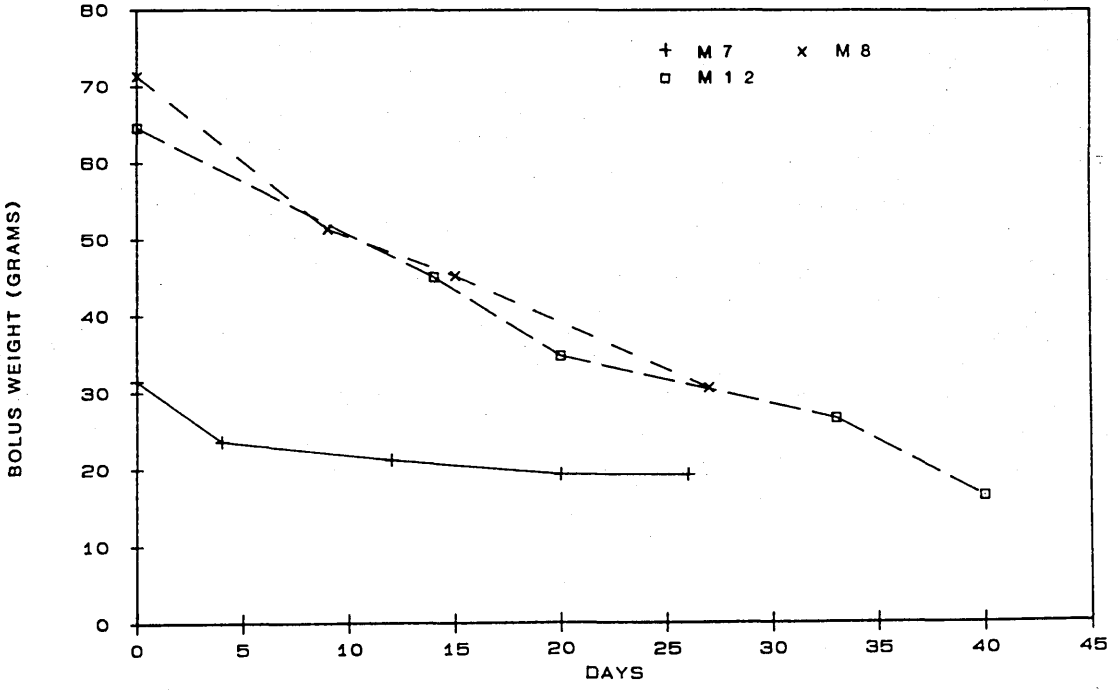


FIG.34



4. Morantel Tartrate and Citrate

INTRODUCTION

(a) Tartrate

The broad spectrum anthelmintic, morantel, currently present in the Paratect bolus (Pfizer Ltd.) was tested as a possible additive to the existing mineral bolus. At present, 13.5 g morantel tartrate is contained within the steel sleeve of the Paratect device for gradual elution through a semipermeable membrane over the grazing season. The major advantage of developing the Glasgow bolus as an alternative carrier would be that it has been designed to be completely degradable. While Paratect has been shown to be efficacious (Jacobs et al, 1982; Jones, 1981), there have been problems in abbatoirs with steel tube residues damaging machinery (Parkins, personal communication).

Morantel is available in two forms - the tartrate and citrate. The morantel tartrate powder which is currently available contains 59.6% active material. It is yellow in colour, and is soluble in water, after an initial phase when it appears to expand in the presence of water.

The recommended dose is 0.15 g active material per day which totals 22.5 g for ninety days. It was intended that two boluses would be supplied per animal, each containing 12 g morantel tartrate.

MATERIALS AND METHODS

In the first instance, the material was very difficult to compact in the mould, and boluses tended to crumble as they emerged from pressing. Boluses which had crumbled were carefuly rebroken into small crumb-like fragments, and pressed again. Generally the second pressing produced a satisfactory bolus. Further problems also arose in pressing the mixture in the 25 mm diameter mould.

Because of the extremely find grade of powder and the tolerance limit between plunger and mould, a layer of morantel became lodged between the two causing jamming of the plunger. This problem was partially resolved by fitting a new plunger with 0.001" limits of tolerance. Though there was still some problem with morantel tartrate clogging, it was possible to satisfactorily manufacture the boluses.

The bullets were lightly sanded before coating with three layers of glass fibre resin. Three bullets T1, T2 and T3, were administered to a fistulated cow.

The composition of T1, T2 and T3 is shown on Table 39.

TABLE 39. Composition of three morantel tartrate boluses

Bolus No.	Mineral Mix (g)	Copper Oxide Needles (g)	Diameter (mm)	Morantel Tartrate (g)
T1	30	35	25	12
T3	25	35	25	12
T6	20	28	25	12

RESULTS AND DISCUSSION

After 48 hours the bullets were recovered and weighed. Each bullet had lost at least half of its total weight within that period. However, the most critical observation was that the exposed active surface of the bullet was soft to the touch, exuding liquid when gentle pressure was applied. The resin coating appeared to be uncharacteristically soft and unsupported and had split in places.

On halving one bullet, it was seen that liquid had seeped into the matrix of the compacted mixture

and had been absorbed to a depth of about 1 cm from the top surface. This retention of liquid had caused expansion of the bullet contents and splitting of the coating.

It is likely that the bullet residue contained less morantel than would be expected from weighing, since spaces could be seen in the longitudinally sectioned bullet which probably had held particles of morantel.

Unfortunately these morantel bullets behaved in a manner quite unlike other bullets manufactured in this way. Normally, in an erodable bolus, the coating remained fixed and rigid to the same level as the active exposed surface, and there was no penetration of water below 1 mm depth.

(b) Citrate

EXPERIMENTAL

Research was then conducted into the behaviour of morantel citrate administered in a mineral bolus base. It was very similar in appearance to morantel tartrate and appeared to have similar physical properties in water. In order to make a more detailed study of the behaviour of the material, the boluses were individually

placed in small nylon bags on a nylon cord and suspended in the reticulum. C3 was initially examined at two hourly intervals. The formulation and results of testing of these boluses is shown on Table 40. Plate 9 shows examples of these boluses photographed at between five and twenty-four hours after administration.

DISCUSSION

It is obvious from these results that morantel is a substance which is entirely unsuited for administration in this type of bolus. The properties of morantel override those of any other constituent. It appears to act as a wick in drawing water into the bolus causing it to expand, with the resultant disruption of the integrity of the coat, and the subsequent swift disintegration of the bolus. Unsuccessful attempts were made to recrystallise morantel in water in an attempt to alter its properties before this approach was abandoned as unworkable.

TABLE 40. Composition of morantel citrate boluses

Bolus No.	Formula	Diameter	Weight Loss (g) of Boluses after period shown			
			2 hr.	4 hr.	14 hr.	24 hr.
C3	12 g morantel citrate 25 g mineral mix 32 g CuO(n)	2.5 cm	7.279	13.608	30.354	
C1	12 g morantel citrate 30 g mineral mix 35 g CuO(n)	2.5 cm				78.416
C4	12 g morantel citrate 25 g mineral mix 32 g CuO(n)	2.5 cm				70.397
C9	6 g morantel citrate 6 g ZnO 4 g mineral mix 14 g CuO(n)	17 mm				split glass fibre resin shell recovered
C7	6 g morantel citrate 10 g mineral mix 14 g CuO(n)	17 mm				glass fibre resin shell recovered

PLATE 9. Morantel citrate boluses photographed 5-24 hours post administration

5. Ivermectin Bolus Development

INTRODUCTION

The stage had been reached in bolus development where reasonable prediction could be made for the daily release rates and consequent bolus life, and this could be altered, within reason by adjustment of certain components of the formulation. It was at this point that it was felt that as well as being a useful device for the supply of nutrients, this bolus could be utilised as a vehicle for sustained drug release such as anthelmintics for long term prophylactic or therapeutic medication. The boluses already described which had been manufactured in Glasgow incorporating medicated ingredients, had in general been promising, providing the additional material was not strongly hydrophobic or hydrophilic, as there was then the risk of dramatically altering the properties of the mineral bolus. An ideal drug for administration via the slow release bolus would be efficacious in small doses, which would minimise its rate of inclusion in the bolus. There would thus be less likelihood of the drug interfering with the erosion rate of the basic bolus. One such material which may be suitable for long term slow release in a bolus would be ivermectin (MK 933).

The avermectins are a family of structurally

related compounds produced by the newly described actinomycete Streptomyces avermitilis. There are eight major avermectins, each having small structural differences. A chemical modification of avermectin B_1 was selected for commercial development. It is known as 22, 23 dihydroavermectin B_1 or ivermectin.

Ivermectin has a wide antiparasite and insecticidal effect when administered in quantities of 100 or 200 mcg/kg to cattle or sheep (Armour et al, 1980; Lloyd et al, 1980; Leamaster and Wescott, 1980; Meleney et al, 1980) by subcutaneous injection or oral dosing. The possibility of developing a slow release method for long term administration of ivermectin was particularly exciting because of the difficulty in controlling ectoparasites in many areas of the world such as Africa and South America, as well as endoparasites. Since the drug was to be administered continuously, it was estimated that 40 µg/kg/d would be sufficient to control parasitism. The total amount of ivermectin required for a ninety day period in a 200 kg beast would therefore be only 0.72 g, a quantity ideal for this method of administration, since it was not expected to dramatically alter the properties of the mineral bolus.

A prolonged release system of drug administration has many advantages. Considerable economies can be made in labour during the grazing season if no

anthelmintic treatment is required beyond that at turnout. In a study of comparative cost of treatment, Jones and Bliss (1983) showed that the Paratect anthelmintic bolus led to considerable financial savings compared to conventional anthelmintic routines. The bolus treatment also had the great advantage of "cleaning up" pastures by eliminating the parasites at an early stage in the season, and thereby interrupting the reproductive cycle thus preventing the midsummer rise in parasitic numbers, and reducing the possibility of infection in the subsequent year.

MATERIALS AND METHODS

Boluses were manufactured from standard laboratory mineral mixes as described in Sections 2:2 and 2:5, to which copper oxide needles had been added at a rate of 45% of the total. To ensure an even mix, the total amount of ivermectin required for a number of boluses was weighed out. Mineral mix was then added gradually in increasing amounts and thoroughly mixed until the correct concentration of ivermectin was present. The method of manufacture and coating was as previously detailed in Sections 2:2 and 2:3 respectively.

Preliminary screening of different ivermectin inclusion formulations and different bolus sizes was

performed in fistulated cows, initially for V1 and V2 on indoor diets and subsequently for V3-V14 at grass. Table 41 details the formulation and physical characteristics of the different mixes V1-V4

TABLE 41. Formulations and specifications of boluses V1-V14

Bolus No.	Mineral (g)	CuO(n)	Ivermectin (g)	Diameter (mm)	Zinc Oxide (g)
V1 ≠	27*	22.5	0.6	19	-
V2 ≠	27	22.5	0.6	19	-
V3	40*	35	0.9	25	-
V4	40*	35	0.9	25	-
V5	35*	38	0.9	25	10
V6	35	38	0.9	25	7
V7	26*	22.5	0.9	19	1.12
V8	25*	22.5	0.9	19	2
V9	27	22.5	0.9	19	-
V10	27*	22.5	0.9	19	-
V11	27	22.5	0.9	19	-
V12	27*	22.5	0.9	19	-
V13	27	22.5	0.9	19	-
V14	27	22.5	0.9	19	-

* no zinc sulphate heptahydrate

≠ tested indoors

All pellets coated to leave one open face

RESULTS AND DISCUSSION

With reference to Figures 35 and 36, it is clearly demonstrated that boluses with no zinc sulphate included in the formula in general eroded quickly at the start, in some cases not being recovered after a few days, for example V9 and V10. In others, erosion became slow and ultimately almost ceased in boluses surviving the initial surge, particularly in those formulations with added zinc oxide, i.e. Nos. V5, V6, V7 and V8. When both zinc sulphate and zinc oxide were present in one bolus (as in V6) the erosion pattern was more akin to zinc oxide containing boluses (such as V5) than zinc sulphate containing boluses such as V11, V13 and V14.

The most promising release rates were found with boluses composed of basic mineral mix (Section 2:5) which included zinc sulphate, ivermectin and no other ingredients: for example V2, over a period of thirty-seven days lost an average of 0.13 g/d. Had erosion continued at this rate, the bolus would have lasted for a total of 390 days. Boluses V11, V13 and V14 were tested in fistulated cows at grass, and despite the coating remaining intact, all three boluses had eroded by day 45. The average release rate was 1.2 g/d for V11 and V13 and 1.3 g/d for V14.

FIGURE 35. Erosion pattern of boluses V1-V7 of varying formulations which contained ivermectin as detailed on Table 41. V1, V2 and V7 were 19 mm in diameter, and V3-V6 were 25 mm diameter. Boluses were tested in fistulated cows.

FIGURE 36. Erosion pattern of boluses V8-V14 which contained 0.9 g ivermectin, 22.5 g copper oxide needles and 27 g of mineral mix with or without the inclusion of zinc sulphate (see Table 41). All boluses were 19 mm in diameter.

FIG.35 Erosion pattern of boluses containing Ivermectin

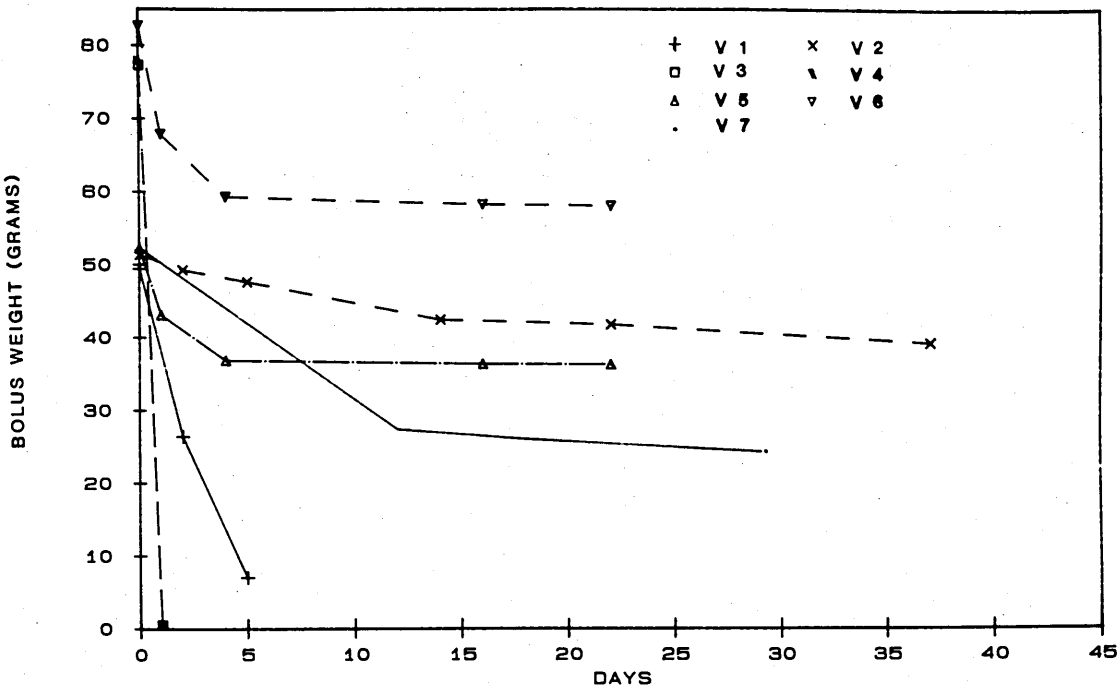
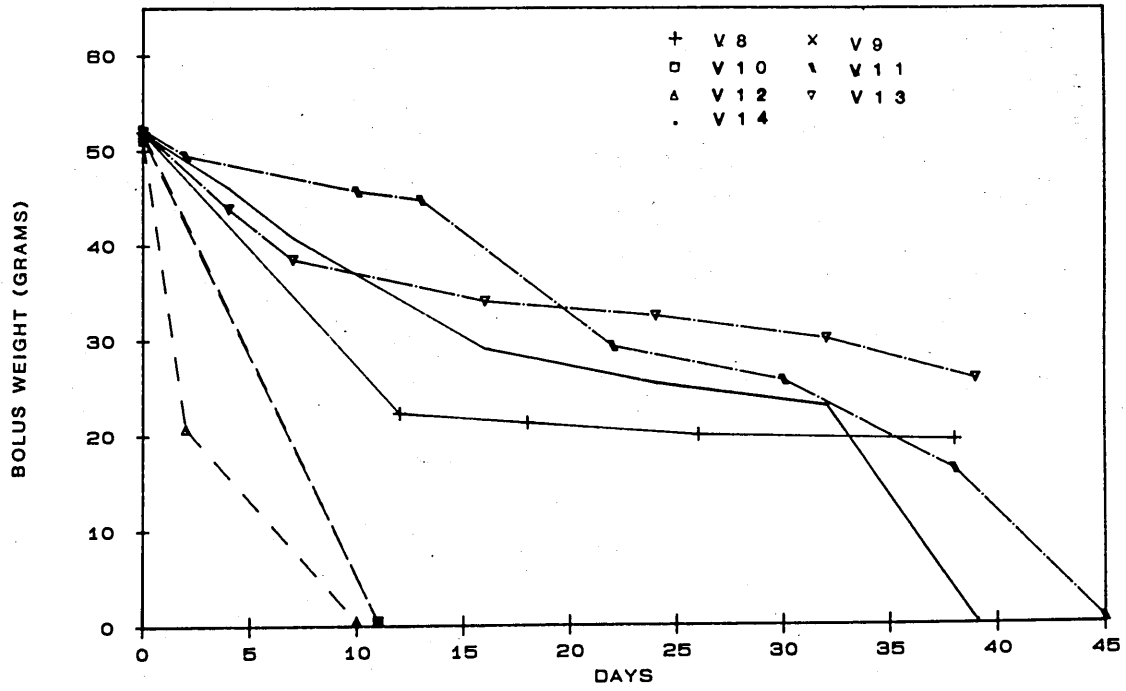


FIG.36



Ivermectin Bolus Development 2

INTRODUCTION

The most successful formulation from the previous trial used in boluses V11, V13 and V14, was then investigated more fully. It was intended to test several replicates of different sizes of bolus outdoors, as a further step towards the development of a commercially applicable ivermectin bolus.

It had been suggested that there may be some commercial advantage in developing a standard treatment regime consisting of one medicated and one non-medicated bolus. For calves, or where a lower dosage was required, one medicated bolus could be administered in conjunction with a mineral bolus. For higher dosage rates, animals could be given two medicated boluses.

MATERIALS AND METHODS

Boluses were made to the formulae shown on Table 42 by the method outlined in Section 2:2. However, some difficulty was experienced in the coating of the bolus. The presence of ivermectin seemed to cause the repelling of the coating to some degree, making it more difficult to apply a smooth even cover. Four coats of resin were applied, and one face was left exposed in each bolus. Boluses were paired so that

one composed of mineral alone, and one containing ivermectin were administered to each fistulated cow at grass. Two sizes were tested: 17 mm diameter and 19 mm diameter.

RESULTS AND DISCUSSION

The average daily weight loss of the boluses over the test period is shown on Table 42, and individual results are plotted on Figures 37 and 38. There were some problems with coating detaching prematurely, and where this occurred, it has been noted.

TABLE 42. Specification and average release rates of ivermectin and mineral boluses tested in cows at grass

Cow No.	Bolus No.	Bolus Formulation	Bolus Diameter (mm)	Average Weight loss/day (g) 0-21	21-40	40-
34	V24 ≠	25 g min 20.5 g CuO(n) 0.4 g IV	19	0.69		
	181 ≠	25 g min 20.5 g CuO(n)	19	0.71		
39	V26	18 g min 15 g CuO(n) 0.4 g IV	17	0.41	0.25	
	183	18 g min 15 g CuO(n)	17	0.14	0.27	
37	V28	20 g min 17 g CuO(n) 0.4 g IV	17	0.14	0.18	0.17
	185	20 g min 17 g CuO(n)	17	0.20	0.20	0.12
41	V29 ≠	26 g min 23 g CuO(n) 0.4 g IV	19	0.36	0.75	
	186	26 g min 23 g CuO(n)	19	0.26	0.28	

≠ coating detached during erosion

Boluses V24 and 181

After fifteen days the coating began to flake badly in both 181 and V24 so that they were removed after twenty-nine days. There was no apparent difference between the bolus containing ivermectin and the one which had mineral alone.

Boluses 183 and V26

When the coating was fully intact in bolus 183 (up to day 34), the erosion mean was 0.141 $\pm$ 0.036 g/day. The ideal rate for a bolus of this size was 0.38 g/d. After day 34, bolus 183 began to uncoat and part of the mineral matrix became exposed and unsupported. Because of its frangibility this portion accidentally broke off during handling of the boluses at weighing. Thereafter there was a resulting rapid loss of weight leading to complete disappearance by day 56. Bolus V26 also dissolved within fifty-six days although its pattern of erosion was slightly different from 183. V26 began to uncoat after the first weighing, so that between that and the second weighing (at days 6-13) a large amount was lost (about 3 x that required). Thereafter until around day 35, weight losses were small and steady and comparable to the same period in bolus 183. However, deep splits began appearing in the coat around day 34 so that by day 43 there was little coating left. The increased abrasion from two other objects (the two broken

pieces of 183), instead of only one probably also contributed to the very rapid disappearance of V26 by day 56.

Boluses 186 and V29

186 remained relatively intact throughout the experimental period. The average weight loss was 0.246 $\pm$ 0.076 g/d, based on the averages of each observation at weighing. This was approximately 50% of the ideal rate. Because of the intactness of the coating throughout testing, there were no large changes in gradient, corresponding to coating loss as with other bullets. V29, the partner initially dissolved at a comparable rate to 186, but very quickly began to lose the coating so that by day 36 only the base of the bolus had any coat remaining. The exposed mineral matrix then eroded very rapidly until it became "hardened" by the rumen environment, after which small steady losses of weight were noted. After the rapid period of erosion, the bolus became cone-shaped.

Boluses 185 and V28

These were 17 mm diameter boluses which could be directly compared to 183 and V26. The coating remained relatively intact throughout the experimental period, which is reflected by a slow steady weight loss by both boluses. The erosion was about 40% of the required rate:

$$V28 = 0.136 \pm 0.086 \text{ g/d}$$

$$185 = 0.17 \pm 0.072 \text{ g/d}$$

It is interesting to note that although the actual daily rates of each bolus's erosion was slightly different, there was a broad relationship between the two. That is, when V28 had a slightly decreased rate of dissolution between two weighings, 185 tended to show the same pattern - increased or decreased erosion rates occurred within the same period. It is possible that rumenoreticular motility patterns or small rumn pH changes could be implicated in determining erosion patterns.

CONCLUSIONS

Boluses have generally eroded at a slower rate than expected, at between 50% and 25% of the target amount. However, the relatively steady pattern of release when the coating remained intact, proved that this bolus is a viable system for drug administration. No real differences were detected in erosion between ivermectin containing boluses and mineral boluses except in cases where coating factors were involved, which proves that the addition of small quantities of material does not dramatically alter the properties of the mineral core. The close similarity between release rates of boluses in different cows which had a similar exposed surface

area, proves that the results are repeatable although it is necessary to ensure that the coating remains perfectly intact. It is however, encouraging to note that even when the coating became completely detached, it did not result in the instantaneous disintegration of the bolus. When the larger (19 mm) bolus remained intact, it appeared to erode approximately 75% faster than those with the smaller diameter (17 mm). Since the 19 mm bolus had a mass 75% greater than the 17 mm bolus, their projected lifespans were similar at around 200 days.

To increase the ivermectin dose to the intended level one could:

(a) double the quantity of ivermectin in the existing boluses so that they last up to approximately 200 days while delivering the required amount of ivermectin;

(b) halve the length of the boluses but keep the same diameter and the same quantity of ivermectin - these should last about 90 days;

(c) attempt to increase the daily erosion rate by incorporating an increased amount of denatured levamisole or some similar compound;

(d) both ends of the bolus could be exposed but from previous experience, this would be likely to increase the erosion rate by a factor of four rather than two (See Section 2:8);

(e) increase the diameter of the bolus to 25 mm.

It was felt that the constant handling and weighing of boluses throughout the trial had contributed to the coating damage, so it was uncertain to what extent this was likely to be a problem in intact animals. Since the formulations tested in this trial were relatively successful in that they delivered the material at a steady rate, it was decided that they should be further investigated in intact cattle, and their erosion pattern traced via blood ivermectin levels.

FIGURE 37 Erosion pattern of boluses V24, V29, 181 and 186 which were 19 mm in diameter. V24 and V29 contained 26 g mineral, 23 g copper oxide needles and 0.4 g ivermectin while 181 and 186 were identical but had no ivermectin component. Boluses were tested in fistulated cows at grass.

FIGURE 38. Erosion pattern of boluses V26, V28, 183 and 185 which were 17 mm in diameter. V26 and V28 contained 20 g mineral, 17 g copper oxide needles and 0.4 g ivermectin while 183 and 185 were identical but had no ivermectin component. Boluses were tested in fistulated cows at grass.

FIG.37 Erosion of boluses V24,V29,181and 186

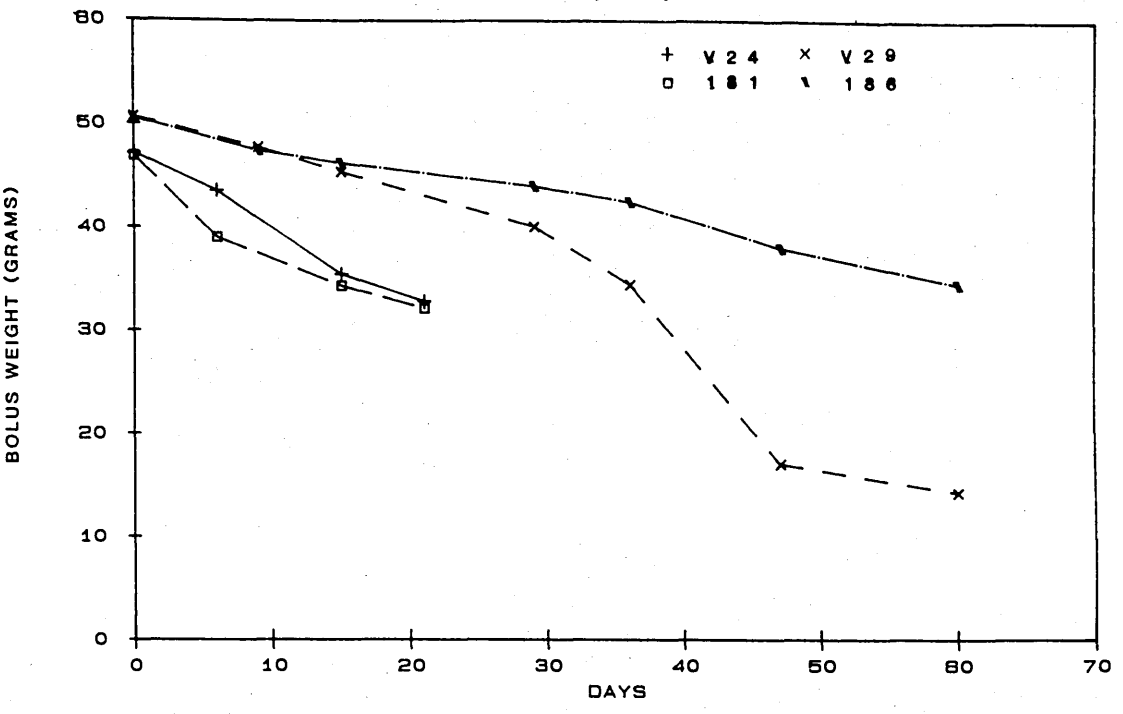
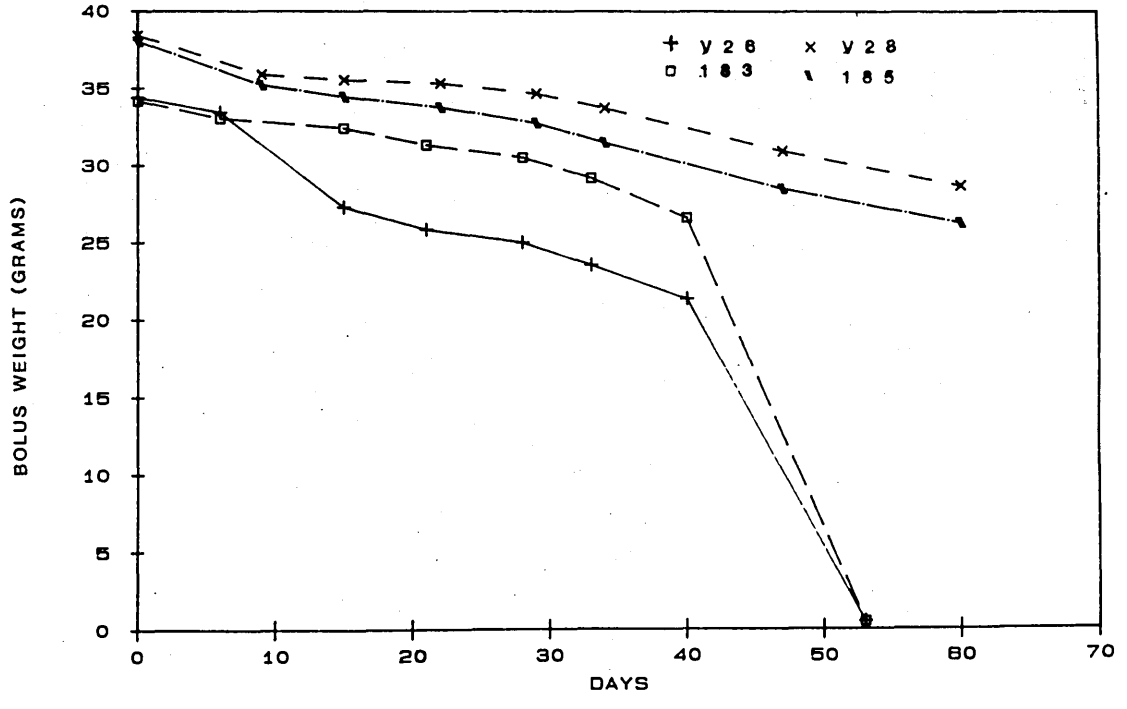


FIG.38 Erosion of boluses V26,V28,183 and 185



SECTION V

1. Introduction to Parasitic Studies

It was proposed that the mineral bolus containing ivermectin should be tested in intact growing calves in a housed and field situation. It was essential to establish whether there was any loss of anthelmintic activity of ivermectin administered intraruminally, and it was also important to detect whether there were any differences between erosion rate outdoors and indoors and between intact calves and fistulated cows. The main target of the ivermectin bolus, if commercially produced, would be in the prevention of parasitic gastroenteritis in calves at grass, which arises mainly as a result of Ostertagia ostertagi infection. O. ostertagi is the most important cause of bovine parasitic gastritis in the temperate areas of the world.

Life Cycle of O. ostertagi

All Ostertagia species have a direct life cycle with two distinct phases (see Figure 39). In the free-living phase, eggs passed in the faeces develop and hatch to become first-stage larvae (L_1) which feed, grow and moult to second stage larvae (L_2). The same process is repeated by L_2 larvae to reach the third and infective stage (L_3). The latter retains the outer sheath of the L_2 and therefore does not feed. It is

the most resistant of the free-living stages to adverse climatic conditions. All of this development occurs in the faecal pats and under suitably moist conditions the L_3 then migrate onto the herbage.

The parasitic stage begins after the ingestion of the L_3 with herbage. It exsheaths in the rumen and enters the tubular gastric glands of the abomasum, especially those in the fundic region.

Moulting to the early fourth stage larvae (L_4) takes place by four days post infection, after which larval development usually continues without delay, taking about ten days to reach the L_5 stage. These grow, and around eighteen days from first infection emerge from the glands onto the surface of the abomasal mucosa to become mature adults.

Another possibility is for the early fourth stage larvae (EL_4) to interrupt their development for several months and then recommence development and maturation. In the Northern Hemisphere this seems to occur primarily in larvae ingested during autumn and winter.

Fertilised adult female worms lay eggs 70-80 μm long and 40-50 μm wide, which are passed out in the faeces at the morula stage.

The life cycle is summarised in Figure 39.

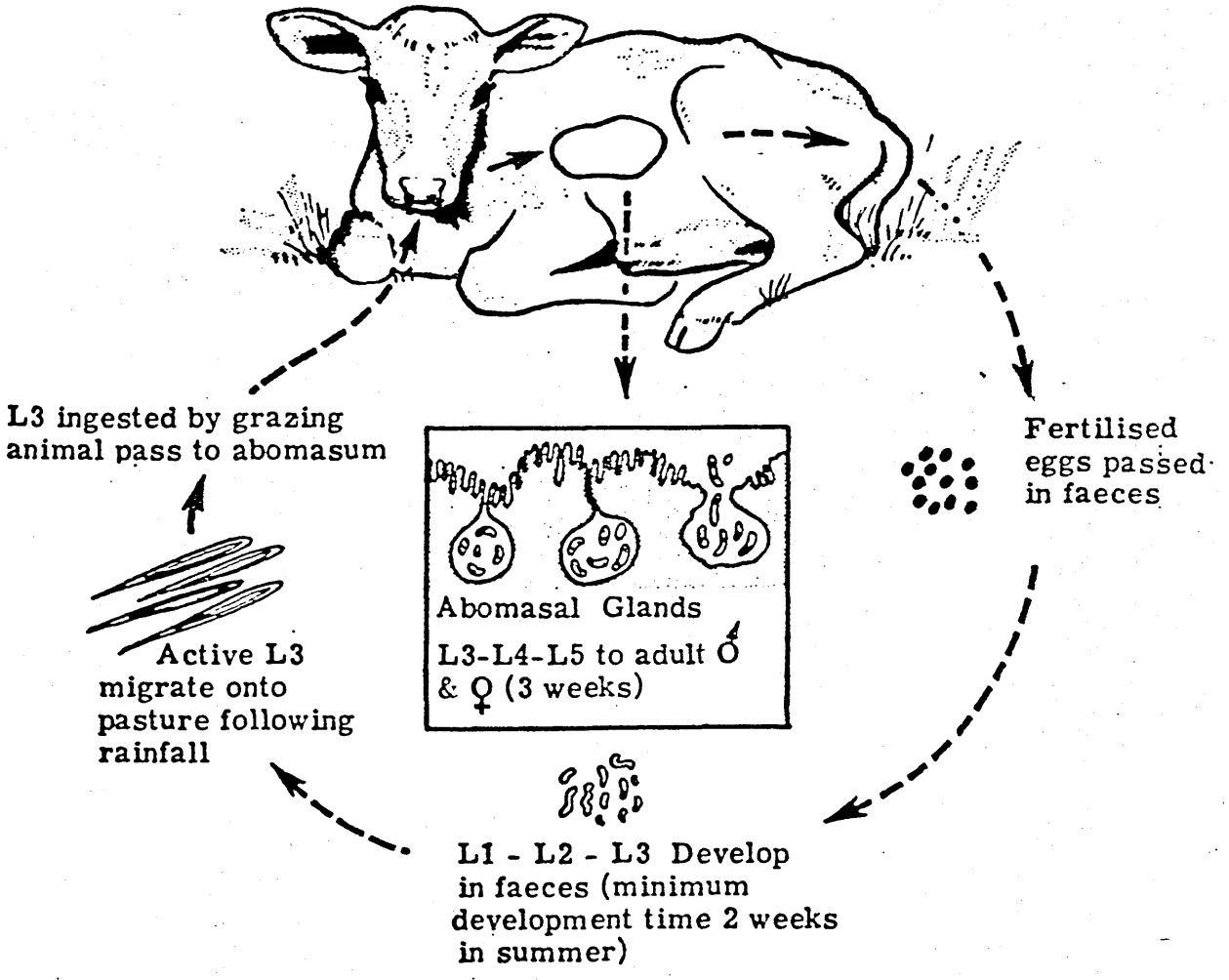
The Clinical Disease

Parasitic gastritis was recognised in England in the 1940's (Stewart and Crofton, 1941; Binford and Fincham, 1945) and later in Scotland (Martin, Thomas and Urquhart, 1957) and Northern Ireland (Gracey, 1960). More recent field studies (Anderson, Armour, Jowett, Jennings, Ritchie and Urquhart, 1965b; Michel, 1969a) have confirmed that Ostertagia parasites are the most important cause of parasite gastritis in Britain. It is also an important parasite throughout Europe and in countries with a subtropical climate if there is winter rainfall, e.g. Southern Australia and the temperate areas of Argentina.

Three phases of the disease have been recognised, two of which are clinically evident, namely type I and type II disease.

The first, type I, is seen in young cattle during the summer, principally between July and October, and is characterised by weight loss and profuse green diarrhoea. This type I clinical entity apparently results from the rapid acquisition of large numbers of larvae which complete their development to the pathogenic L_5 and adult stages in three to four weeks.

FIGURE 39. The life cycle of Ostertagia ostertagi



The other phase, pre-type II ostertagiasis, is regarded as being asymptomatic or showing only mild symptoms and preceding the clinical type II disease. At the pre-type II stage the worm population consists almost entirely of EL_4 stages which are apparently arrested in development and have been acquired as L_3 in the previous autumn. The EL_4 are considered to be relatively non-pathogenic.

The clinical type II disease occurs in the late winter or early spring (March to May), usually in cattle housed after their first grazing season but is also seen in cattle which have grazed during the winter. Inappetence and marked loss of weight are typical signs and diarrhoea, which ranges from being intermittent to profuse and prolonged, is present. Sometimes it is difficult to detect the intermittent diarrhoea if the diet is largely dry forage.

Submandibular oedema, moderate anaemia and hypoalbuminaemia are also often seen in type II disease.

The clinical nature of the type II phase is thought to occur when large numbers of the relatively non-pathogenic EL_4 develop synchronously to the pathogenic L_5 and adult stage.

Cases among older cattle are less common but type II

outbreaks have been seen in milking cows, predominantly heifers at first calving in New Zealand (Wedderburn, 1970), Canada (Smith and Perreault, 1972) and Scotland (Petrie, Armour and Stevenson, 1984).

In beef cattle two outbreaks from Scotland were described (Selman, Reid, Armour and Jennings, 1976), one in an autumn-calving herd and the other in a spring-calving herd where the clinical biochemical, haematological, parasitological and pathological findings were similar to those characteristic of type II ostertagiasis in immature dairy cattle. In southern temperate environments, type II disease has been seen in a small percentage of animals from two to four years of age, mainly in cows calving in the autumn (Hotson, 1967; Anderson, Donald and Waller, 1983).

Pathogenesis

In general the severity of the damage in ostertagiasis is related to the number of parasites which reach the adult stage within a short period of time, and the persistence of these parasites in the abomasum. If the numbers of larvae accumulate slowly, it allows the animal time to acquire an immunity and to repair and compensate for the damage inflicted by the parasite, a process which is accelerated in older animals.

There are two distinct phases in the parasitic part of the life cycle. The first begins immediately after infection when the larvae are developing in the gastric glands and any cellular changes are confined to the parasitized glands; there are no significant alterations in the biochemical values of either the abomasal fluid or the blood at this time, and clinical signs are not detectable. The second phase occurs from about eighteen days after infection when the young adult worms begin to emerge from the gastic glands and marked cellular changes appear; microscopically there is hyperplasia and loss of cellular differentiation, particularly of the hydrochloric acid-producing parietal cells. This happens not only in the parasitized gland but also in the surrounding glands.

In heavy infections, the pH of the abomasal fluid rises to above 7, which is well above the level required for normal digestion (4.5). In such infections there is elevated levels of plasma pepsinogen because the pepsinogen which is secreted into the gastric rumen is not activated at these high pH levels and enters the circulation via the open epithelial cell junctions.

In heavy infections the clinical consequences of the pathogenic changes are:

(i) loss of appetite

(ii) impaired abomasal digestion because pepsinogen activity is negligible above a pH of 4.5 leading to weight loss

(iii) diarrhoea

Epidemiology

Studies on the epidemiology of bovine ostertagiasis have centred on three main areas, namely, the population dynamics of the free living larval stages; arrested larval development at the EL_4 state; and the acquisition of immunity by cattle.

Population Dynamics of the Free-Living Larval Stages

Michel (1969b) made a most important development in epidemiological knowledge by demonstrating that the numbers of free-living L_3 of O. ostertagi fluctuate seasonally on the herbage. In Britain, and indeed the Northern Hemisphere, a considerable increase in larval numbers is noticed sometime during the second half of the recognised grazing season, from July onwards, declining during the subsequent winter months, to reach low levels by the following spring, and around the zero mark by the next June.

This seasonal pattern appears to be a constant one and is accounted for by two important facts. Firstly, the increase of L_3 during the latter half of the

recognised grazing season originates from infection deposited as eggs on the pasture, in the early grazing period, i.e. spring or early summer, by grazing animals. Thus research by Michel showed that eggs of O. ostertagi deposited in faeces during the late spring and early summer first appear as L_3 sometime during mid-summer; thereafter development of eggs to L_3 slows, with little or no development occurring after September. This would allow one, or at most two, generations of Ostertagia spp. to occur annually with the important contamination period being in the spring or early summer (April, May, June).

Secondly, once established on the pasture in summer or autumn, L_3 can survive until the following spring and thus infect the next season's grazing calves. This pattern has been confirmed in many areas of the world.

Arrested Larval Development

Arrested larval development (or hypobiosis) may be defined as the temporary cessation of development of a nematode at a precise point in its early parasitic life, this being the EL_4 stage in Ostertagia spp.

It was originally suggested that type II outbreaks of parasitic gastritis in housed animals was caused by the maturation of worms which had been ingested as L_3 during the previous grazing season and then arrested in

their development in the EL_4 stage, presumably because of an immunity acquired by the host as a result of exposure to larval challenge during the grazing season.

However, a series of experiments by Anderson et al (1965) demonstrated that cold conditioning of L_3 larvae at $4^{o}C$ induced them to become EL_4 on ingestion in calves kept in housed winter conditions. In further studies, larvae were found to begin maturing spontaneously and synchronously after four months in arrested development (Armour and Bruce, 1974).

Immunity

Three main findings have emerged from studies on the acquisition of immunity by the host in ostertagiasis.

Firstly, both field and experiment studies would suggest that there is no absolute age immunity against O. ostertagi. For example, outbreaks of clinical osteragiasis have been noted in U.S.A. and Britain when adult cattle have been moved from areas where the causal parasite does not occur to areas where outbreaks are common (Bailey and Herlich, 1953; Selman et al, 1976). Although experimental studies in U.S.A. and Britain have shown a greater resistance in adult cattle to the debilitating effects of ostertagiasis, with slower worm development and lower egg production, considerable numbers of worms do develop with

pathological changes being similar to those in young, naive calves (Herlich, 1960; Armour, 1967). Speculation that adult cattle will acquire immunity more rapidly than young stock has, however, not yet been substantiated.

Secondly, young cattle acquire immunity relatively slowly and outbreaks may still occur in animals after three to four months constant exposure. The results of British studies showed that calves at the end of their first full grazing season (May to October), displayed a good immunity to experimental challenge infection (Ross and Dow, 1965; Armour, 1967) but following a winter housing period there was a considerable decrease in the level of immunity by the following April (Armour, 1967). The result of further studies of young cattle over two consecutive summer grazing seasons, with winter housing intervening, showed that there was a markedly lower faecal egg count and worm burden in the cattle in the second grazing season (Armour et al, 1979). However, although young cattle acquire a good immunity by the second grazing season, sufficient burdens are established to maintain contamination of the pasture, albeit at a reduced level.

Thirdly, although a certain state of immunity may be established by the end of the second grazing season, such a state is not necessarily permanent. This is

especially so for the heifer cow around calving and during early lactation, as shown by Michel, Lancaster and Hong (1979). Petrie et al (1984) recently described an outbreak of type II ostertagiasis in dairy heifers which calved in November/December and became ill in the following May.

Control

The control of ostertagiasis can conveniently be divided into the treatment of existing infections and their prevention or prophylaxis.

Treatment

The wide-spectrum anthelmintics currently available for treatment come from four main chemical groupings:

(i) the benzimidazoles and pro-benzimidazoles;

(ii) the tetramisoles;

(iii) the pyrantel group;

(iv) the avermectins.

Most are effective against developing larval stages and adults of Ostertagia spp. and some are also effective against the arrested larval stages. The efficiency of the group (i) anthelmintics is high but only the less soluble compounds are highly effective

against arrested larvae (fenbendazole, oxfendazole and albendazole and febantel). The tetramisoles also possess a high degree of efficiency against the developing larvae and adult parasites but neither these nor the pyrantel group are effective against the arrested larvae stage. Studies with the newly found avermectins, in particular the natural B_{1A} component, ivermectin, have shown a wide spectrum of activity against all stages of Ostertagia, including arrested larvae (Armour, Bairden and Preston, 1980; Williams et al, 1981).

Prophylaxis

Prevention of ostertagiasis is applied mainly to yearling stock in the herd by a variety of methods. The seasonal fluctuation in numbers of L_3 on herbage has provided the basis for the application of the control methods. Thus the Weybridge 'dose and move' method (Michel, 1969), as used in the Northern Hemisphere, relies on two main factors. Firstly, that young cattle grazed in spring rarely acquire sufficient amounts of L_3 to be seriously affected although significant numbers of eggs may be deposited on the pasture within three to four weeks. Secondly, since it takes until mid-July for the newly deposited eggs to mature to L_3, a move early in July to pasture ungrazed by cattle since the previous autumn, should result in only light infection being subsequently acquired. If

the move is accompanied by an effective anthelmintic treatment, the stock should remain relatively worm-free for the rest of the grazing season (Michel, 1969). Spedding (1969) however pointed out that although the helminth disease may be eradicated it is not possible to eradicate the helminths themselves.

Rotational grazing of cattle, alternate grazing of cattle with different host species or integrated rotational grazing of different age groups of cattle are also used to combat bovine ostertagiasis. The straightforward rotational system involving solely cattle has proved to be less effective than set-stocking of cattle on pasture, since the rotation may return the cattle to an area at a time when the L_3 are accumulating, e.g. in July and August (Levin and Clark, 1961; Michel, 1969). Furthermore, the luxuriant herbage cover in rotated pasture encourages larval survival whereas the sparse herbage in set-stocked areas mitigates against survival.

Better control has been achieved by methods which involve either grazing mixed host species together (Arundel and Hamilton, 1975) or alternate grazing of different host species (Barger and Southcott, 1975; Southcott and Barger, 1975; Rutter, 1975). The success of both systems depending on the host specificity of Ostertagia spp.

Finally, although anthelmintic treatment of the young cattle set-stocked on permanent pasture has been shown to be economically beneficial (Cornwell, Jones and Pott, 1973) regular reinfection occurs and several treatments are necessary to maintain production levels; this is undesirable from the management viewpoint particularly when labour costs are ever increasing. To overcome the first of these problems it has been suggested that treatment should be concentrated in the early part of the grazing season (Pott, Jones and Cornwell, 1974; Armour, 1978; Herd and Heider, 1980). By limiting pasture contamination to a sufficiently low level in the spring and early summer, the expected increase in L_3 numbers from mid-July onwards would be considerably reduced and economically significant weight gains achieved.

In an attempt to overcome some of the practical difficulties associated with repeated treatments, it was suggested that the provision of continuous anthelmintic medication in feed or water could be beneficial (Downey, O'Shea and Spillane, 1974; Jones, Pott and Cornwell, 1978; Pott , Jones and Cornwell, 1979). However, although it overcame the management problem of regular treatment, it has been difficult to regulate individual consumption of the anthelmintic on a daily basis under field conditions. A more attractive development is that of sustained-release

devices which are delivered into the rumen via a special drenching gun (Jones, 1981). One such device was designed to provide a sustained release of the anthelmintic morantel tartrate over ninety days and so prevent the establishment of infective larvae in the alimentary tract during this period. Under conditions existing in Western Europe, the application of this device in the spring had been shown to limit the acquisition of overwintered O. ostertagi L_3 and so reduce significantly the contamination of pasture for the vital three months of April, May and June with liveweight gain benefits over untreated controls of up to 41 kg being achieved over one grazing season (Jones, 1981; Armour et al, 1981; Jacobs et al, 1981; Tharaldsen and Helle, 1982; Helle and Tharaldsen, 1982).

A similar type of device to the morantel sustained release bolus containing ivermectin would have the additional benefit of controlling EL_4 stage, if it could be made to last for a sufficiently long period. The practice of preventing parasitic disease rather than controlling it, would lead to similar advantages in treated animals and would reduce parasite levels on pasture.

2. Trial of Boluses Containing Ivermectin in Housed Calves

INTRODUCTION

The boluses from the previous trial (Section 4:5) appeared to erode slowly over a prolonged period in fistulated cows. However, the integrity of the bolus coating became disrupted in some cases, partly perhaps as a result of repeated drying and weighing. Therefore, it was thought necessary to test the same formulae as previously in a controlled situation in intact animals in order to assess the variability of bolus erosion, and the efficacy of the boluses against an experimentally induced challenge of Ostertagia ostertagi.

The principle of administering one medicated and non-medicated bolus was again applied in this trial. The purpose behind this idea is that there would be a two tier more flexible dosage system. For lower dosage regimes, one medicated and one non-medicated bolus would be administered. Where a higher level of medication was necessary, two ivermectin containing boluses would be administered simultaneously.

The same two sizes of boluses as previously, 19 mm and 17 mm diameter, were tested in order to compare

their relative efficacy in intact animals. The boluses were designed to release approximately 40 mcg ivermectin per kg bodyweight daily for ninety days when administered to a calf of 200 kg.

MATERIALS AND METHODS

Animals

Eighteen male Friesian calves, purchased from local farms where they had been reared worm-free, were used for the study. At the time of treatment (day 0) they were approximately four to six months of age and weighed between 140 and 182 kg.

Replicates

There were six replicates.

Identification

Each animal was individually identified by means of a numbered ear tag.

Management

The calves were housed in pens within roofed buildings, with unmedicated control animals penned separately from medicated animals. Straw bedding was provided over the concrete flooring. It was considered that the conditions were such as to preclude accidental infection with nematode parasites.

Feed and Water

Hay was fed to appetite, supplemented with a proprietary 16% protein concentrate ration ("Calfmaker 16 Pencils", Eastern Counties Farmers Ltd., Ipswich, Suffolk) containing virginiamycin 40 ppm. Water was freely available from mains-fed drinkers.

Infections

Commencing seven days after treatment and continuing at weekly intervals for a total of eight weeks (day 7 to day 56), each calf was infected orally with approximately 2,500 infective third stage larvae of Ostertagia ostertagi and 2,500 infective larvae of Cooperia oncophora by mouth. Individual doses containing the required number of larvae were prepared by taking aliquots from bulk suspensions of larvae of each parasite.

Bodyweights

Calves were individually weighed on the day of treatment and weekly thereafter.

Allocation

The calves were divided into two batches of nine to reduce the work load on the day of termination. The same allocation procedure was adopted for each batch: calves were ranked in order of bodyweight and the first three heaviest formed the first replicate,

the next three heaviest the second replicate, and the last three the third replicate. Within each replicate, animals were randomly assigned to treatment by lottery.

The first three replicates were treated on 2nd November, 1983 and the second three weeks later on 23rd November, 1983.

Boluses

Boluses were produced by the standard pressing procedure outlined in Section 2:2, and were coated four times with Plastic Padding glass fibre resin, leaving one face exposed.

Treatments

Group 1. Unmedicated, infected controls.

Group 2. One ivermectin bolus, total weight 51.3 g (0.72 g ivermectin; 23 g copper oxide needles, 26 g mineral mix, 1.6 g resin coat; ¾ inch diameter) plus one 'blank' bolus, total weight 50.5 g (23 g copper oxide, 26 g mineral mix, 1.6 g resin coat; ¾ inch diameter).

Group 3. One ivermectin bolus total weight 38.9 g (0.72 g ivermectin; 17 g copper oxide needles, 20 g mineral mix, 1.2 g resin coat; ½ inch diameter) plus one 'blank' bolus, total weight 37.9 g (17 g copper oxide, 20 g mineral mix, 1.2 g resin coat; ½ inch diameter).

Boluses were given orally, using a balling gun.

Difficulty was experienced in administering the ivermectin bolus to the first animal, Calf 710, because the rubber sleeve placed round the balling gun made the gun too wide to pass over the back of the tongue. This bolus was partially chewed by the calf before being swallowed. The rubber sleeve was therefore removed and no further dosing problems were experienced.

Duration

The calves were killed seventy-seven days after treatment for estimation of worm burdens.

Blood Samples

Venous blood samples from all animals were collected into heparinized containers for recovery and estimation of ivermectin concentration on days 0, 1, 2, 3 and 7, and once weekly thereafter. Plasma samples were retained deep frozen (-20^{o}C) until required.

Assay Method

The procedure used multi-step extraction by solvent partition, followed by fluorogenic derivatization with acetic anhydride/1-methyl imidazole/DMF, and finally high performance liquid chromatography. Lowest detectable concentration of ivermectin by this method varied between 0.2 and 0.5 ppb.

Faecal Samples

At weekly intervals, commencing on day 28, faecal samples were collected from each calf for estimation of nematode egg counts. A modified McMaster salt

flotation technique was used.

Necropsy Procedures

At necropsy, the remains of boluses were recovered and weights recorded. In addition samples of liver and fat were collected from calves in Replicates 1, 2 and 3 for assay of ivermectin residues.

The abomasum and upper small intestine were removed from each calf and their contents retained. In addition, the abomasum was digested in a 1% hydrochloric acid-pepsin solution at 37^{o}C overnight for recovery of mucosal parasites. Worm burdens were estimated from counts of 1/10th samples.

Adverse Reactions

All animals were observed daily for signs of adverse reaction.

Daily Log

A daily log of scheduled and non-scheduled events was maintained.

Statistical Analysis

Total counts of each parasite were transformed to the natural logarithm of (count +1) for calculation of geometric means. Pairwise treatment comparisons were made using Wilcoxon's Rank Sum statistic in a

randomisation procedure which leads to an exact probability of finding a test statistic as extreme as or more extreme than the one observed. A two-sided significance level of $p = 0.05$ was used for each comparison. Results for two calves have been omitted from the statistical analysis. These were Calf 727 (Group 2) whose medicated bolus was found at necropsy to be missing most of its coating and Calf 710 (Group 3) which partially chewed its medicated bolus before swallowing it.

RESULTS

No adverse reactions to treatment occurred.

Records of calf bodyweights are shown in Table 43. The faecal nematode egg counts are shown in Table 44. Apart from positive egg counts in two treated calves on day 28, which are considered to be suspect and possibly the result of labelling errors, patency developed in only one treated calf. This animal, Calf 710, had a count of 50 epg on days 56 and 63. This was the calf which had chewed the ivermectin bolus while it was being administered, and plasma concentrations were down to 1 ppb by this time. At termination Calf 710 also had more nematode parasites than any of the other ivermectin treated calves.

Necropsy worm burdens for each calf are shown in Table 45a. There was relatively little within-group variation in the various parasite counts, although there were more O. ostertagi larval stages in the first batch of control calves, Replicates 1, 2 and 3, than in corresponding animals in the second batch, Replicates 4, 5 and 6. The geometric mean values for total C. oncophora and total O. ostertagi, together with the percentage efficacies for the two treatment groups compared with control, are also shown in Table 45a. The two-sided probability values for treatment comparisons are shown in Table 45b. The mean counts for animals in both medicated groups were significantly lower ($p < 0.01$) than for controls both for total C. oncophora and total O. ostertagi. No significant differences ($p < 0.10$) were found between the two medicated groups for either parasite.

Results of assays for plasma concentration of ivermectin are shown in Table 46. High values were recorded for Calf 710 during the first twenty-one days of the study which was consistent with the fact that this calf had chewed the ivermectin bolus before swallowing it. Despite this, and the failure to recover any of the bolus from the reticulum post-mortem, ivermectin was still detectable in the plasma at termination seventy-seven days after administration of the bolus. A few other animals, e.g. Calves 667 and

735 had relatively high plasma ivermectin concentrations in the early part of the study, which subsequently declined. With one animal, Calf 727, the concentration steadily increased for fourteen days and although it subsequently declined, it remained relatively high throughout the remainder of the study. This was the animal in which most of the coating of the ivermectin bolus was found to be absent post-mortem.

The weights of the boluses recovered from the reticulum are shown in Table 47 and the calculated erosion rates for the ivermectin boluses are shown in Table 48 although data for the boluses which were broken or had lost their coating have been omitted from the latter table. It is interesting that the mean rate of ivermectin release was almost identical for the two types of boluses, although the rate of release was only approximately one quarter of that which had been anticipated. It appears from Table 48, that the presence of ivermectin in the boluses reduced the rate of erosion. Although 55 and 50 per cent of the ½ inch and ¾ inch unmedicated boluses were eroded only 22 per cent of both types of ivermectin containing boluses were eroded during the seventy-seven day experimental period.

DISCUSSION

The ivermectin intraruminal bolus treatment was found to be most satisfactory in the control of O. ostertagi and C. oncophora. Ivermectin was detected in the blood of 11/12 animals after seventy-seven days, and despite providing about 25% of the target dosage, the bolus was shown to have maintained its efficacy throughout the seventy-seven day period. Apart from in the animal which chewed the ivermectin bolus on administration, faecal egg counts were uniformly zero from day 28 onwards, and worm burdens at necropsy significantly reduced by the presence of the ivermectin bolus. The mean erosion rate of the larger 19 mm bolus was 75% greater than the erosion rate of the 17 mm bolus, which is the same factor of variance between the two, as was found in the previous trial (section 4:5), although in this trial, the overall release rate was reduced. A fact not obvious from previous work was the slowing of the release rate of boluses containing ivermectin, compared to mineral boluses. The projected lifespan of ivermectin boluses was approximately 350 days for both sizes, as compared with approximately 150 and 175 days for ¾" and ½" non-medicated boluses respectively. The most obvious problem which this poses is that, should the mineral bolus not be present as an abraiding surface, the ivermectin bolus may fail completely to erode. It is possible that this system of administration where two

boluses are non-identical may have to be altered in case varying ingredients show up erosion differences over prolonged periods.

There were far fewer problems with flaking coating than in the previous trial, which highlights the difference between using intact animals as compared with fistulated cows in testing. Obviously, economically, fistulated cows are necessary, but the possible limitations of this system of testing must be borne in mind.

It is interesting to note that one quarter of the normally recommended dose of ivermectin is capable of controlling parasitism when administered in a sustained release system, although ideally the bolus would release at a higher rate to prevent any tendency to select for ivermectin-resistant parasites. Uniformity of results between the different experimental replicates of handmade boluses is an encouragement to future commercial development. If the bolus could be made faster in erosion and produced on a commercial scale, it would seem to indicate that it would prove to be a very effective antiparasitic device.

TABLE 43. Calf bodyweights (kg)

	Group	Calf No.	Rep.+ No.	Day of Trial 0	7	14	21	28	35	42	49	56	63	70	77
1.	Control	675	2	161	164	174	180	185	193	198	198	200	218	213	219
		713	3	152	152	162	170	173	184	192	195	189	204	208	205
		714	1	182	187	193	199	202	214	219	225	226	243	252	252
		729	4	160	162	171	184	189	185	201	205	201	214	214	220
		730	6	148	151	157	163	170	167	175	175	175	174	182	199
		731	5	153	157	165	173	175	180	195	192	192	209	215	210
2.	¾" bolus*	667	2	158	160	166	174	182	183	194	201	208	208	217	219
		711	3	156	151	150	157	165	167	179	188	185	195	202	200
		715	1	167	179	190	199	198	208	213	224	225	235	247	240
		727	4	155	170	176	181	190	187	197	208	208	215	227	224
		734	5	153	163	167	180	186	190	196	201	207	208	226	214
		735	6	140	142	153	156	163	165	174	180	177	182	190	184
3.	½" bolus*	710	3	154	152	150	156	168	171	178	188	186	197	204	206
		712	2	159	159	168	177	178	186	190	196	196	205	215	216
		716	1	177	186	192	189	202	210	220	227	227	237	248	250
		728	6	143	144	155	160	165	167	173	176	182	183	190	182
		732	5	154	156	166	175	181	180	185	194	198	200	211	202
		733	4	164	173	178	184	191	193	203	213	215	213	223	215

* One medicated bolus (0.72 g ivermectin) plus one unmedicated bolus.

+ Rep. = Replicate

TABLE 44. Faecal egg counts

Group	Animal No.	Day of Trial 28	35	42	49	56	63	70	77
1. Control	675	50	100	550	1650	550	450	50	900
	713	100	350	450	900	850	1700	1150	900
	714	0	450	600	350	750	700	550	300
	729	0	50	200	750	450	550	550	850
	730	0	0	400	500	550	650	1600	1000
	731	200	200	150	800	450	550	1000	1350
2. ¾" bolus*	667	0	0	0	0	0	0	0	0
	711	0	0	0	0	0	0	0	0
	715	0	0	0	0	0	0	0	0
	727	250	0	0	0	0	0	0	0
	734	0	0	0	0	0	0	0	0
	735	0	0	0	0	0	0	0	0
3. ½" bolus*	710	0	0	0	0	50	50	0	0
	712	0	0	0	0	0	0	0	0
	716	0	0	0	0	0	0	0	0
	728	0	0	0	0	0	0	0	0
	732	NS	0	0	0	0	0	0	0
	733	100	0	0	0	0	0	0	0

* One medicated bolus (0.72 g ivermectin) plus one unmedicated bolus.

NS = No Sample

TABLE 45a Individual worm counts at necropsy, plus group geometric means and efficacy percentages

	Group	Animal No.	C. oncophora	O. Ostertagi E4	L4	L5	Adults	Total Ost.
1.	Control	675	13,510	870	190	220	5,160	6,440
		713	15,800	800	200	110	8,380	9,490
		714	11,830	230	110	120	8,080	8,540
		729	11,610	0	0	20	7,730	7,750
		730	13,040	0	0	30	8,720	8,750
		731	11,200	0	40	130	6,890	7,060
	Geometric Mean		12,743					7,936
2.	¾" bolus*	667	140	0	0	10	10	20
		711	0	0	0	0	0	0
		715	150	0	0	0	0	0
		727	0	0	0	0	0	0
		734	0	0	0	0	0	0
		735	0	0	0	0	0	0
	Geometric Mean		6.3					0.8
	% Efficacy		99					99
3.	½" bolus*	710	460	0	0	30	10	40
		712	60	0	0	0	0	0
		716	170	0	0	0	10	10
		728	0	0	0	0	20	20
		732	100	0	0	0	10	10
		733	320	0	0	0	30	30
	Geometric Mean		50					8.5
	% Efficacy		99					99

C. oncophora = Cooperia oncophora
O. ostertagi = Ostertagia ostertagi

* One medicated bolus (0.72 g ivermectin) plus one unmedicated bolus.

TABLE 45b Necropsy worm counts. Two-sided probability values for treatment comparisons

	Treatment Comparisons		
	Control v ¾" ivermectin bolus	Control v ½" ivermectin bolus	¾" v ½" ivermectin bolus
C. oncophora (total)	0.002	0.002	0.10
C. ostertagi (total)	0.002	0.002	0.10

TABLE 46. Calf plasma Ivermectin Assays (ppb)

	Group	Animal No.	0	1	2	3	7	14	21	28	35	42	49	56	63	70	77
1.	Control	675	0+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		713	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		714	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		729	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		730	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		731	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2.	¾" bolus*	667	0	10	17	11	2	3	3	3	1	1	0	2	1	1	1.5
		711	0	11	2	4	3	2	3	2	1.5	0.5	5	1	2	0.5	2
		715	0	0.5	1	2	3	2	3	2	0.5	1	2	4	2.5	1.5	1.5
		727	0	0	6	6	10	24	6	6	4	4	2	6	8	1	3
		734	0	7	9	3	0	2	4	2	2	2	0.5	1	2	2	2
		735	0	14	21	11	3	3	4	3	2	3	0.5	2.5	4.5	1.5	3
3.	½" bolus*	710	0	290	271	230	16	5	6	1	1	1	1	2	1.5	1.5	0.5
		712	0	3	3	3	3	2	3	2	2	2	2	3	3	1	2
		716	0	0	1.5	2	1.5	1	1	2	1.5	0.5	2	3	2	1.5	1
		728	0	4	2	2	0	1	3	3	3	2	1	2.5	1.5	0	0
		732	0	4	1	1	0	2	0.5	3	2	2	1	1	2	1	1
		733	0	2	2	2	1	3	0.5	1	2	2	2	1.5	0.5	1.5	1.5

* One medicated bolus (0.72 g ivermectin) plus one unmedicated bolus

+0 = Non detected, i.e. 0.5 - 1.0 ppb

TABLE 47. Weights of ivermectin and blank boluses at day 77

Group	Animal No.	Weight of Ivermectin Bolus (g)≠	Weight of Blank Bolus (g)
2. ¾" bolus	667	38.4	23.2
	711	37.9	20.9
	715	42.3	27.7
	727	(22.7)*	25.3
	734	42.5	28.6
	735	39.3	27.7
	Mean	40.08	25.57
	S.D.	2.18	3.03
	Mean % of boluses eroded	21.9%	50.2%
3. ½" bolus	710	broken	30.9+
	712	27.1	18.6
	716	29.3	23.6
	728	32.4	30.5
	732	33.8	15.1
	733	29.7	(16.9)*
	Mean	30.46	21.95
	S.D.	2.65	6.68
	Mean % of boluses eroded	21.7%	43.6%

≠ Mean initial weights ¾" bolus 51.3 g
½" bolus 38.9 g

* Coating lost (excluded from mean)

+ Partner of broken bolus (excluded from mean)

TABLE 48. Ivermectin erosion rates/daily release rates

Group	Animal No.	Erosion Rate g/day	Dose Rate Ivermectin kg/day*
2. ¾" bolus	667	0.168	11.7
	711	0.174	12.2
	715	0.117	8.18
	727	-	-
	734	0.114	8.0
	735	0.156	10.9
	Mean	0.146	10.2
	S.D.	0.028	1.98
3. ½" bolus	710	-	-
	712	0.153	14.2
	716	0.125	11.5
	728	0.084	7.81
	732	0.066	6.13
	733	0.119	11.1
	Mean	0.109	10.1
	S.D.	0.035	3.19

- bolus broken or coating lost

S.D. = Standard deviation

*A nominal calf bodyweight of 200 kg has been assumed in this calculation

3. Ivermectin Bolus Trial in Calves at Pasture

INTRODUCTION

Following the success in an indoor trial of the treatment of one ivermectin and one non-medicated mineral bolus against experimentally induced O. ostertagi and C. oncophora infection in a group of calves of approximately 200 kg, a similar experiment was planned outdoors where infection would be naturally acquired. The indoor trial had shown that almost complete parasite control of C. oncophora and O. ostertagi was possible in an experimental situation, but it was necessary to prove that this could be reproduced in the field under normal grazing conditions where the parasite challenge would be more sporadic. In the indoor situation, the bolus erosion rate was very slow, particularly that of the ivermectin bolus, but for comparativity it was thought that 19 mm diameter boluses identical to those used in the indoor trial should be administered because it seemed likely that they would last for the entire grazing season, and would constitute a more complete study of these boluses. It was considered a possibility that their erosion rate at grass may be different from that indoors in intact calves.

O. ostertagi and C. oncophora larvae are generally

ingested by susceptible first season calves when they are turned out in spring as a result of the development on the pasture of overwintered larvae excreted as eggs during the previous grazing season. As the larvae develop in the abomasum (and small intestine in the case of C. oncophora), they complete their life cycles, becoming adults, reproducing, and excreting eggs which are passed onto the pasture in the faeces where they develop quickly into infective L_3 larvae thereby multiplying the initial challenge. The cycle continues until pasture larvae levels reach a critical peak midsummer (know as the midsummer rise) when first season calves are particularly susceptible to clinical symptoms of parasitic gastroenteritis.

The advantage of anthelmintic bolus treatment is that it interrupts the parasite life cycle by acting on initially ingested larvae, thereby preventing the development to adult and the subsequent release of eggs. In this way, the pasture can be "cleaned up" in the early part of the grazing season. It is not necessary for the bolus to last for the whole grazing season in order to prevent disease; it is sufficient to kill ingested parasites in the first few months after turnout.

Thus it was intended in this trial to make a study of bolused and control calves at grass with respect to

blood ivermectin levels in calves, faecal egg output, plasma pepsinogen levels, necropsy data and pasture larvae levels. Weight differences in bolused and control animals were also to be investigated.

MATERIALS AND METHODS

(a) Animals

A total of twenty-five male Friesian calves were involved in the experiment. Thirteen were bought from a local calf dealer. They were late autumn-born, reared in a helminth-free environment and weighed 118-140 kg at the start of the experiment. A second group of twelve calves were from Cochno Farm, Duntocher. These were also autumn-born but spanning a greater period and therefore had a wider liveweight range of 137-210 kg. They had already been grazed briefly outdoors prior to being incorporated into the experimental programme. The latter group of calves were intended to be used solely for liveweight measurements since they were not helminth-naive.

(b) Pasture

The grazing area at Glasgow University Veterinary School which was known to be contaminated with *Ostertagia ostertagi* in previous seasons consisted of one large field of 6.2 h a which was

purposely divided into five separate plots (see Diagram 4a). Plots 2, 3, 4 and 5 were about 1.2 ha each in area. Plot 1 had an effective 1.4 ha of grazing area. Plots 3 and 5 were space separated from plots 2 and 4 by a double fence 1.5 m apart. Plot 6 was situated on an adjacent but not adjoining site on the Veterinary School Estate.

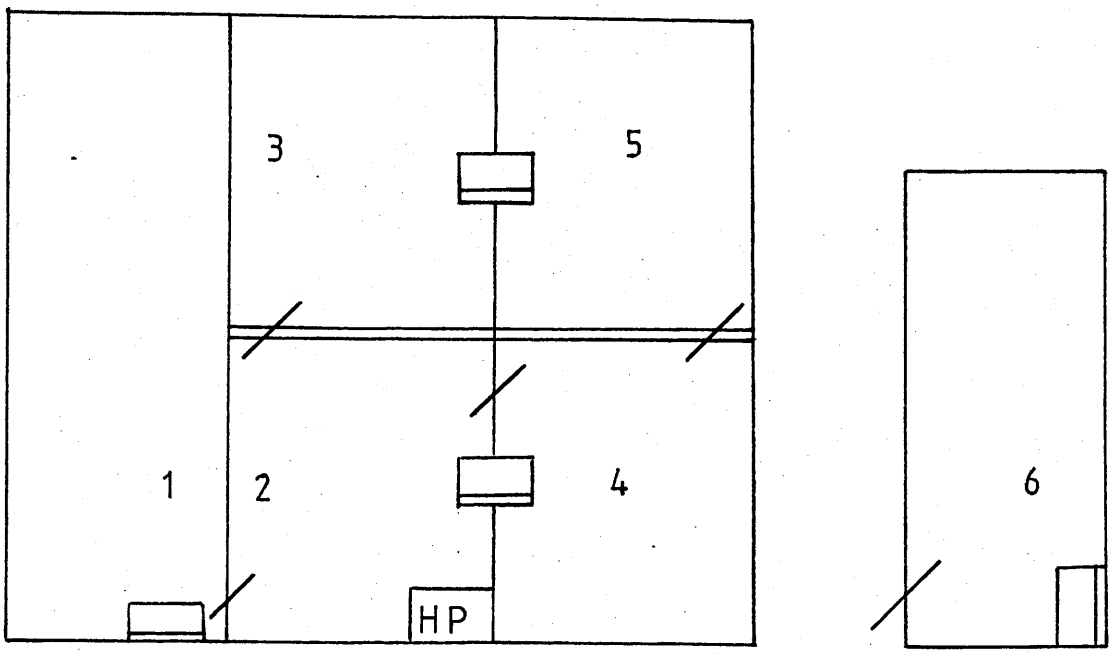
(c) Pasture infection

Turnout to grazing was during mid May after an abnormally long dry spell. Since the parasite counts on the herbage of plots 1-6 were found to be relatively low at the start of the experiment, having been grazed in the previous year by twenty month old cattle grazing for their second year, it was decided to enhance the levels artificially by watering the pasture with infective L_3 larvae manually to ensure a strong challenge. In addition, the twelve Cochno calves were given an oral dosage, diluted from a bulk suspension of 2,500 *O. ostertagi* L_3 larvae.

(d) Bolus manufacture

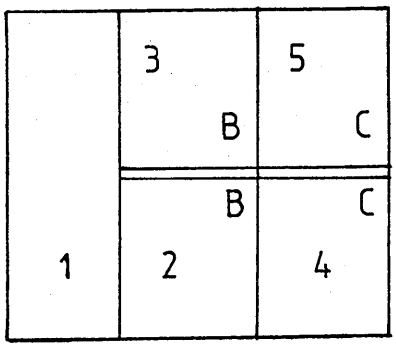
The boluses were manufactured as previously described in Section 2:2. They were cylindrical in shape of 19 mm diameter. The formulation of the ivermectin bolus was as follows: 0.72 g

DIAGRAM 4(a) Schematic representation of grazing paddocks

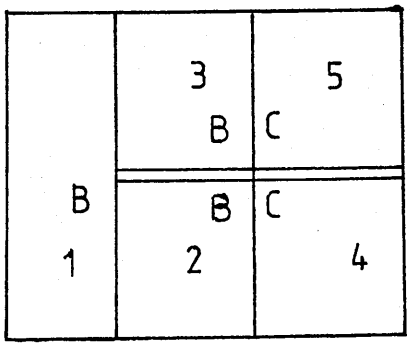


H.P = Handling pen / = gate = water trough

DIAGRAM 4(b)



Aug. '84



Sept. '84

ivermectin, 23 g copper oxide needles and 26 g of the standard mineral mix described in Section 2:5. The mineral bolus partner was the same except that no ivermectin was included. Four coats of resin (Plastic Padding Ltd.) were applied to each bolus type leaving one exposed end which added approximately 1.4 g to the weight. The total approximate weight of each was 51.3 g for ivermectin boluses and 50.5 g for mineral boluses.

(e) Treatment group

There were four experimental groups:-

Group 1 - 5 untreated control calves

Group 2 - 8 bolus treated calves

Group 3 - 6 untreated control Cochno calves

Group 4 - 6 bolus treated Cochno calves

Assignment to the different groups was done entirely at random. The disparity in the size of Groups 1 and 2 was to allow for the serial slaughter of the three extra calves in Group 2 during the experimental period so that data on bolus erosion rate could be assimilated.

(f) Treatment administration

The fourteen animals in Groups 2 and 4 had one ivermectin and one mineral bolus administered on day 0 of the experiment, using a standard oral balling gun. On several occasions, boluses were chewed during administration. Calves 3, 25, 9 and 18 damaged the ivermectin bolus on administration by chewing off the coated end, so that two faces were exposed instead of only one. Part of the reason was that the only standard balling gun for intraruminal administration which was available was an adapted balling gun for 1" boluses, which left some space between the 19 mm bolus and gun sleeve, so that occasionally the bolus slipped out into the animal's mouth before the gun was placed down the back of the throat. It was not anticipated that administration would cause any problems if a properly sized balling gun was used.

(g) Management

It was intended at turnout to set stock the calves on the same paddock throughout the whole season. Observations could then be made on any differences between pasture larval counts bolused and control calf grazing.

At turnout -

Untreated control Group 1	-	grazed plot 6
Bolused Group 2	-	grazed plot 1
Untreated control Group 3	-	grazed plot 4
Bolused Group 4	-	grazed plot 2

However, the whole grazing season was abnormally dry and it became regrettably necessary to move the calves around to make efficient use of the pasture.

After eight weeks grazing on plots 1, 2, and 4 here had been little new regrowth and yet the grazing density had not been high enough to keep the grass cropped short, and long fibrous seed heads were dispersed throughout paddocks 2 and 4. Little grass was left on paddock 1. It was decided at this point to "top" the grass on paddocks 3 and 5. The two bolused groups (Groups2 and 4) were amalgamated and grazed on paddock 3 while the control group of Cochno calves (Group 3) was grazed on paddock 5. The control Group 1 remained throughout the experiment on plot 6. Fertilizer was applied at topping. Following one month of growth, animals were allowed to graze paddocks 2 and 4 again, in

addition to 3 and 5. The bolused animals (Groups 2 and 4) remained thereafter as one single group, and the six Cochno control calves as another.

Because of the persistent very dry weather, the pasture was below normal standards from mid July to mid September. In mid September the bolused animals were also given the run of paddock 1 (Diagram 4(b)).

(h) Sampling

Grass samples were taken from the pastures two weeks prior to turnout, at turnout and thereafter at two or three week intervals until the end of the grazing season for numerical estimation of infective trichostrongyle larvae.

Venous blood samples were collected into heparinized "Vacutainers" on day 0, day 9, day 21, and thereafter at two or three week intervals. The plasma fraction was separated off, blood ivermectin assays and plasma pepsinogen estimation performed.

Faecal samples were collected on day 0, 9 and 21 and thereafter at two or three week intervals and processed to evaluate the number of strongyle

eggs being excreted, using a modified McMaster salt flotation technique.

The liveweights of the Cochno calves (Groups 3 and 4) were recorded at approximately fortnightly intervals throughout the experiment.

(i) Necropsy

In mid August (day 93) two of the bolus treated group calves were slaughtered.

Four of the remaining six bolused Group 2 calves were slaughtered at the end of the grazing season, and all five of the control Group 1. Worm burdens were estimated, and the appearance of the abomasal mucosa was noted and the presence of lesions described. A search was made of the reticulum and rumen for any bolus residues.

RESULTS

(a) Liveweights

The liveweights of bolused and control Cochno calves (Groups 3 and 4) is shown on Table 49. The average liveweight gains per day between the measurement intervals are also detailed on the table. From the start of the experiment until the middle of July, the average accumulated weight

weight increase per day of the bolused group was slightly higher than that of the control animals (1.45 kg/day as compared with 1.20 kg/day. Between days 56 and 64, the margin of gain by bolused animals was statistically significant at $p < 0.002$. After day 64 there was a marked change in pattern, and at each measurement thereafter, control animals had a higher average daily weight gain. At days 77 and 134, this was significant at $p < 0.02$ and at day 119, significant at $p < 0.05$.

(b) Plasma pepsinogen activity

The individual plasma pepsinogen activity levels of the calves in Groups 1 and 2 are given in Table 50 and plotted graphically on Figure 40. Statistical comparisons of the measurements between Groups 1 and 2 are also given in the table. Values for the bolused group remained consistently low at around 1.0 iu tyrosine for approximately 110 days, but then began to climb steadily to a value of 2.8 iu by day 147. The control group registered levels around 2.8 iu throughout the experimental period with peaks at 35 and 149 days of 3.8 and 4.0 iu tyrosine respectively. After 21 days there was a statistically significant difference between Groups 1 and 2 comparing the approximately

fortnightly measurements, until day 119. Thereafter there was no significant difference in the tyrosine values.

(c) Faecal egg counts

The faecal egg output for Groups 1 and 2 calves are detailed in Table 51 and shown graphically on Figure 41. Statistical comparisons indicating the level of significance between Groups 1 and 2 at approximately fortnightly intervals are also shown on Table 51. Trichostrongyle eggs were completely undetectable up to day 56 in bolused animals, and thereafter were present in relatively low numbers until late October, except in cases where calves had crunched the bolus on administration. One animal (No. 5) apparently excreted no eggs throughout the entire experimental period. By contrast, the untreated control group were found to have comparatively high faecal egg counts from day 21 onwards. An average peak count of 1900 was observed at 35 days followed by another smaller peak of 900 epg on day 149. From day 21 onwards, until towards the end of the experimental period, when average faecal egg outputs were compared over the same interval in Groups 1 and 2, they were statistically significantly different.

(d) Pasture larval counts

These are detailed in Table 52. The pasture grazed by the untreated control group calves showed fairly low L_3/kg DM levels until late July when numbers began to rise, and reached a peak of 23,233 O. ostertagi and 4,511 cooperia spp by September 12 (Table 52). The pasture grazed by bolus Group2 calves had fairly low larval levels until mid September when they markedly increased to an average of approximately 5,000 ostertagia per kg DM and 2,124 cooperia per kg DM between plots 1, 2 and 3. In general, the pattern seemed to be about six weeks behind that of plot 6 grazed by the untreated animals. There were no differences between the paddocks grazed by bolused or control Cochno calves (Groups 3 and 4) but the movement and amalgamation of calves, would mask any real effects.

(e) Blood ivermectin levels

Blood ivermectin levels are shown on Table 53. By day 8 after bolus administration, the levels were fairly high in most cases (mean level 6.6 $\pm$ 5.5 ng/ml) then decreased gradually, until by day 56, only low concentrations (0.062 $\pm$ 0.44 ng/ml) were detected in the blood. After 77 days, levels were virtually zero, and presumably corresponded with the total erosion of the ivermectin-containing bolus.

(f) Post-mortem examination

(i) Calves slaughtered mid August.

a. Bolus recovery

Neither calf had been recorded as chewing either bolus at administration. An intact mineral bolus residue weighing 37.073 g was recovered from animal No.10. No residues were recovered from animal 16.

b. Pathological observation

On post mortem examination of the abomasum there was evidence of a tissue reaction in response to a low parasite challenge in both animals. The small intestine was normal in both cases.

c. Parasitological findings

Calf No. 10 was found to be completely clear of O. ostertagia and any other helminth parasite, which is in accordance with the zero egg counts recorded and low plasma pepsinogen. O. ostertagia was found in calf 16 - there were 100 adults present, which represented a very low level of infection. The abomasal pH was 3.2 in animal No. 10 and 4.9 in No. 16.

(ii) Calves slaughtered at the end of the trial.

a. Bolus recovery

An intact mineral bolus residue was recovered from calf 11 weighing 36.421 g. Nothing was recovered from any other animal.

b. Pathological observation

At post-mortem, classical lesions of ostertagiasis, i.e. coalescence of nodules, hyperaemia and oedema were clearly evident in the abomasa of the untreated control animals of Group 1. One of these (No. 54) was destroyed in extremis on 28.8.84.

Of the four calves slaughtered from bolused Group 2, calf 11 showed some signs of O. ostertagi infection, the abomasum being swollen and oedematous and there was also some evidence of Trichostrongyle axei infection. Calf 8 and 25 showed some evidence of T. axei thumbprint lesions, and some O. ostertagi lesions. The abomasum of calf 5 was in relatively healthy condition.

c. Parasitological findings

Table 54 details the worm burdens of O. ostertagi and Cooperia species of the control and ivermectin bolus treated calves necropsied during and after the grazing season. The average total worm burdens of the control group at the end of the trial was 63,600±33,926 parasites per animal, and that of the treated group, the lower value of 27,400±8,775 parasites which was not statistically significantly different. For each type of parasite, adult or L_4 there were fewer found in the group which had been bolused. There were significantly fewer (p 0.001) Cooperia adults in the treated group; the mean was 100 parasites as compared with 4,850 in the control group. The numbers of adult Ostertagia were not statistically different between the two groups although the average value of 8,625±5,835 parasites in the treated group was much less than the 21,000±12,043 Ostertagia adults found in the control group. Ostertagia L_4 levels were more similar with treated and control averaging 16,575±8,246 and 34,850±28,079 parasites respectively.

(g) Statistical evaluation

The data for the various parameters assessed in this experiment were examined to determine whether there was a degree of correlation between sets of data. In particular the blood ivermectin levels were compared with the plasma pepsinogen levels and faecal egg outputs for any possible inter-relationships. No statistically valid relationships were established, although there was a broad correlation between high blood ivermectin levels and low plasma pepsinogen concentrations and zero faecal egg outputs.

DISCUSSION

The weather during this experimental trial was drier than that of previous years from mid June until late August, followed by fairly heavy and consistent rain from early September. Numbers of infective L_3 pasture larvae on control grazing followed the typical pattern of previous years' recording (Entrocasso, 1984) in this area. Paddocks 2 and 4, having been grazed by bolused animals, had a later rise in L_3 numbers which was much reduced in magnitude compared to control levels and closely resembled the pasture L_3 counts of grazing in the same location by calves treated with a morantel sustained release bolus (Pfizer Ltd., Sandwich, Kent) (Entrocasso, 1984). Following ingestion of

these larvae by the calves, eggs first appeared in the faeces three weeks after turnout in the control group and increased steadily over the following months. The larvae resulting from these eggs reached the herbage from late July onwards, eventually reaching a peak 27,744 L_3/kg dh in mid September. Coincident with the increasing levels of O. ostertagi larvae on the pasture, clinical type 1 ostertagiasis occurred in the control group which resulted in one calf (754) being necropsied in extremis on 28.8.84. Faecal egg counts and plasma pepsinogen values rose steeply, reflecting the degree of parasitism.

Administration of the ivermectin bolus altered the pattern of faecal egg excretion and significantly reduced plasma pepsinogen levels. From blood ivermectin assays, it appeared that effective levels of anthelmintic were circulating until approximately day 35. Between day 35-day 56 the ivermectin concentration of the majority of the calves began to decline so that only trace amounts were detectable by day 77. This can be taken as evidence of the complete erosion of the ivermectin containing bolus during this period. The treatment was intended to last 90-120 days but had shortfallen this target, probably by about forty days.

Two boluses recovered by necropsy during and after

the trial were of similar type. Despite being retrieved from animals two months apart, their weights were closely similar. Probably their ivermectin companion bolus eroded relatively quickly for some unknown reason, leaving the mineral bolus without a "grinder". Boluses administered singly are known to be much more resistant to erosion. These results are in contrast to the previous trial (Section 5:2) where the ivermectin bolus eroded more slowly than was expected and was, in fact, slower than the mineral bolus.

It can only be speculated that some component or property of the grass altered the expected behaviour of the ivermectin bolus. The hydrophobic nature of ivermectin in the bolus matrix tends to cause the coating to be repelled, so it may have been that weaknesses in the coating which were not important in a housed situation were more vulnerable to damage at grass because of the ingestion of small stones or gravel, although nothing of that nature was recorded at necropsy. The previous trial was conducted with calves with an approximately similar size of reticulum, so this factor should not have influenced the erosion rate. Further research and development work on improving the longevity of the ivermectin bolus in intact animals at grass is obviously required.

Despite a bolus life of approximately 7-8 weeks, the treatment was reasonably effective. Low (50 and 100 epg) faecal egg counts were only recorded in two animals which had intact boluses by mid July. Three calves excreted no eggs until mid September, and one calf necropsied mid August, produced no eggs throughout the trial and on post mortem was found to be helminth free. There was evidence in the abomasum that the animal had been challenged, but since ivermectin acts against the tissue stages of the parasite, this is to be expected. Towards the end of the trial, faecal egg output began to climb. Plasma pepsinogen values in the bolused group began to increase towards the end of September, and before necropsy both groups had similar values. Over the same period, it was noticed that the calves had lost some condition. After necropsy, there was no statistically significant difference between the total worm burden of bolused and control animals although the mean burden was lower in the control group.

The movement of the bolused calves for grazing purposes, probably coincided with the complete erosion of the ivermectin bolus. It has been stated by Spedding (1969) that to move calves after anthelmintic treatment to pasture which has been ungrazed since the previous winter, prevents the development of ostertagiasis. The rationale is that parasites which are initially ingested on the first pasture in spring

in relatively low numbers, complete their life cycle and multiply. By mid to late July large numbers of infective L_3 are present on the grass. If calves can be prevented from ingesting these larvae, this should prevent the development of clinical ostertagiasis. The predicted pattern of L_3 larval numbers rising towards late July was demonstrated on the pasture grazed by control calves. Bolused animals were moved to a different pasture for one month in mid July which was registering zero L_3 O. ostertagi counts. This is further verified by the necropsy of two calves in mid August which were virtually parasite free. After this they were allowed access to their original paddock plus another pasture which had also been grazed by a bolused group of calves until mid July. Technically, therefore, the development of the heavy worm burdens found at necropsy should not have occurred.

The upsurge in pasture L_3 parasites in September was also associated with the end of two months of dry weather and the beginning of a prolonged wet spell. Large reservoirs of L_3 (5-10 M/ha) have been found consistently in soil examined on a regular basis (Armour et al, 1980). It is suggested therefore that during the dry weather a proportion of parasites were unavailable being within the soil and only migrated to the surface when the weather became wet at the beginning of September. It appeared that there were

sufficient numbers of L_3 in September to cause fairly heavy parasitic burdens by late October. Plasma pepsinogens began to show a marked increase, only in late September. If indeed this is the true reason for the helminth numbers present at necropsy, it points to the possible drawbacks of slow release anthelmintic treatments which do not last for the full grazing season. Under this particular combination of weather patterns, animals remained vulnerable relatively late in the grazing season, and although bolus treatment may have "cleaned up" the L_3 present on the pasture early in the season, sufficient numbers were still present later to mount a reasonable challenge.

Production data from the Cochno calves showed that initially bolused animals were advantaged with respect to weight gain for the first two months, which corresponded with the predicted active life of the bolus. Thereafter control animals were growing faster.

This pattern coincides with the movement of the calves in attempts to maximise grazing conditions. As stated in the Methods Section in mid July, bolused calves were by necessity herded into a single group, so obviously the stocking rate was much higher than in the control group (10.8/ha compared with 4.16/ha). Because the pasture was so poor after persistent dry sunny weather, there was a discrepancy in weight gains

as a result. When additional grazing was again available in mid August, the LWG in the experimental group began to improve again but failed to catch up with the control group, probably through lack of time.

FIG.40 Plasma pepsinogen activity in bolused and control calves

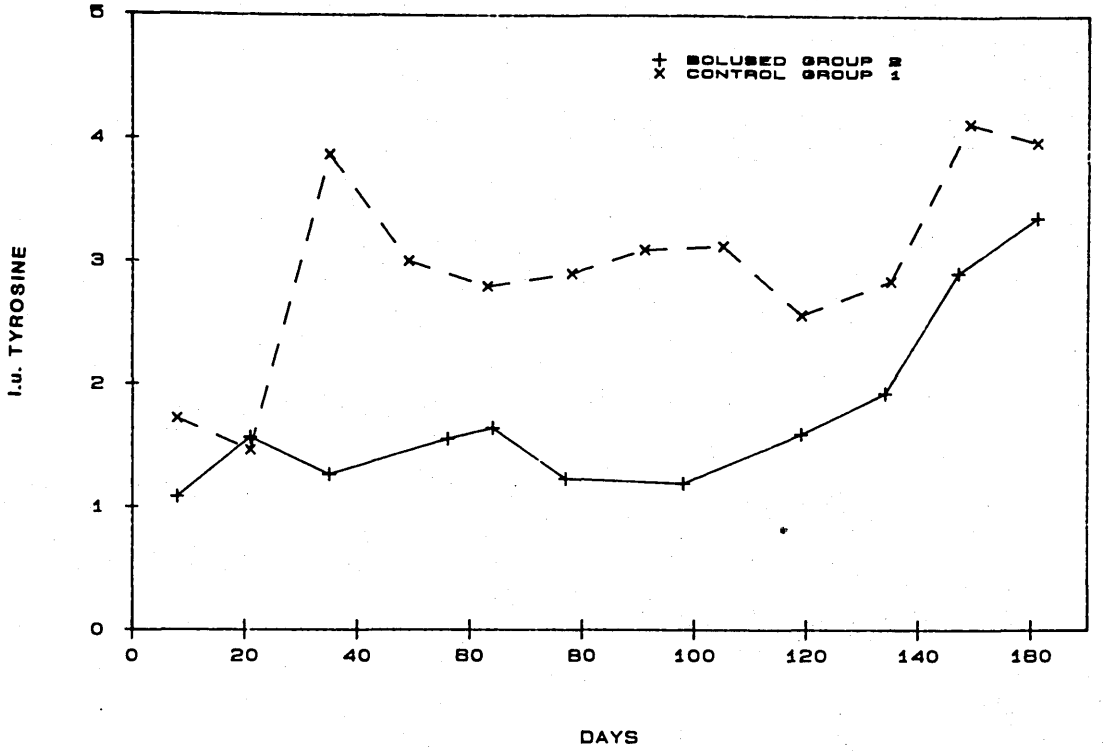


FIG.41 Faecal egg output of bolused and control calves

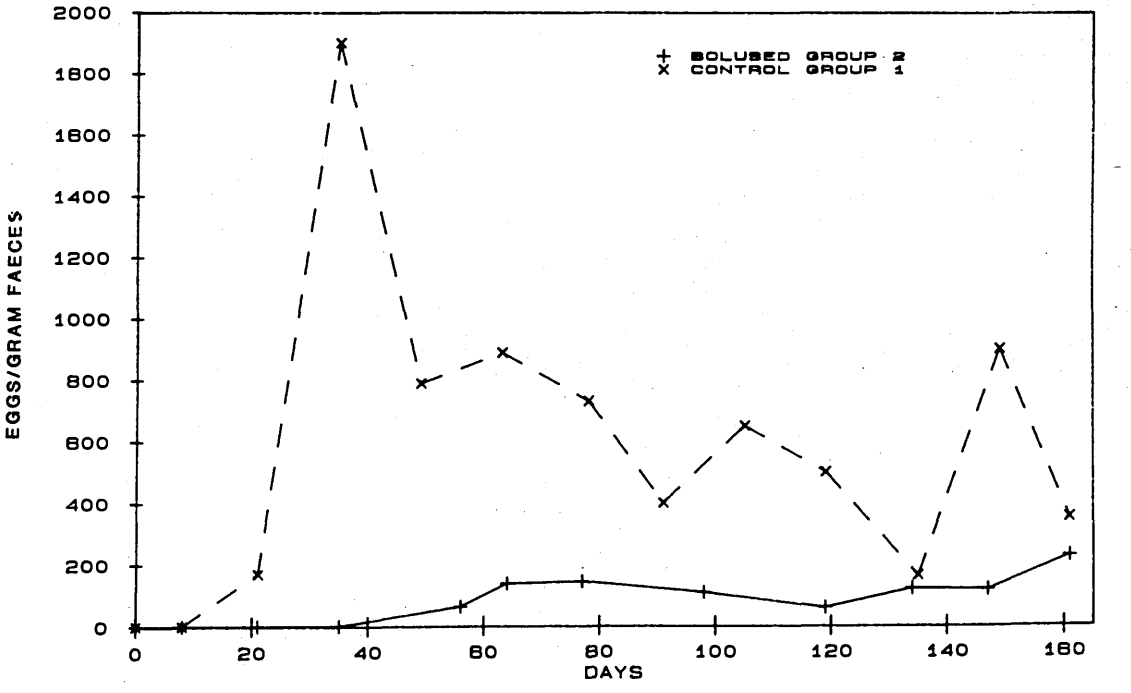


TABLE 49. Groups 3 and 4 calves liveweight (kg)

Calf No. Control Gp. 3	Date	15/5	23/5	4/6	18/6	9/7	17/7	30/7	20/8	10/9	25/9	8/10	22/10	Weight Change Over 161 d
	Day	0	8	21	35	56	64	77	98	119	134	147	161	
2		180	225	220	235	265	267	283	297	325	330	335	340	160
4		142	165	185	200	225	225	240	260	275	290	295	310	168
13		165	195	210	220	240	250	255	280	290	300	305	315	150
15		210	250	270	280	315	315	325	350	368	370	380	385	175
22		150	170	190	205	230	235	240	255	275	275	287	300	150
24		210	245	245	260	290	295	305	315	325	345	355	360	150
	Mean Liveweight Gain/d (kg)		4.02± 1.21	0.96± 0.76	0.95± 0.18	1.31± 0.25	0.46± 0.48	0.78± 0.36	0.87± 0.29	0.80± 0.32	0.58± 0.52	0.60± 0.25	0.63± 0.32	

Calf No. Bolused Gp. 4	Date	15/5	23/5	4/6	18/6	9/7	17/7	30/7	20/8	10/9	25/9	8/10	22/10	Weight Change Over 161 d
	Day	0	8	21	35	56	64	77	98	119	134	147	161	
9		207	225	250	265	295	310	315	320	330	330	338	345	138
12		165	195	215	235	265	275	280	300	305	305	310	320	155
14		195	215	240	245	275	285	290	305	315	320	300	300	105
18		137	155	175	185	220	230	230	245	245	245	245	255	118
20		173	195	215	225	252	265	270	290	295	295	302	305	132
23		182	220	230	240	270	280	285	295	305	305	310	330	148
	Mean Liveweight Gain/d (kg)		3.04± 1.01	1.54± 0.42	0.83± 0.37	1.44± 0.12	1.42± 0.27	0.32± 0.15	0.67± 0.28	0.32± 0.19	0.056± 0.14	0.32± 0.26	0.60± 0.50	

TABLE 50. Plasma pepsinogens (i.u. tyrosine) groups 1 and 2

Calf No. Control Gp. 1	Date	8/5	21/5	4/6	18/6	2/7	17/7	30/7	13/8	27/8	12/9	26/9	8/10
	Day	8	21	35	49	63	78	91	105	119	135	149	161
Y52		2.343	1.355	4.106	2.835	3.071	3.987	3.253	2.795	1.958	1.885	1.564	2.198
Y54*		0.840	1.041	4.534	3.203	2.684	2.989	2.508	2.129	2.213			
Y57		0.902	1.279	2.244	1.734	1.436	1.575	1.698	1.827	1.319	2.028	1.970	2.436
Y88		2.073	1.205	3.641	3.727	3.420	2.729	1.80	1.70	1.972	2.822	7.163	5.922
Y90		0.802	0.664	4.244	2.504	2.260	2.172	5.260	6.244	4.145	3.771	5.440	4.910
Mean + Std. Deviation Plasma Pepsinogen		$1.392^{\pm}$ 0.752	$1.109^{\pm}$ 0.274	$3.754^{\pm}$ 0.904	$2.801^{\pm}$ 0.750	$2.574^{\pm}$ 0.769	$2.690^{\pm}$ 0.906	$2.904^{\pm}$ 1.458	$2.939^{\pm}$ 1.895	$2.321^{\pm}$ 1.072	$2.627^{\pm}$ 0.867	$4.034^{\pm}$ 2.716	$3.867^{\pm}$ 1.839

Calf No. Bolused Gp. 2	Date	23/5	4/6	18/6	9/7	17/7	30/7	20/8	10/9	25/9	8/10	22/10
	Day	8	21	35	56	64	77	98	119	134	147	161
3/		0.615	1.416	0.972	1.251	1.392	0.899	0.857	0.967	1.440	2.077	2.312
5		0.761	1.359	0.785	0.823	0.688	0.664	0.682	0.978	1.200	1.266	1.470
6		0.642	1.294	1.151	1.170	1.388	0.804	0.790	1.294	2.452	1.576	2.125
8		0.835	1.207	0.903	1.299	X	0.790	0.788	1.016	1.453	2.444	3.314
10**		0.748	1.151	0.804	0.826	X	0.665					
11		X	1.151	0.821	1.187	1.354	0.883	0.873	1.687	2.185	5.020	4.930
16**		0.756	1.334	0.864	1.604	1.490	0.886					
25/		0.522	0.853	0.821	1.492	1.516	1.213	0.874	1.578	1.00	3.825	5.024
Mean + Std. Deviation Plasma Pepsinogen		$0.697^{\pm}$ 0.108	$1.221^{\pm}$ 0.178	$0.890^{\pm}$ 0.122	$1.207^{\pm}$ 0.279	$1.305^{\pm}$ 0.309	$0.851^{\pm}$ 0.174	$0.811^{\pm}$ 0.074	$1.253^{\pm}$ 0.319	$1.622^{\pm}$ 0.571	$2.701^{\pm}$ 1.443	$3.196^{\pm}$ 1.501
Degree of Significance Btwn. Gps.1 & 2		$p<0.05$	NS	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.01$	$p<0.05$	NS	NS	NS

* died in extremis 28/8
/ crunched ivermectin bolus at administration
** slaughtered on 15/8 for bolus recovery

X = no sample
NS = not statistically significant

TABLE 51. Faecal egg count/g groups 1 and 2

Calf No. Control Gp. 1	Date	8/5	21/5	4/6	18/6	2/7	17/7	30/7	13/8	27/8	12/9	26/9	8/10
	Day	8	21	35	49	63	78	91	105	119	135	149	161
Y52		0	250	2600	1200	450	0	300	400	150	350	750	350
Y54*		0	250	2500	800	2350	1560	700	1950	850			
Y57		0	50	2500	900	1000	1350	450	500	1050	50	950	400
Y88		0	100	450	200	250	0	50	150	100	200	150	150
Y90		0	200	1450	850	400	750	500	250	350	50	1750	500
Mean ± Std. Deviation faecal egg count		0	170± 90.83	1900± 937.42	790± 364.69	890± 864.15	732± 731.35	400± 242.38	650± 739.09	500± 427.20	162.5± 143.62	900± 660.81	350± 147.20

Calf No. Bolused Gp. 2	Date	23/5	4/6	18/6	9/7	17/7	30/7	20/8		10/9	25/9	8/10	22/10
	Day	8	21	35	56	64	77	98		119	134	147	161
3/		0	0	0	200	150	350	350		350	150	0	0
5		0	0	0	0	0	0	0		0	X	0	0
6		0	0	0	0	0	0	0		0	0	0	100
8		0	0	0	0	0	0	0		0	100	500	400
10**		0	0	0	0	0	0						
11		0	0	0	50	0	50	50		0	350	200	750
16**		0	0	0	50	100	400						
25/		0	0	0	200	850	350	250		0	0	0	50
Mean ± Std. Deviation faecal egg count		0	0	0	62.5± 87.63	137.5± 293.68	143.75± 186.01	108.33± 153.03		58.33± 142.87	120± 144.05	116.67± 204.12	225± 294.53
Degree of Significance Btwn. Gps. 1 & 2		NS	p 0.001	p 0.001	p 0.001	p 0.05	p 0.05	p 0.05		p 0.05	NS	p 0.02	NS

/ crunched ivermectin bolus on administration

** slaughtered 15/8 for bolus recovery

* died in extremis 28.8.84

X = no sample

TABLE 52. Parasite count on herbage at Garscube (per kg DM)

Field No.	Date	8/5	16/5	21/5	4/6	18/6	9/7	17/7	30/7	20/8	10/9	25/9	8/10	22/10
	Day	8	0	5	19	33	46	54	67	88	109	124	137	151
1		120 coop	0	252 ost	0	217 ost	377 ost	0	0	71 ost	0	178 ost	1129 ost	208 ost
2		0	0	206 ost	0	67 ost	608 ost	0	0	0	0	0	5581 ost 2325 coop	4722 ost 1944 coop
3		0	0	0	0	259 ost	30 ost	0	0	0	1308 ost	3061 ost	8333 ost 4048 coop	3962 ost 2452 coop
4		0	0	0	135 ost	185 ost	344 ost	0	0	149 ost	416 ost	2093 ost	277 ost	833 ost
5		0	0	0	0	452 ost	40 ost	0	0	0	178 ost	784 ost	3611 ost	857 ost

Field No.	Date	8/5	16/5	21/5	4/6	18/6	2/7	17/7	30/7	13/8	27/8	3/9	12/9	26/9
	Day	8	16	21	35	49	63	78	91	5	119	124	135	149
6		563 ost	NS	526 ost 175 coop	0	185 ost	292 ost 58 coop	169 ost	1125 ost 125 coop	2424 ost 303 coop	7692 ost 769 coop	1739 ost 725 coop	23233 ost 4511 coop	16000 ost 4000 coop

TABLE 53. Blood ivermectin assay (ng/1) bolused group 2

Calf No.	Date / Day	15/5 / 0	23/5 / 8	4/6 / 21	18/6 / 35	9/7 / 56	17/7 / 64	30/7 / 77
3		0	14.8	3.8	0	0.5	1.0	0.5
5		0	4.0	1.6	0.6	0.5	0.7	0.5
6		0	12.8	3.8	2.7	0.5	1.0	NS
8		0	11.4	7.5	0.7	1.5	NS	0.5
10		0	1.9	2.8	1.7	1.0	NS	0
11		0	1.0	7.8	1.0	0.5	0.9	0
16		0	2.2	1.8	1.7	0.5	0.5	0
25		0	4.4	0.7	0.4	0	0.5	0.5
Mean ivermectin (ng/1)		0	6.56 ± 5.52	3.73 ± 2.65	1.1 ± 0.88	0.63 ± 0.44	0.77 ± 0.23	0.29 ± 0.27

TABLE 54. Calf necropsy data

Calf No.	Date of Necropsy	Ostertagia Adult	Ostertagia L_4	Cooperia Adult	Cooperia L_4	Total Helminth Burden	
E10*	15. 8.84	0	0	0	0	0	
E16	15. 8.84	100	0	0	0	100	
C54	28. 8.84	14,800	1,000	3,200	100	19,100	
C52	17.10.84	10,000	4,200	12,800	7,200	34,200	
C57	17.10.84	12,900	18,400	2,900	400	34,600	x worm burden = 63,600 $\pm$ 33,926
C88	17.10.84	24,800	63,200	200	0	88,200	
C90	17.10.84	36,300	53,600	3,500	4,000	97,400	
E25≠	25.10.84	10,700	25,200	0	0	36,400	
E5	25.10.84	400	19,800	0	1,200	21,400	
E8	25.10.84	11,000	15,600	400	6,300	33,300	x worm burden = 27,400 $\pm$ 8,775
E11*	25.10.84	12,400	5,700	0	400	18,500	

≠ crunched ivermectin bolus on administration
* recovered mineral bolus

C = control group 1
E = bolused group 2

SECTION VI

Continued Commercial Development

GENERAL INTRODUCTION

Following the relative success of laboratory manufactured boluses in fistulated cows and field studies involving intact calves, renewed attempts were made, which followed several different directions, to produce a bolus on a commercial scale with similar properties. The prime objective was to produce a bolus containing anthelmintic suitable for sustained treatment over the entire grazing season. Such a bolus would ideally last 90-120 days, a longer period than the originally developed mineral bolus. Commercial production processes are subject to constraints which do not apply to small scale laboratory bolus manufacture. There were three factors which, for practical reasons, were subject to consideration and testing, with a view to possibly adapting the original bolus design for ease of commercial production. The three factors for study were:

(a) the use of pelletting additives

(b) the use of copper oxide powder as a replacement for needles

(c) horizontally pressed boluses

(a) The addition of a lubricating agent such as magnesium stearate to the bolus formula was known to be a necessity for the smooth functioning of large presses. However, since such additives would most probably affect the erosion properties of the mineral mix, many studies were involved in testing the effect of additives of different natures on bolus erosion.

(b) Prior granulation of material before compaction is essential in commercial operations, otherwise settlement of ingredients, in particular of dense particles, would occur as the machine vibrated during operation. Even after granulation, some settlement would tend to occur of the relatively large copper oxide needles. It was thought that a better more even dispersion could be produced by using copper oxide powder, therefore this was investigated in some detail.

(c) While cylindrically shaped boluses of up to 60 mm in length can be produced successfully in the laboratory hydraulic press, it proved impossible to locate any commercial tabletting machine in Britain which was capable of compacting a cylindrical bolus to that length (see Section Thomson and Capper boluses). An alternative method of producing boluses would be to use a

mould where the bolus is compressed side on, rather than from end to end. By this means, a longer bolus of any size and length could be produced on existing machinery.

Much of the investigative work on these three factors was carried out concurrently and was difficult to segregate into discrete sections. Certain experimental boluses were shown to be unsuitable almost immediately so that the trials were then prematurely terminated.

Pregranulation, which is a prerequisite for commercial processing, could not be carried out in the laboratory at Glasgow. Therefore it was arranged that Merck, Sharp and Dohme should carry out this process at Hoddesdon and despatch the granulated material to Glasgow for compaction and testing.

1. Additives in Ivermectin Boluses

INTRODUCTION

It was anticipated that granulation of the mineral salts base and the addition of a lubricating agent would alter the erosion characteristics of the bolus. Granulation causes particles to aggregate until the powder assumes a crumb-like texture. Therefore it was thought possible that this may cause the erosion rate of the bolus to increase, because the basic particle size had increased, and more space than in a fine powdered mix would exist between particles on compaction through which fluid could permeate. Magnesium stearate the lubricant of choice was known to be hydrophobic, which may have offset the anticipated influence of granulation. Different additives, initially binders intended to slow the erosion rate, PVPK90, corn starch and Klucel LF were added to basic granulated mixes with magnesium stearate in order to assess the influence of lubricant, granulation processes, and different binders.

MATERIALS AND METHODS

Five different mixes were granulated by Merck, Sharp and Dohme and sent to Glasgow for pressing into 52 g compacts. Mixes were composed as shown on Table 55.

TABLE 55. Constituents of mixes 1-5 bolus formulae

	% Copper Oxide Needles	* % Mineral Mix	% Ivermectin	% Mg Stearate	% Other Additive
1	46.26	52.3	1.45	-	
2	45.35	51.26	1.42	1.96	
3	44.80	50.64	1.40	1.94	1.23% PVPK90
4	44.90	50.78	1.40	1.94	0.95% corn starch
5	44.75	50.58	1.40	1.94	1.29% Klucel LF

* as detailed in Section 2:5

Boluses were pressed in a 19 mm diameter cylindrical mould in the laboratory and coated four times with glass fibre resin (Plastic Padding Ltd.), leaving one end exposed. Paired boluses were placed in the reticula of fistulated cows and examined at intervals. At days 5, 12 and 18 the boluses were removed from the cows, washed, dried, weighed and replaced.

RESULTS AND DISCUSSION

The results are plotted on Figure 42 and the average release rates over the test period shown on Table 56. It was difficult to draw any definite conclusion from the results as there was

considerable variation between boluses of the same formulation. Broadly, the granulated mix with no additives (mix 1) surprisingly eroded at the slowest rate, and the addition of magnesium stearate to the granulated mix (mix 2) appeared to increase the rate of erosion. Further additions of binders (mixes 3-5) did not appear to superficially alter erosion very much, except for corn starch binder which slowed the rate by varying degrees. The wide variability in results was thought to be due to either settlement of some ingredients during manufacture, or to the random pairing of formulae. Previous testing procedures usually involved two boluses of the same formulation being paired. It could have been in this case that "harder" bolus formulae caused more erodable boluses to dissolve more quickly than they would have done, had they been paired with boluses of their own type. After this trial, attention was paid to the pairing of identical formulae. It is uncertain whether any settlement of ingredients could have been involved. The fact that mix 1 had no additives and that these boluses produced the most similar erosion rates may apparently support this theory.

More detailed observations and a greater number of replicates were required in the investigation of each individual additive. Corn starch binder was thought, from this trial to have exerted the most definite effect on erosion, seeming to steady it

compared to the addition of PVPK90 and Klucel LF. It was therefore chosen for further investigation.

TABLE 56. Average release rates of boluses of mixes 1-5 over an 18 day test period

Bolus Mix	Bolus No.	Average Release Over 18 d period (g/d)
Type 1	01-2	0.123
Type 1	01-3	0.205
Type 2	02-1	0.959
Type 2	02-2	0.463
Type 2	02-3*	0.086
Type 3	03-1	1.572
Type 3	03-2	0.705
Type 3	03-3	1.242
Type 4	04-1	0.065
Type 4	04-2	0.509
Type 4	04-3	0.259
Type 5	05-1	0.340
Type 5	05-2	0.549
Type 5	05-3	1.339

* located in rumen d.12

FIGURE 42 (i) Handmade boluses of O1 code containing pregranulated mix with 46% copper oxide needles, 52% mineral mix and 1.45% ivermectin and O2 code boluses containing the same proportions of ingredients plus 1.96% Mg stearate. Boluses were tested in fistulated cows.

FIGURE 42 (ii) Erosion pattern of handmade boluses containing pregranulated mix with 45% copper oxide needles, 50.6% mineral mix, 1.4% ivermectin, 1.94% Mg stearate and 1.23% PVPK90 tested in fistulated cows.

FIG.42(i) Additives in Ivermectin bolus

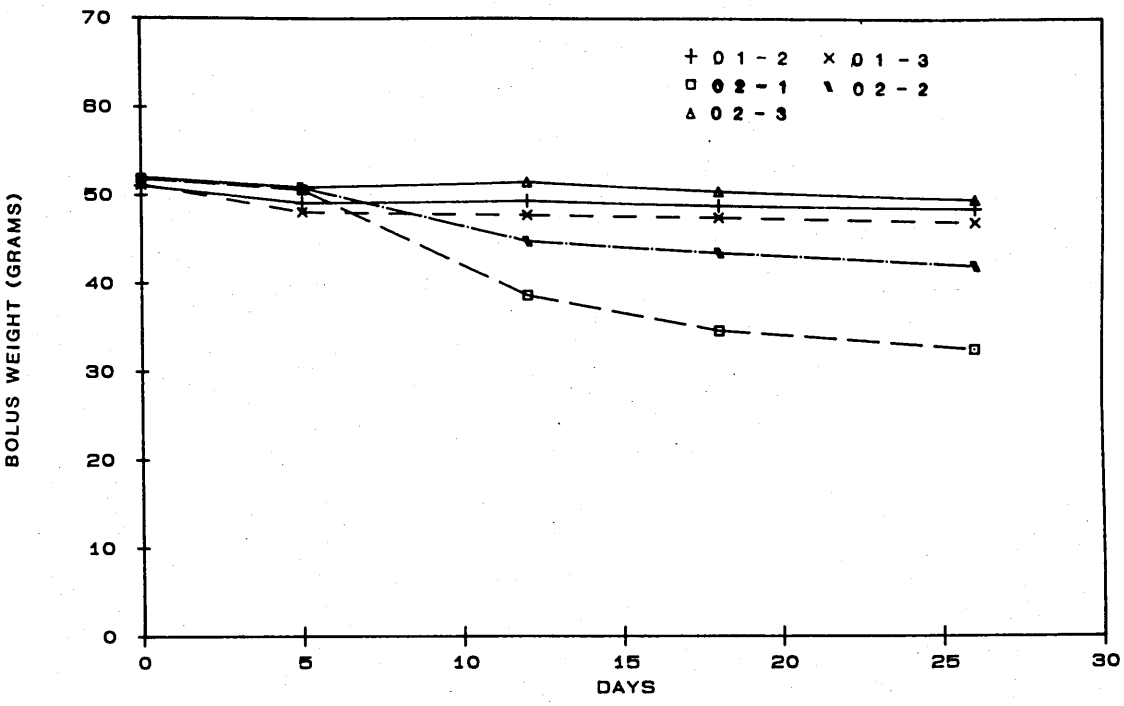


FIG.42(ii)

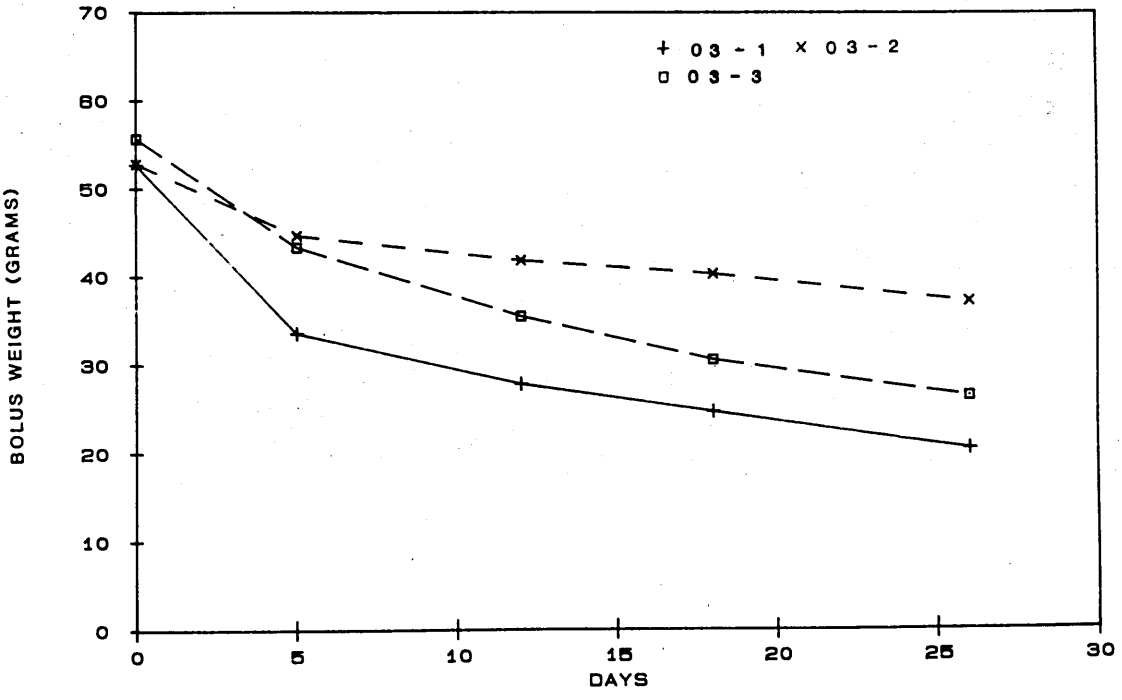


FIGURE 42 (iii) Erosion pattern of handmade boluses containing pregranulated mix 44.9% copper oxide needles, 50.8% mineral mix, 1.4% ivermectin, 1.94% Mg stearate, and 0.95% corn starch, tested in fistulated cows.

FIGURE 42 (iv) Erosion pattern of handmade boluses containing pregranulated mix with 44.75% copper oxide needles, 50.9% mineral mix, 1.4% ivermectin, 1.94% Mg stearate and 1.29% Klucel LF, tested in fistulated cows.

FIG.42(III)

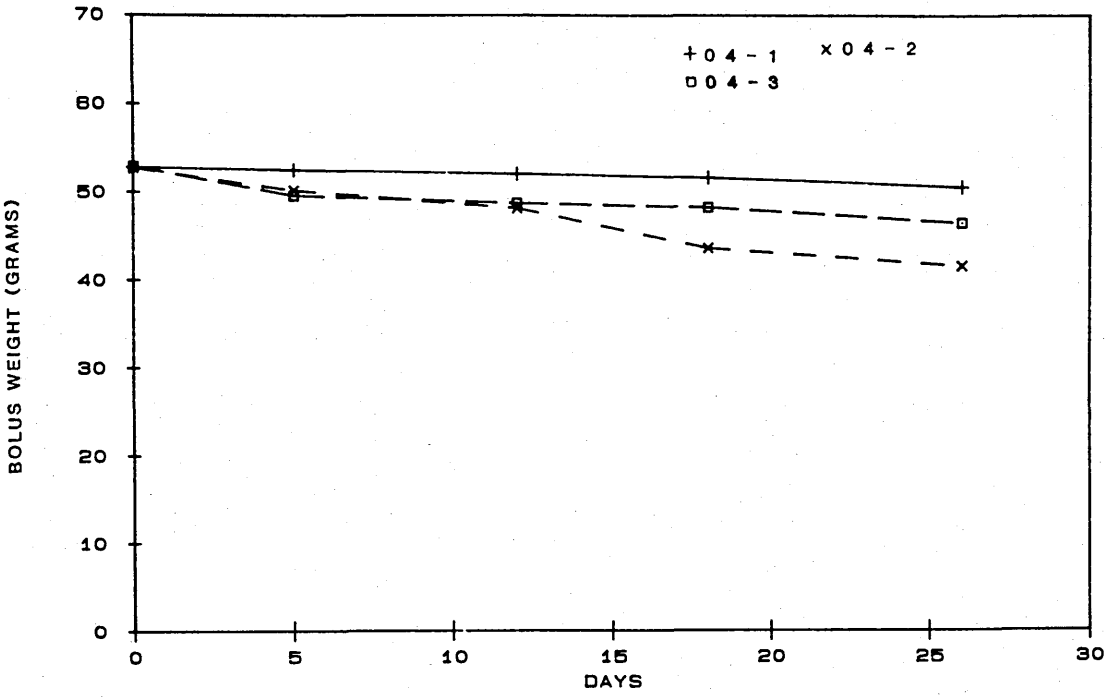
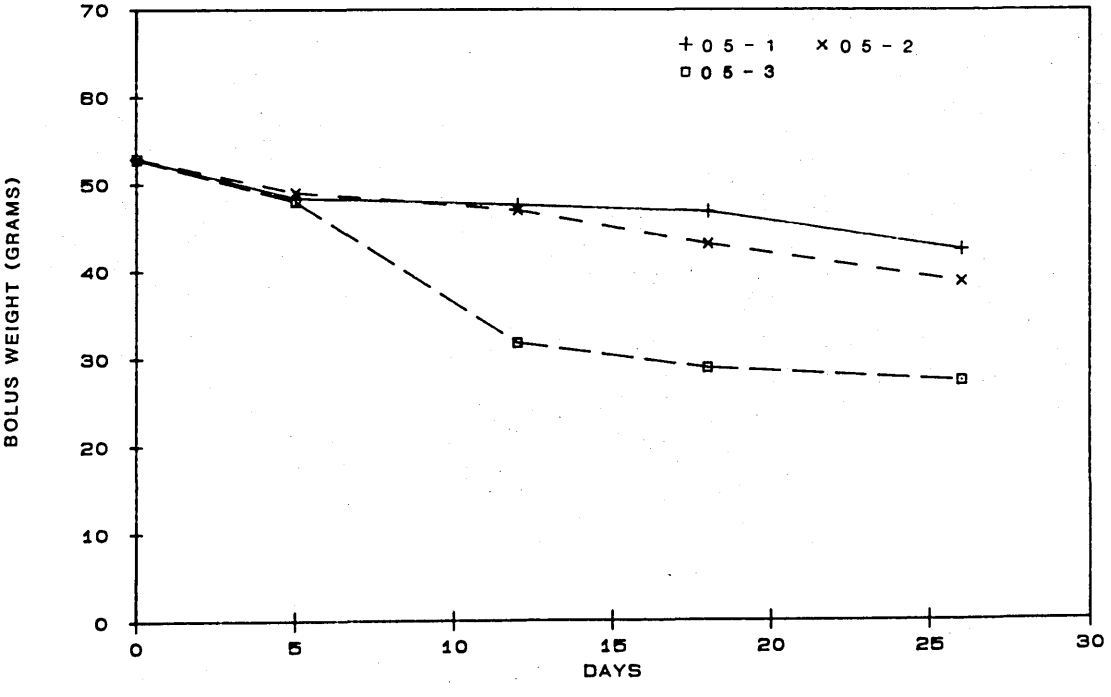


FIG.42(IV)



2. Alternatives to Copper Oxide Needles

INTRODUCTION

Copper oxide needles 2-4 mm in length, traditionally composed 45% of the basic bolus mix. However, it was felt that some investigation should be made into their replacement with material which could be more evenly dispersed throughout the mix during pregranulation. Even after granulation procedures, copper oxide needles still tended to settle out of the mix. Some difficulty was also being experienced in obtaining copper oxide needles on a large scale. An alternative should still supply the ruminant requirements for copper, therefore copper oxide powder was thought possibly to be a more suitable material. It is more readily available commercially than copper oxide needles, is very much cheaper, and is produced in a variety of grades from small particles (50 mesh size) to a fine sooty powder (200 mesh). An experiment was set up, involving the use of coarse powder (50 mesh) compared with copper oxide needles (200 mesh).

MATERIALS AND METHODS

Two formulations were mixed and pregranulated as follows:

(a) 1.94% magnesium stearate
44.92% copper oxide needles
0.95% maize starch binder
50.78% standard mineral mix (Section 2:5)
1.41% ivermectin

(b) 1.94% magnesium stearate
44.92% copper oxide power (50 mesh)
0.95% maize starch binder
50.78% standard mineral mix (Section 2:5)
1.41% ivermectin

These mixes were handpressed into eight cylindrical boluses of each formulation, 19 mm in diameter, 50 mm in length, 52 g in weight, and were coated with four layers of glass fibre resin leaving one exposed face. They were administered to fistulated cows indoors, two per animal, and were examined at regular intervals.

RESULTS AND DISCUSSION

The results are plotted graphically on Figures 43 and 44 and the average daily release rates between measurement intervals, shown on Table 57. Over the first fifteen days, the boluses containing coarse powder had widely varying release rates, which regularised to some degree between d.15-d.36. Boluses containing needles were less variable, although by day

36, bolus residues ranged from 21-36 g in weight.

Between days 15 and 36, the mean weight loss of boluses containing needles was:

0.217 ± 0.149 g/d with a projected bolus life of approximately 173 ± 29 days

Boluses containing powder had, between days 15 and 36, lost on average:

0.178 ± 0.087 g/d with a projected bolus life of approximately 185 ± 53 days

The projected life of both bolus types were much longer than being aimed for, but the main area of concern was the variability in erosion pattern between boluses of apparently identical composition. Part of the variability was thought to have possibly arisen from hand production where inevitable small inconsistencies in manufacture may have led to variability in erosion. Therefore it was decided as a priority that further work be carried out with machine manufactured boluses.

TABLE 57. Erosion data on needles and powder boluses

Bolus No.	Erosion rate (g/day)		
	0-8	8-15	15-36
N1	2.014	1.675	0.231
N2	1.621	1.386	NR
N3	1.459	1.469	0.024
N4	1.042	1.154	0.059
N5	0.212	1.372	0.402
N6	0.289	0.787	0.405
N7	1.860	0.702	0.224
N8	2.222	1.085	0.176
P1	0.83	0.416	0.099
P2	1.231	2.183	0.179
P3	2.378	1.070	0.147
P4	1.825	1.752	0.244
P5	2.093	0.693	0.134
P6	2.065	0.992	0.170
P7	0.189	0.073	0.091
P8	0.478	0.919	0.358

NR = not recovered

FIGURE 43. Erosion pattern of handmade boluses containing pregranulated copper oxide powder (50 mesh), mineral mix, Mg stearate, maize starch binder and ivermectin tested in fistulated cows.

FIGURE 44. Erosion pattern of handmade boluses containing pregranulated copper oxide needles, mineral mix, Mg stearate, maize starch binder and ivermectin tested in fistulated cows.

FIG. 43 Ivermectin boluses with maize starch and CuO (p)

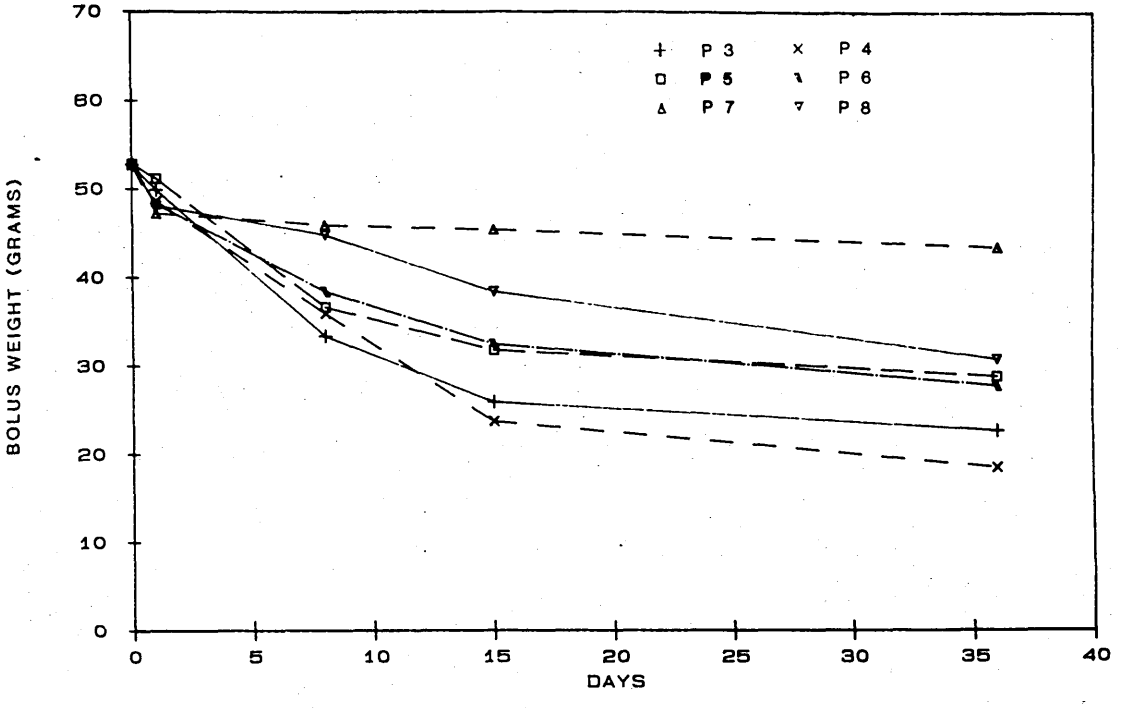
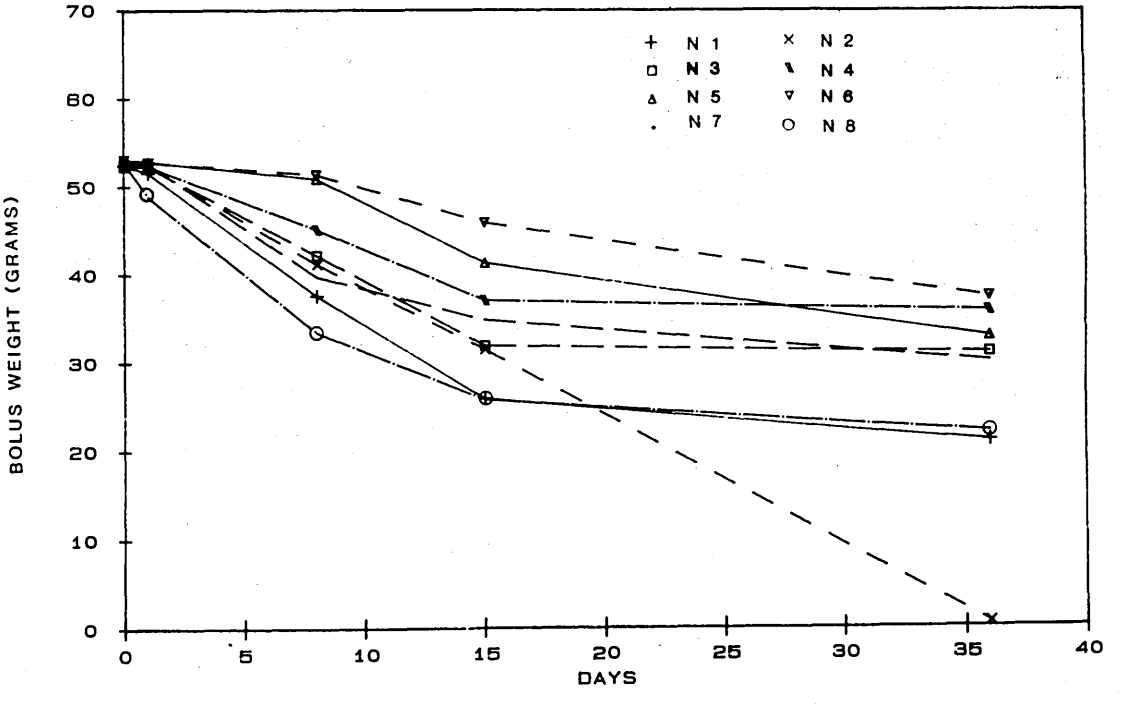


FIG.44 Ivermectin boluses with maize starch and CuO (n)

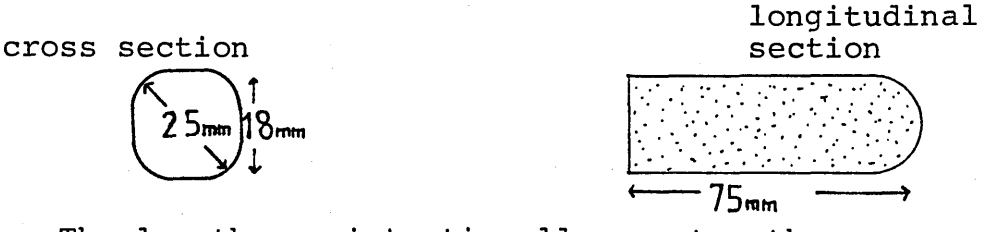


3. Horizontally Pressed Boluses

INTRODUCTION

As was previously discussed both in the general introduction and Section 3:3, informed opinion was that it was virtually impossible to produce a cylindrical bolus in Britain, pressed end to end of the dimensions 19 mm diameter x 60 mm length. It was thought that a mould for a similar horizontally compressed object could be tailored in such a way as to produce a bolus which could be rounded off to a very similar shape as the existing cylindrical bolus. One advantage of horizontal compaction is that pressure is more evenly distributed throughout the bolus compared to end-on pressing. However, the major disadvantage is the inevitable presence of mould flashlines or sharp squared edges which may require to be sanded off.

Specifications were drawn up for a horizontally pressed bolus as follows:



The length was intentionally greater than desired in the finished product. This could be

reduced by milling after compaction to any desired length.

It was planned that the same formulae tested in the last trial, i.e. coarse copper oxide powder and copper oxide needles boluses with maize starch binder should be repeated in horizontally pressed boluses so that their performance could be directly compared.

MATERIALS AND METHODS

Material was pregranulated and horizontally compacted on a Manesty tabletting machine. The mixes were identical to those used in the previous trial (Section 6:1), containing mineral mix (as Section 2:5), copper oxide needles (2-4 mm) or powder (50 mesh), magnesium stearate, ivermectin and maize starch in identical proportions to 6:1. The dimensions were as follows:

(a)	Copper oxide needles plus maize starch	18 x 14.5 x 75.5 mm
(b)	Copper oxide powder plus maize starch	18 x 13 x 75.5 mm

From the dimension measurements it can be deduced that the cross section was not square, being due at this stage to inexperience in judging the amount of

material to correctly fill the mould. Also, the boluses were not nearly as rounded off as expected and were very much closer to a rectangle in shape than the slightly flattened cylinder as was hoped. One end was expected to be rounded off as shown in the diagram, but both ends were in fact flat.

The edges of the boluses were lightly sanded in the laboratory before applying four coats of glass fibre resin, leaving one exposed face. The boluses were administered in pairs to fistulated cows indoors on a hay and concentrate diet and were examined and weighed at intervals over a thirty-five day period.

RESULTS AND CONCLUSIONS

Erosion patterns of boluses containing needles is shown on Figure 45, and those containing coarse powder, on Figure 46. Boluses containing needles had an abnormally wide spread in release rate while those containing coarse powder, apart from those lost or broken, were more uniform. The overall average daily release rate over the test period and the projected bolus life is shown on Table 58.

For three weeks approximately, the coating remained fairly intact on the boluses, but towards the end of the test period it began to appear patchy and

TABLE 58. Erosion data for horizontally compressed mineral boluses

	Bolus No.	Average Daily Erosion Rates (g/day) d0-13	d13-20	d20-35	d0-35	Projected Bolus Life (d)
CuO needle boluses	MN1	1.246	2.044	0.874	1.246	45
	MN2	0.852	0.756	0.275	0.586	92
	MN3	0	0.136	0 ≠	0	
	MN4	0	0.259	0 ≠	0.0137	3664
	MN5	1.10	1.672	0.089	0.782	72
	MN6	0.146	0.418	0	0.130	413
	MN7	1.684	0.664	-	-	
	MN8	1.637	NR			
CuO powder boluses	MP1	0.860	0.881	0.765	0.824	63
	MP2	0.760	0.785	1.053	0.892	56
	MP3	0.053	0.042	0.029	0.041	
	MP4	NR	-	-	-	
	MP5	0.819	1.043	0.111	0.561	87
	MP6	1.075	0.885	0.098	0.619	79
	MP7	0.078	0.086	-	-	
	MP8*	2.855	0.826	-	-	

* bolus broken d.13

NR = not recovered

≠ boluses found in rumen

split at the edges, particularly in the boluses containing copper oxide needles. However, the effect of coating does not account for the wide variation in bolus erosion. Bolus N2 eroded at an average rate of 0.586 g/d and its coating had the same degree of intactness over thirty-five days as bolus N6 which eroded at an average rate of 0.130 g/day.

Of the coarse powder boluses, one was lost entirely after thirteen days, and another was broken. The two partners of these boluses consequently failed to erode. Of the four pairs of boluses, two pairs remained intact and dissolved in a fairly impressive manner, P1 losing an average of 0.824 g/day, and P2 losing 0.892 g/day, although P5 and P6 showed a definite slowing in erosion between days 20 and 35; they lost on average 0.561 g/day and 0.619 g/day.

When the projected lifespan was calculated from the average daily release over thirty-five days, needles boluses with partners were found to vary in predicted lifespan between forty-five days and infinity. Boluses containing coarse powder were found to vary between 56-87 days, a considerably closer margin.

These findings are in marked contrast to the handmade boluses of the previous trial when the predicted lifespan of copper oxide needles boluses was

173 $\pm$ 29 days, and that of coarse powder boluses was the wider range of 185 $\pm$ 53 days.

It can be concluded that the mechanical production of a horizontally compressed bolus has somehow caused the increase in erosion rate of boluses containing coarse powder, yet it has introduced wide variation into the behaviour of boluses containing copper oxide needles, without any obvious reasons.

The differences observed must have arisen because of (a) differences in the consistency of the mix, or (b) coating variations, or (c) between animal variation.

The presence of needles in the granule in the vibrating hopper during compaction may have caused some differential settlement of ingredients which was less marked in the more compact granules containing coarse powder. If this were the case, it would be advantageous to switch to powder permanently in commercial operations, perhaps utilising an even finer grade than 50 mesh, hopefully to lead to granules of more uniform consistency. In the previous trial where boluses were handpressed, thorough handmixing preceded compaction.

All boluses were coated by hand by the same person

using an identical technique. No real correlation could be drawn between coating integrity and erosion characteristics.

Between cow variation may have played some part in influencing the erosion pattern. Since the boluses were long and narrow, it is possible that end to end abrasion, normally assumed to play a significant role in erosion, was difficult except in animals with a large reticulum, which could explain the excessively slow rates in some cows. The shape and length may also have made the boluses more easily transferable to the rumen or to regurgitation. On one occasion boluses were recovered from the rumen, and it would have been possible for them to have been passing back and forward between the reticulum and rumen in this cow throughout testing. If regurgitation of the shape and size of bolus could occur, it may explain the fact that MP8 was broken after administration.

FIGURE 45. Erosion pattern of horizontally compacted boluses MN1-MN8 which contained copper oxide powder (50 mesh), mineral mix, maize starch binder, magnesium stearate and ivermectin tested in pairs in fistulated cows.

FIGURE 46. Erosion pattern of boluses which were horizontally compacted and contained identical ingredients to those detailed above apart from the substitution of copper oxide needles for the 50 mesh powder.

FIG 45 Boluses containing copper oxide needles

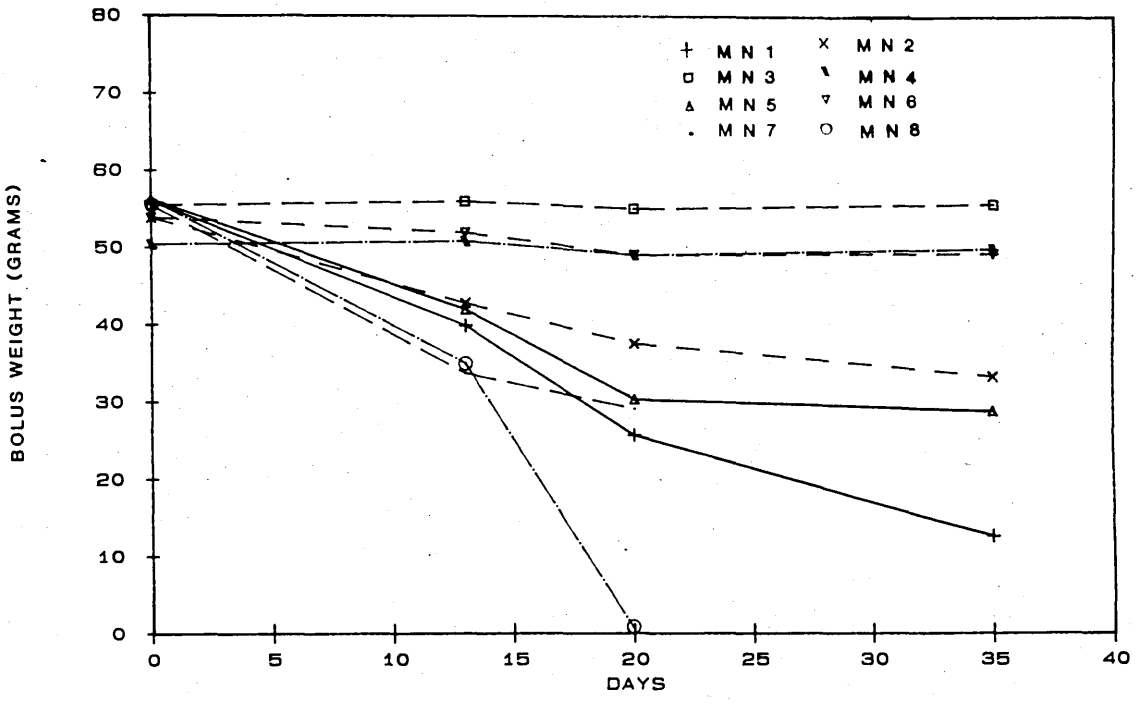
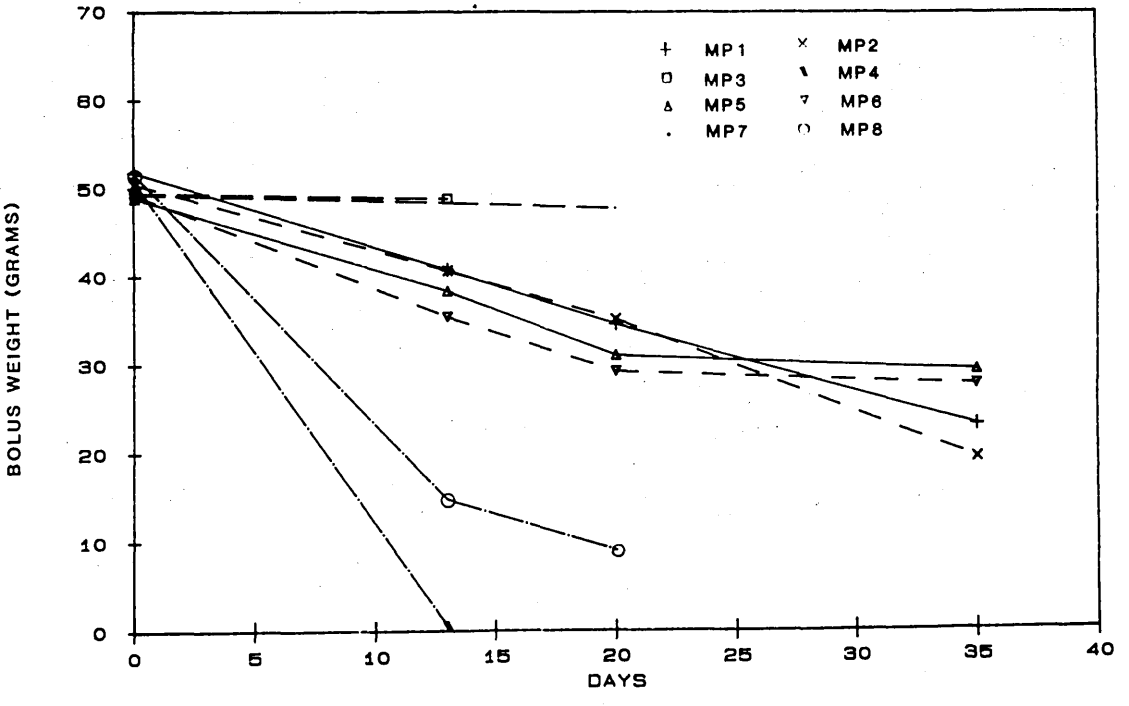


FIG.46 Boluses containing coarse CuO powder



4. Fine Copper Oxide Powder Boluses

INTRODUCTION

The very wide variation in erosion rate of machine pressed boluses containing copper oxide needles in the previous trial and the narrower range of values noted when coarse powder substituted copper oxide needles, implied that small particles of copper oxide produced a more homogeneous mix with more predictable erosion characteristics. Although previously cylindrical hand mixed and pressed boluses containing copper oxide needles eroded in a predictable and repeatable manner, once granulation and horizontal pressing became involved, the release rate became highly variable. Therefore it was proposed that a finer grade of copper oxide powder should be tested in an effort to reduce between bolus variability. Fine copper oxide powder of 200 mesh is the normally available standard grade which has the added advantage of being easily and cheaply available commercially. A previous trial (in Section 2:6) in which handmade boluses of 200 mesh and 50 mesh copper oxide powder were compared showed that boluses with 200 mesh copper oxide powder eroded at a very much slower rate than those with 50 mesh.

Corn starch was retained in the formula with 200 mesh copper oxide powder in the form of a more readily

mixed paste in one bolus batch, and another binder gelatin, added to another batch of boluses for comparison. Since the previous horizontally pressed boluses had in general eroded more quickly than was expected, the proportion of binder was increased.

MATERIALS AND METHODS

The basic formulation used in this trial contained 40.8% copper oxide powder (200 mesh), 1.94% magnesium stearate, 1.29% ivermectin, 46.1% standard mineral mix. Starch and gelatin were added as follows:

Trial A -

1. 10% maize starch compaction pressure 280 lbs psi
2. 15% maize starch compaction pressure 280 lbs psi

Trial B -

3. 5% gelatin compaction pressure 280 lbs psi
4. 10% gelatin compaction pressure 280 lbs psi

The dimensions of both bolus types were 18 mm x 75.5 mm and were therefore square in cross section. Boluses were horizontally compacted and despatched to Glasgow where they were first sanded and then coated with four layers of glass fibre resin. Two boluses of the same type were administered to fistulated cows indoors on a hay and concentrate diet. There were

either four or five replicates of each treatment so that eighteen fistulated cows were involved in the study. Boluses were examined after twenty-four hours, then at weekly intervals.

RESULTS AND DISCUSSION

After twenty-four hours, one pair of each type of bolus was weighed and described. Starch boluses were beginning already to wear at the edges and the resin coating was missing from the four corners of the base. At this point the weight loss was negligible. Gelatin boluses after twenty-four hours were showing slight wear on the edges.

When all the boluses were examined after one week's testing, the glass fibre resin had peeled off the boluses exposing large areas. This effect was particularly marked in the starch inclusion boluses. Little remained of the starch inclusion boluses after a period of fourteen days. Figures 47 and 48 show the erosion pattern of the starch boluses over the test period. Gelatin boluses, despite partial loss of coating in many cases, lost little weight after one week. Only two boluses which contained 10% gelatin out of eight lost any weight. Small weight losses were reported during the second week and thereafter the erosion rate improved. Erosion data for gelatin

boluses is shown on Table 59 and plotted graphically on Figures 49 and 50. During days 0-32 boluses containing 10% gelatin eroded at a slower rate than those with 5%, although the coating condition of the two formulae was similar. Overall the boluses containing 5% gelatin still intact after thirty-two days lost an average of 0.434 ± 0.205 g/d over thirty-two days and those containing 10% gelatin lost on average 0.264 ± 0.153 g/d. Coating after thirty-two days was in varying conditions, but those with an intact coat, marked on the table, lost least over the test period.

Starch boluses were thought to have broken up because of the starch being digested out of the matrix by normal gut action, leaving behind gaps and weaknesses in the bolus structure, making boluses vulnerable to damage. Starch was present in higher quantities than previously (Section 6:3) which is probably why this effect was so marked. Similar results were obtained with both 10% and 15% starch, and obviously no binding function was carried out by the starch paste.

Gelatin appeared to perform a binding function in that boluses in which the coating remained intact eroded relatively slowly. An increase of 5% in gelatin content on average slowed the erosion rate by 40%.

The non-adherence of the resin coating to the

TABLE 59. Average release rate of boluses containing gelatin over 32 d test period

Bolus No.	Bolus Type	Average Weight Loss/Day (g) d0-7	d7-14	d14-32	d0-32
A Gel 1	5% gelatin	0.387	0.383	0.473	0.476
A Gel 2	" "	0.395	0.689	0.408	0.491
A Gel 3	" "	0.037	0.454	1.124	0.800
A Gel 4	" "	0.284	0.268	0.641	0.451
A Gel 5	" "	5.642*	-	-	
A Gel 6	" "	0.428	0.559	0.279	0.371
A Gel 7	" "	+0.122	0.243	0.108	0.122≠
A Gel 8	" "	0.153	0.424	0.257	0.325
A Gel 9	" "	0.297	5.152	-	-
A Gel 10	" "	0.044	0.584	2.186	-
B Gel 1	10% gelatin	+0.044	0.088	0.047	0.085≠
B Gel 2	" "	+0.162	0.139	0.138	0.110≠
B Gel 3	" "	+0.116	0.151	0.263	0.188
B Gel 4	" "	0.387	0.196	0.259	0.277
B Gel 5	" "	+0.408	1.577	0.563	0.560
B Gel 6	" "	+0.235	0.689	0.452	0.352
B Gel 7	" "	0.246	0.203	0.434	0.327
B Gel 8	" "	+0.037	0.140	0.365	0.211

* 2 small pieces
+ gained weight
≠ intact coat

matrix exterior was common throughout this trial and few boluses retained the full shell. It is likely that the powdery exterior of boluses containing fine grade copper oxide powder boluses prevented the coat adhering to the mineral core. When the coat peeled off small surface particles of the mineral matrix were found to be embedded in it. Perhaps boluses could be briefly dipped in a hydrophobic liquid prior to coating which may encourage the resin to penetrate the matrix more deeply. Between the weaknesses of the square edges of the horizontally pressed pellet and the powdery exterior when fine copper oxide powder is present, such difficulties would require investigation in the future.

Results of this trial were more uniform between boluses than previous studies. Particle size does therefore seem to influence homogeneousness, small particles appearing to produce more consistently eroding boluses. It was therefore proposed to further develop copper oxide powder boluses, altering the erosion characteristics with a known range of additives until a suitable formula was found.

FIGURE 47. Erosion pattern of horizontally compacted boluses containing copper oxide powder (200 mesh), magnesium stearate, ivermectin, mineral mix and 10% maize starch, tested in fistulated cows.

FIGURE 48. Boluses of identical formulation and testing to those detailed above, except that 15% maize starch was included in the formulation.

FIG.47 10% maize starch inclusion boluses

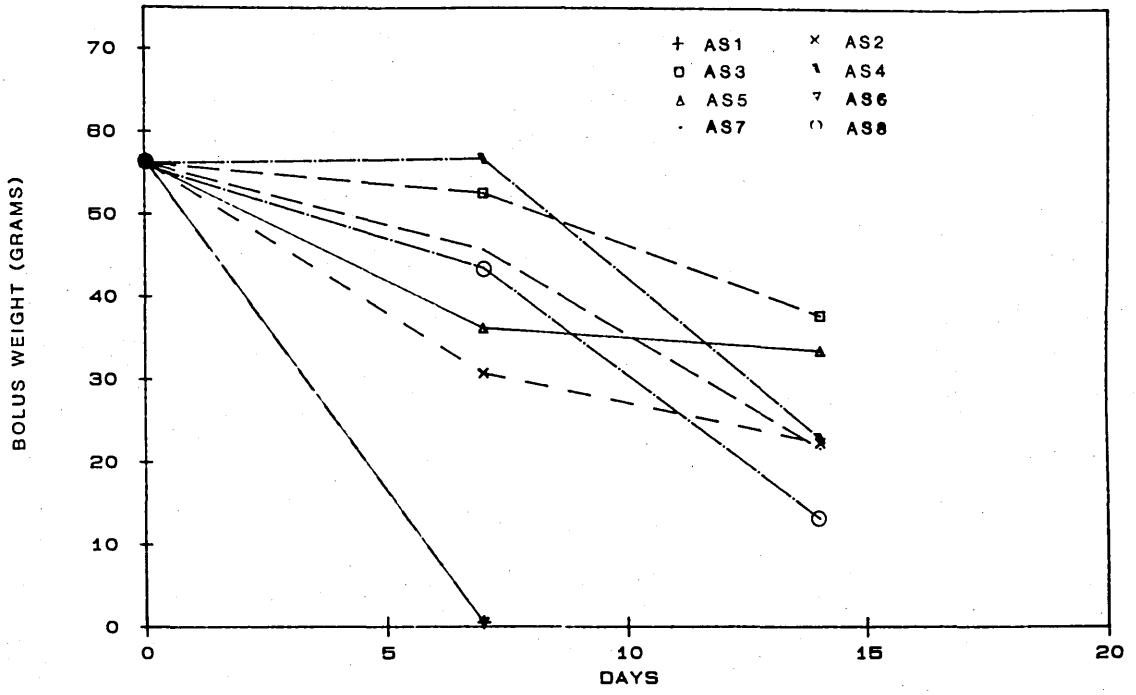


FIG.48 15% maize starch inclusion boluses

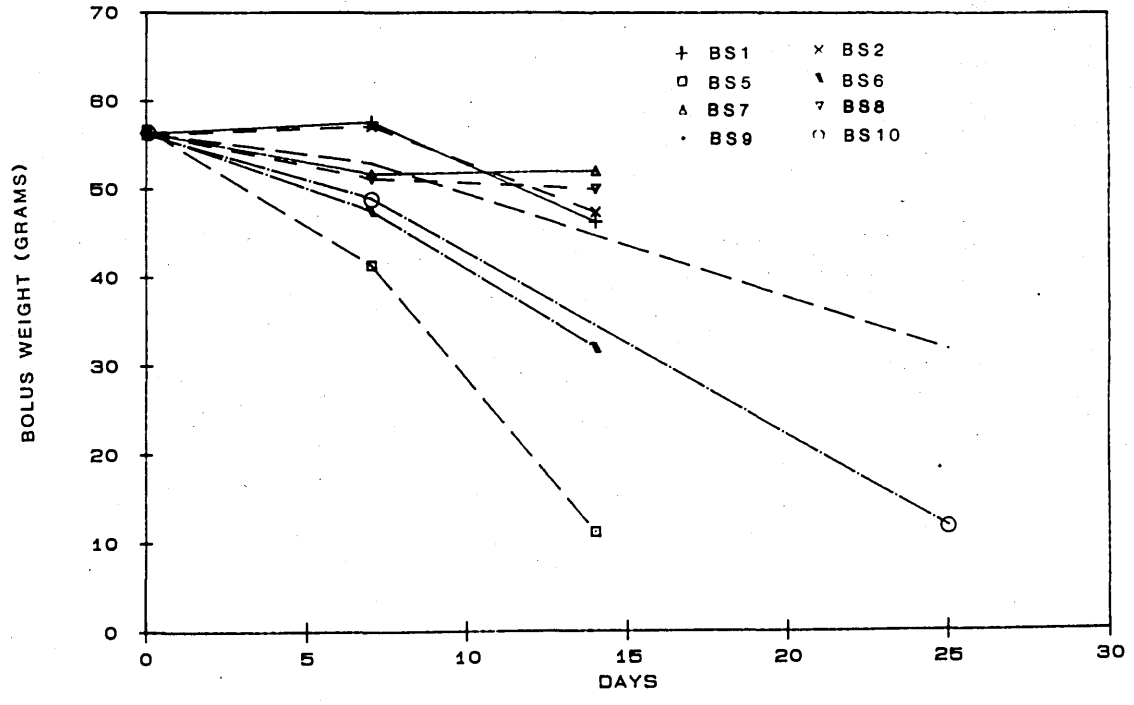


FIGURE 49. The erosion pattern of boluses containing copper oxide powder (200 mesh), magnesium stearate, mineral mix, ivermectin and 5% gelatin, tested in fistulated cows.

FIGURE 50. The erosion pattern of boluses containing the same ingredients as above but with 10% gelatin in the formulation.

FIG.49 5% gelatin inclusion boluses

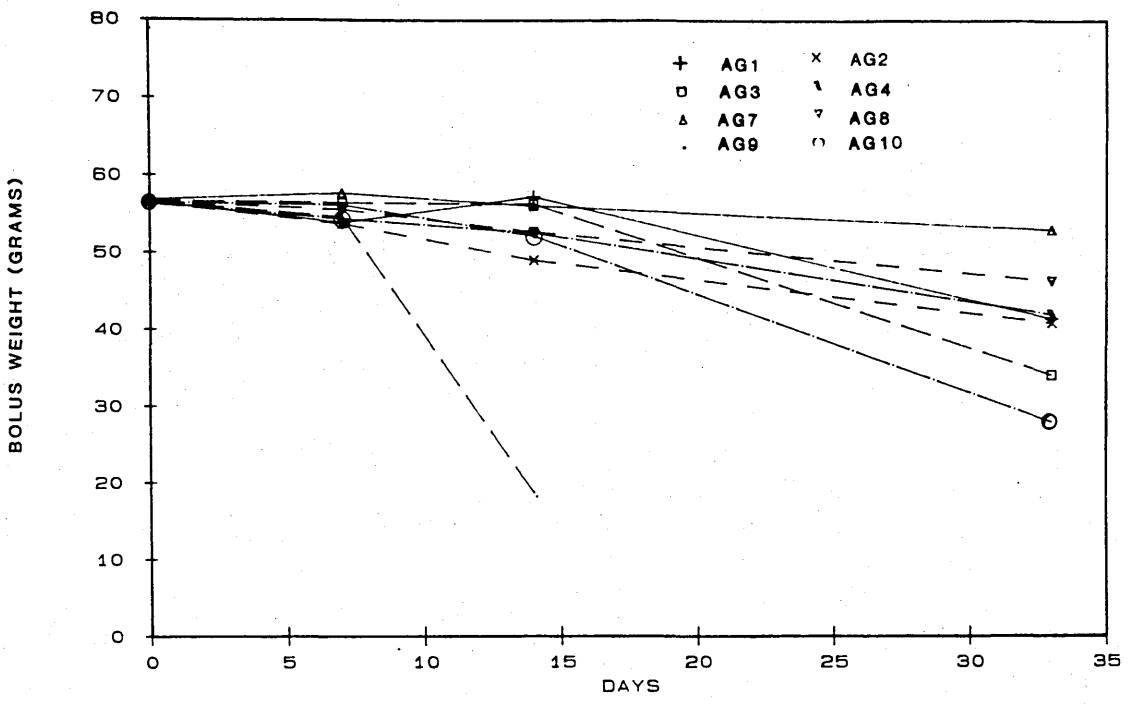
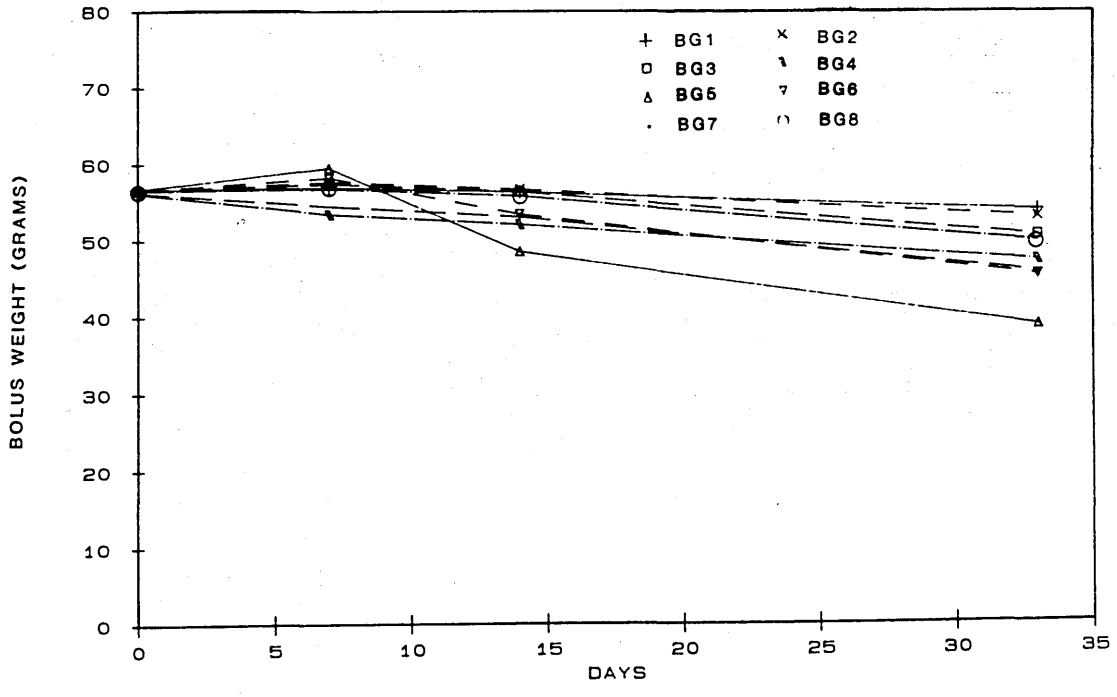


FIG.50 10% gelatin inclusion boluses



5. Addition of Disintegrants to the Mineral Mixture

INTRODUCTION

Following the observation in the previous trial (Section 6:4) that fine copper oxide powder (200 mesh) boluses which fully retained their resin coating eroded at relatively low rates (about one fifth of the desired rate), it was decided to test a range of additives in the boluses which are known to act as disintegrants to speed up erosion rate. Such materials are present in, for example, a soluble aspirin tablet. In general they act by absorbing liquid and then expanding and dissolving out of the matrix causing the particles around them to dissociate and dislodge from the matrix.

The ultimate purpose of testing disintegrants would be to develop a single bolus treatment whose release rate was totally independent of the presence of another bolus. This could in theory be achieved by incorporating a small quantity of a disintegrant which had a linear release rate *in vivo* into the bolus formulation.

The disintegrants proposed for testing were micronized XL-PVP (a different material from PVPK90)

tested earlier as a binder) and amberlite. Both of these materials had been shown to promote a linear release rate *in vitro* in small test boluses, independently of all other factors. Figure 51 shows the *in vitro* effect on erosion of increasing the percentage of amberlite. Initially only small quantities of disintegrant additives were included in the formulae, since such additives are normally effective in low concentrations. All boluses which were to be tested were weighted with 200 mesh copper oxide powder since it had shown better results of consistency between boluses.

It had never been established whether pressure of compaction played any part in the ultimate erosion behaviour of the boluses. Therefore in addition to testing the action of different disintegrants, XL-PVP and amberlite boluses were produced at two different levels of compaction.

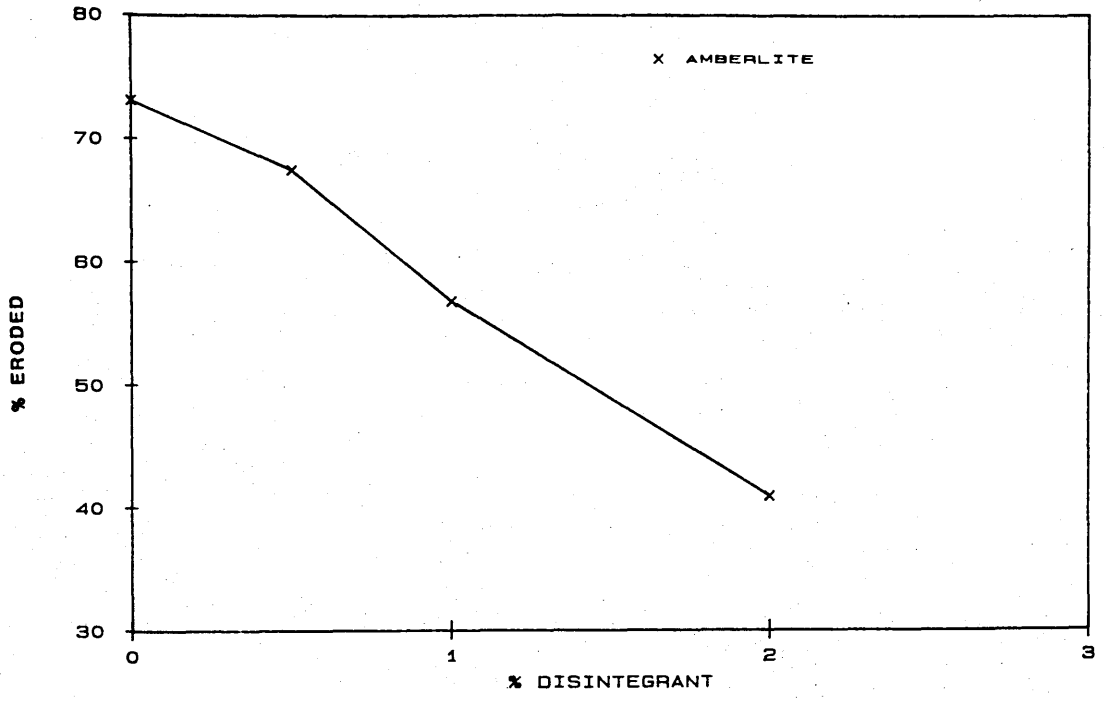
MATERIALS AND METHODS

Three basic formulae were produced as follows:

A1 & A2	1.94%	magnesium stearate
	1.41%	ivermectin
	51%	mineral mix (as Section 2:5)
	45.6%	copper oxide powder (200 mesh)

FIGURE 51. The effect on *in vitro* erosion rate of increasing the percentage of amberlite disintegrant in small test boluses made from mineral mix, copper oxide powder (200 mesh) magnesium stearate and ivermectin.

FIG.51 Amberlite disintegrant in vitro



B1 & B2	1.94%	magnesium stearate
	1.41%	ivermectin
	50.76%	mineral mix (as Section 2:5)
	44.9%	copper oxide powder (200 mesh)
	0.5%	XL-PVP
C1 & C2	1.94%	magnesium stearate
	1.41%	ivermectin
	50.48%	mineral mix (as Section 2:5)
	45.09%	copper oxide powder (200 mesh)
	1%	amberlite

Formulations A1, B1 and C1 were compacted at 13 tons, and A2, B2 and C2 were compacted at 18 tons. Boluses were horizontally compacted and their final dimensions were 1.8 x 1.8 x 7.5 cm. Each was coated four times with glass fibre resin leaving one face exposed. They were paired with another bolus of the same type, and administered to fistulated cows indoors on a hay and concentrate diet.

RESULTS AND DISCUSSION

Average daily erosion rates for the six different bolus types over the test period are shown in Table 60. Figures 52, 53, 54 and 55 show erosion data of representative samples of the bolus tested containing 0.5% PVP and 1% amberlite at 13 and 18 tons compaction

pressure.

Control boluses with no additives eroded very slowly, the highest average erosion rate being between d10-19 in the 13 tons bolus when a mean of 0.133 g/d was lost. This was in general associated with patches of coating becoming detached, which until day 10 had been fully intact. There was a reduction of about 50% in the release rate of the control boluses compacted at 18 tons up until day 10. Thereafter the coating on the 13 tons boluses began to flake consequently increasing the erosion rate whereas the 18 tons boluses remained fully intact and continued to erode at the same constant rate of approximately 0.03 g/day.

A small addition of PVP dramatically increased the average daily weight loss compared to boluses with zero inclusion. The effect tended to be most marked in the first few days, then diminish after fourteen days, particularly in boluses compacted at 18 tons. The coating in both types of bolus was similar at fourteen days and in an intact condition.

Boluses containing amberlite at 1% eroded faster than controls, but tended in cases where the coating was intact to plateau towards d14. The 18 tons boluses apparently eroded more quickly than the 13 tons

TABLE 60. Average erosion rates of control, PVP-XL and Amberlite inclusion boluses compacted at 13 and 18 tons

Bolus Type	Erosion Average (g/day) d0-4	d4-10	d10-14	d10-19
% PVP 13 tons	0.083 ± 0.17	0.057 ± 0.07		0.133 ± 0.07
% PVP 18 tons	0.034 ± 0.06	0.024 ± 0.05		0.030 ± 0.05
0.5% PVP 13 tons	3.266 ± 1.11	1.248 ± 0.54	1.242 ± 0.89	
0.5% PVP 18 tons	3.106 ± 0.97	1.436 ± 0.35	0.684 ± 0.46	

	d0-7	d7-16	d16-23
1% amberlite 13 tons	1.128 ± 0.48	0.507 ± 0.42	0.196 ± 0.18
1% amberlite 18 tons	1.453 ± 0.70	0.675 ± 0.42	1.288 ± 1.19

during days 16-23, but when data on coating integrity was examined, the higher erosion rates in all cases were associated with incomplete coating.

In the first seven days, amberlite appeared to be controlling erosion and the coefficient of variation was less than 50%, but as time went on, the standard deviation became an increasingly large percentage of the mean erosion proving that it was then failing to act as an independent disintegrant. PVP-XL appeared to act as a superior disintegrant in that on average the bolus release rates were a little higher with a smaller standard deviation.

It was noted that PVP-XL had increased the erosion rate by too great a degree, and almost 50% of the bolus weight was lost within ten days. Obviously this is unsuitable for a sustained release system. On the other hand, the inclusion of a disintegrant material is necessary in copper oxide powder (200 mesh) boluses since their erosion rate is negligible in the absence of any such materials. Amberlite did not seem capable of sustaining its disintegrant effect beyond approximately one week in vivo, although its exact effect was difficult to gauge because of coating damage.

FIGURE 52 (i)-(iv) The erosion pattern of pairs of boluses tested in individual fistulated cows. Boluses contained magnesium stearate, ivermectin, mineral mix, copper oxide powder and 0.5% XL-PVP and were horizontally compacted at 13 tons psi

FIG.52(i) 0.5% XL-PVP at13tons psi

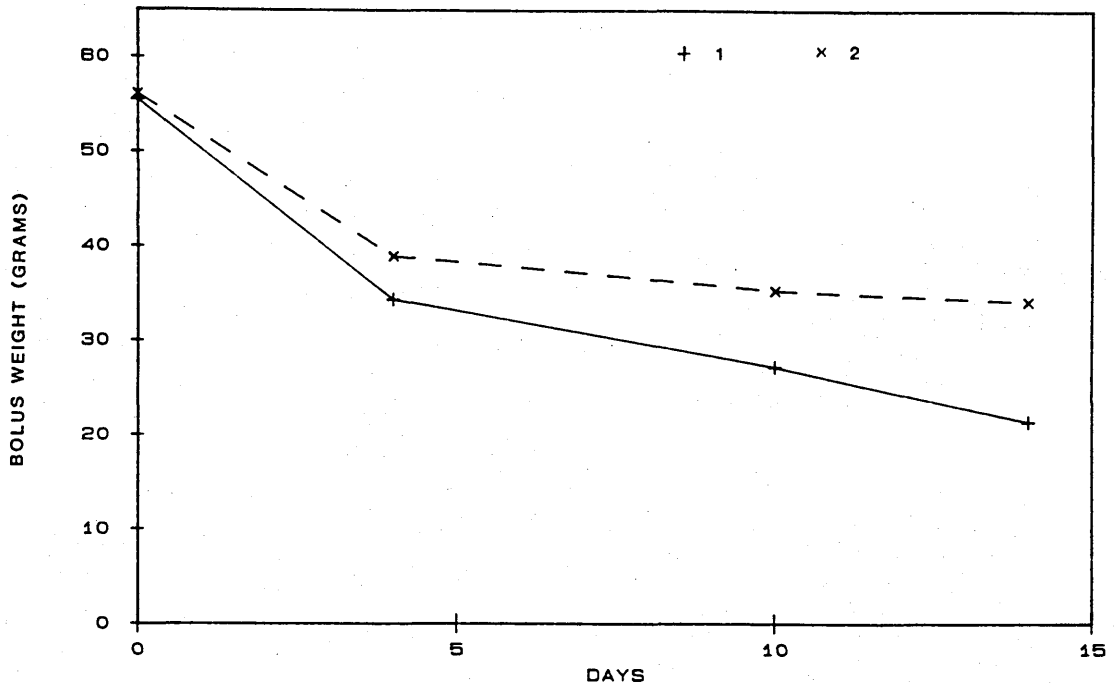


FIG 52(ii)

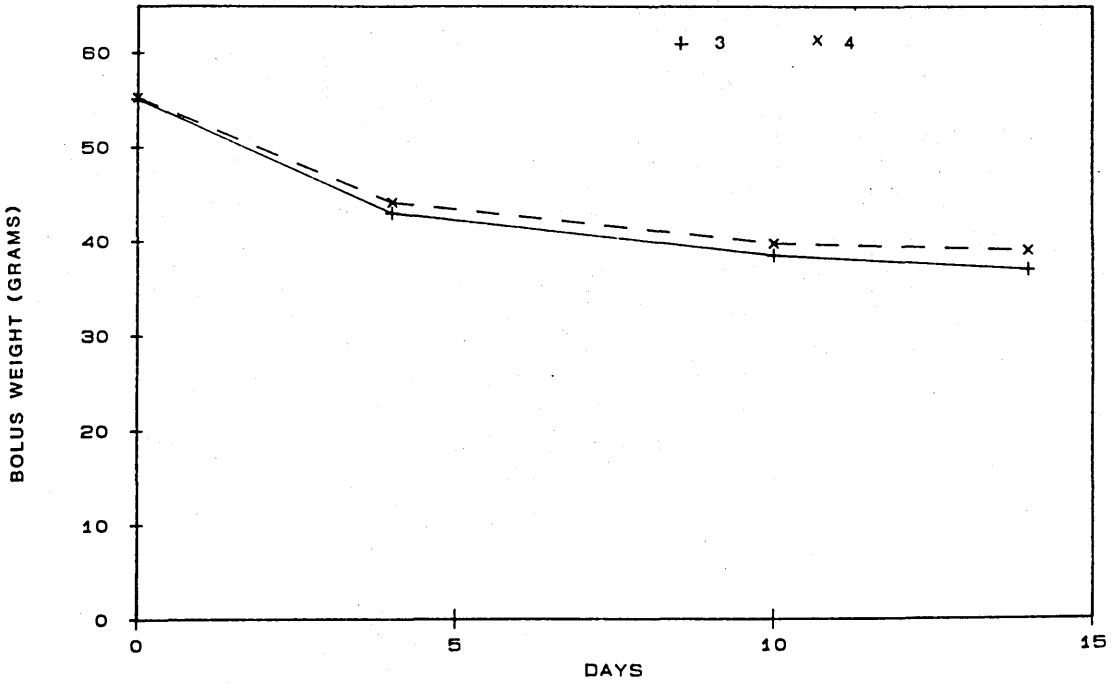


FIG.52(iii)

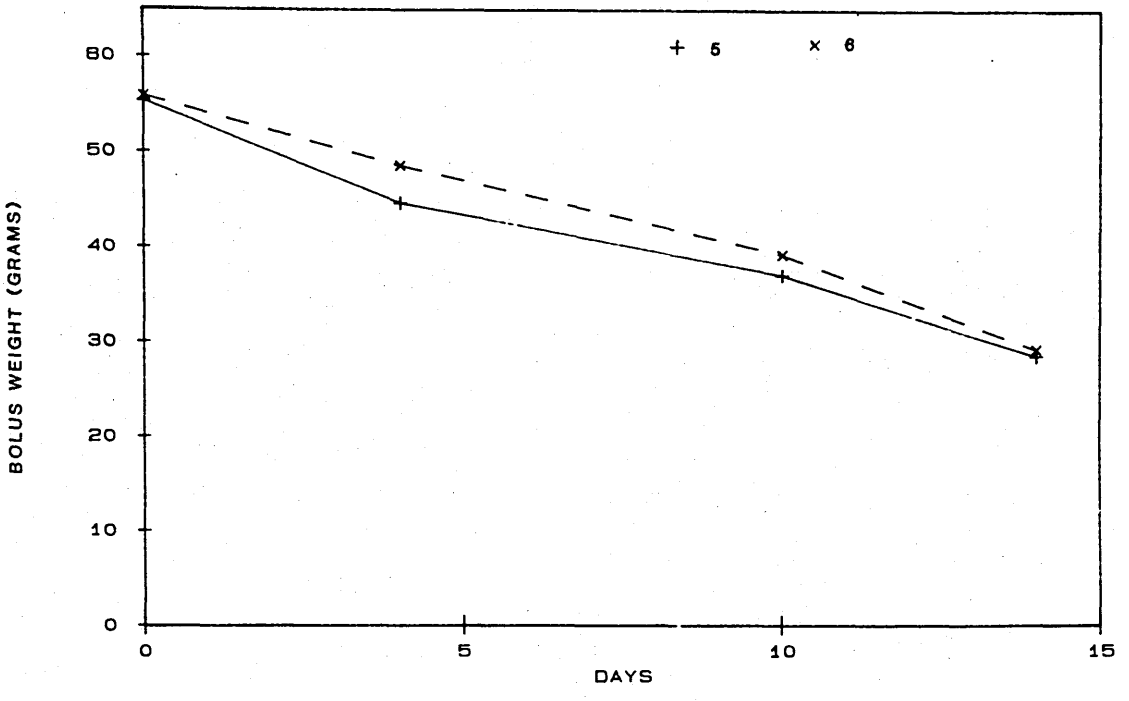


FIG52(iv)

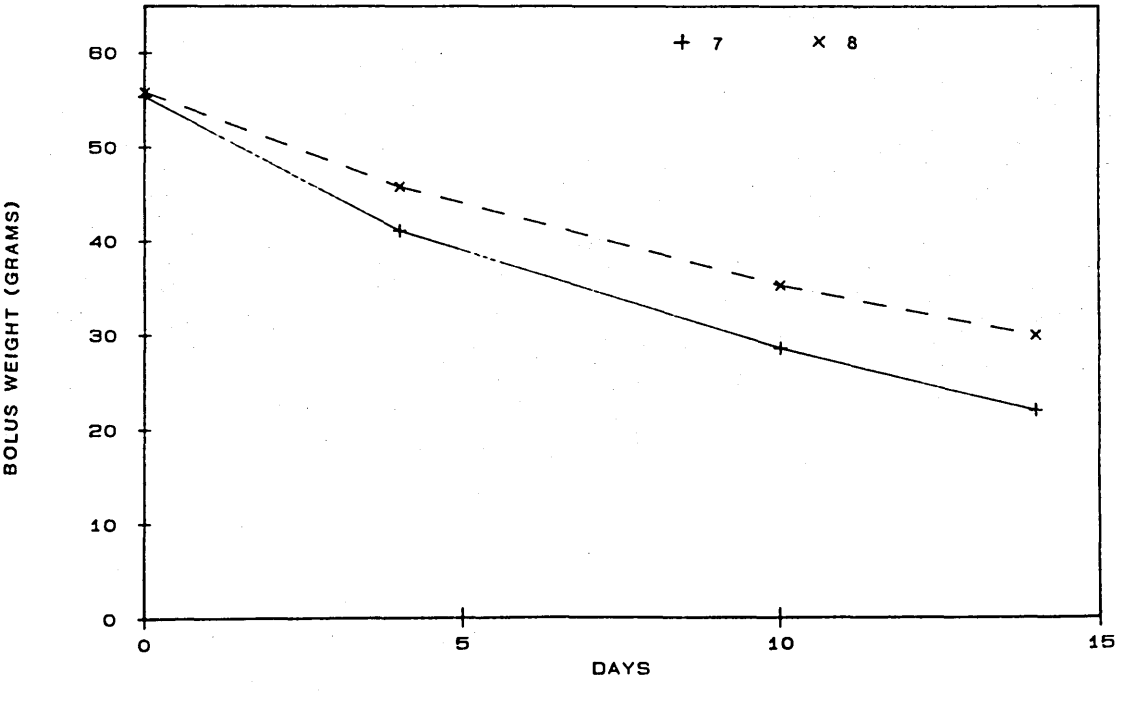


FIGURE 53 (i) - (iv) Erosion pattern of horizontally compacted boluses tested in pairs in individual fistulated cows. Boluses contained magnesium stearate, ivermectin, mineral mix, copper oxide powder (200 mesh), and 0.5% XL-PVP compacted at 18 tons psi

FIG53(i) 0.5% XL-PVP at 18 tons psi

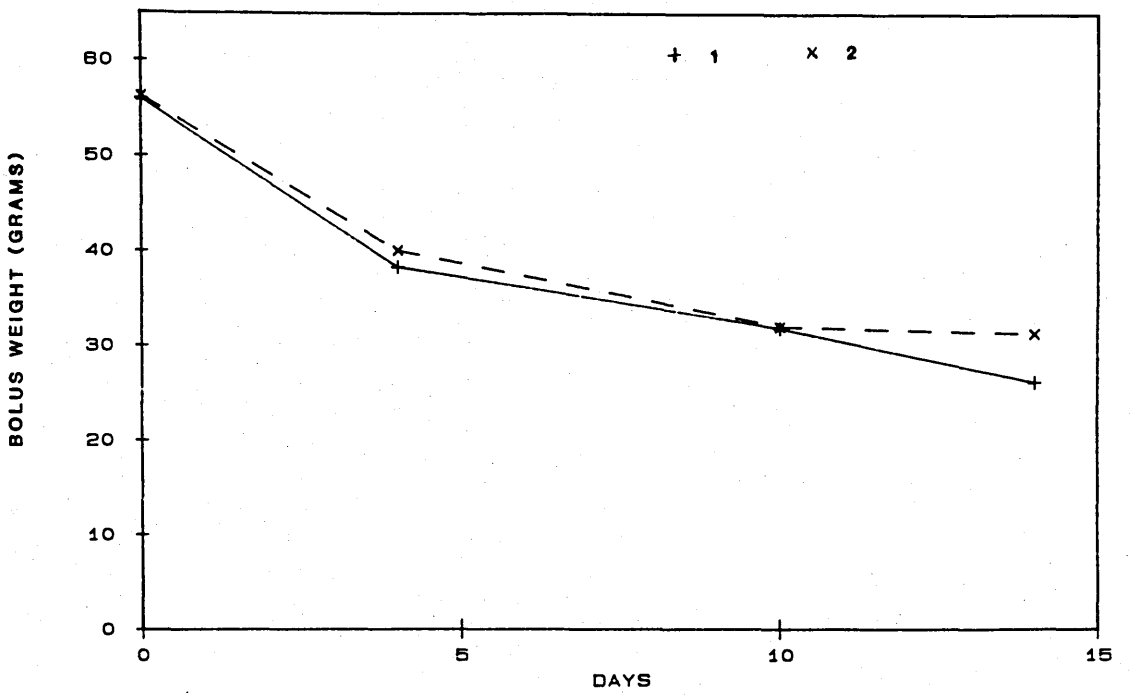


FIG.53(ii)

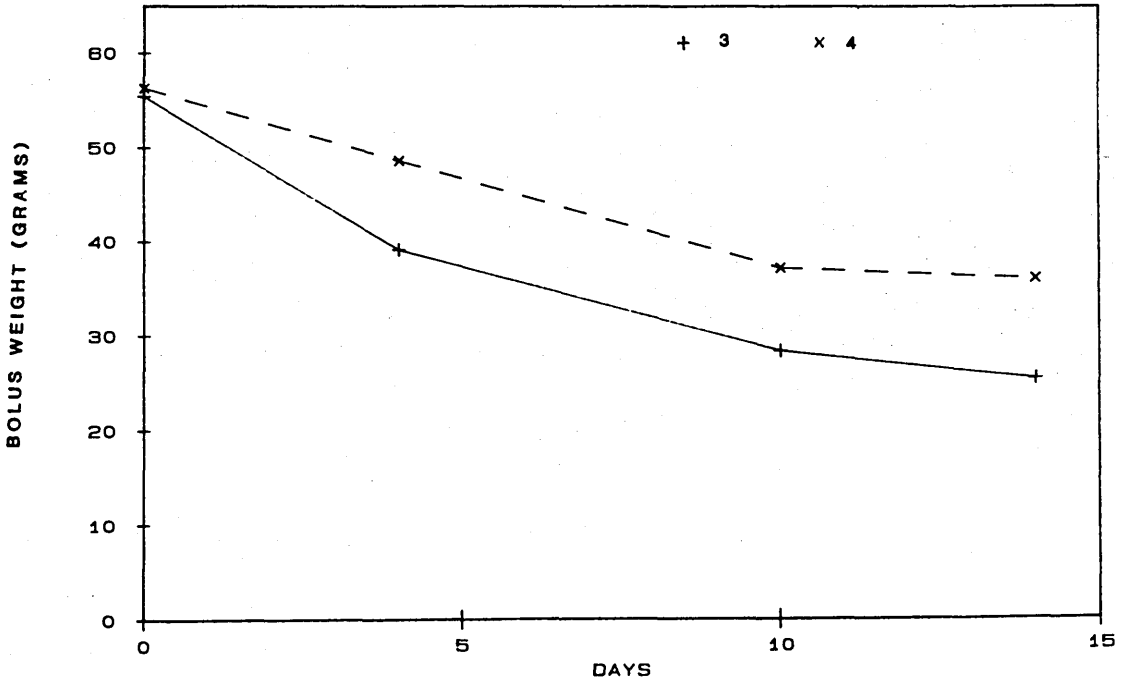


FIG.53(iii)

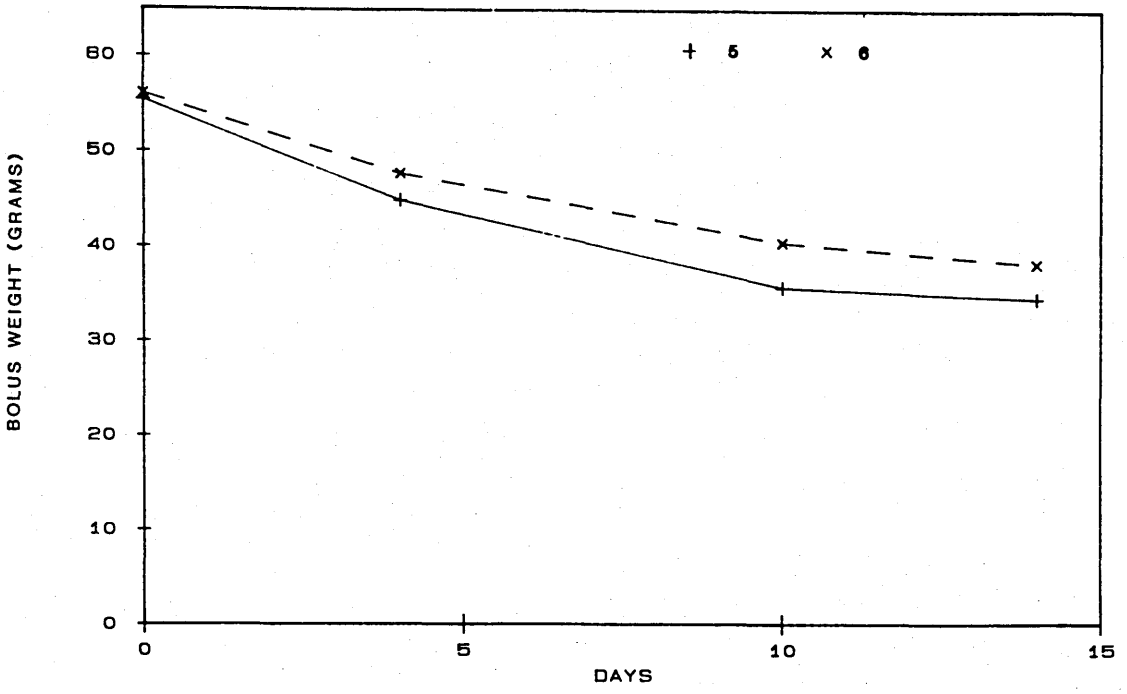


FIG.53(iv)

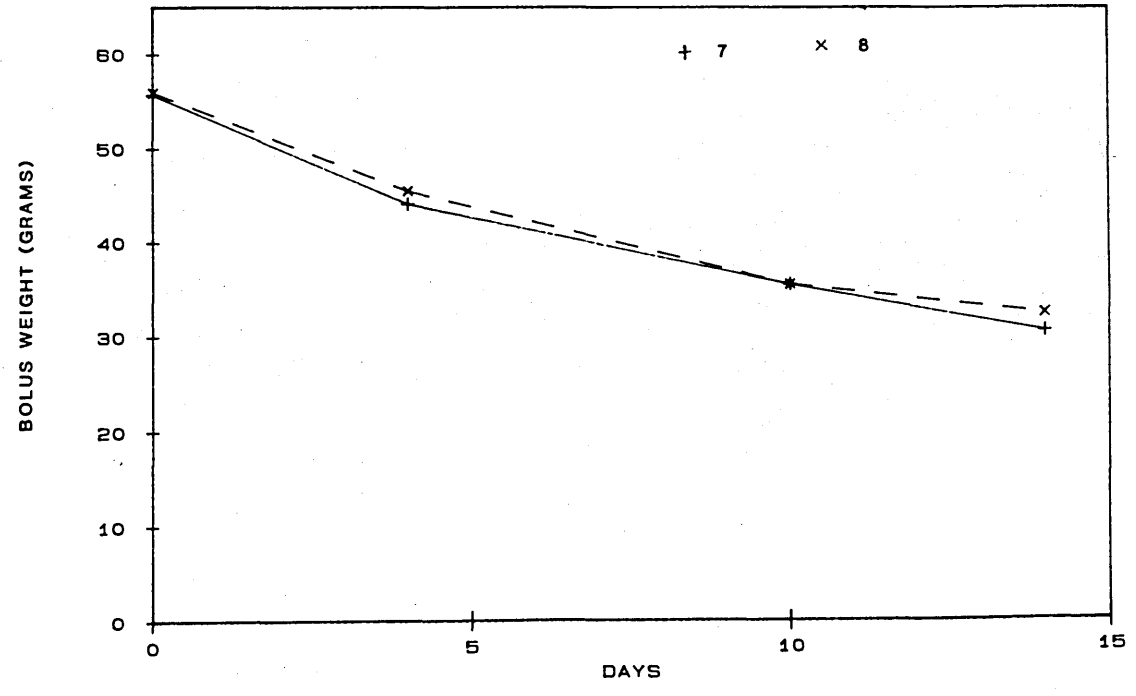


FIGURE 54. Horizontally compacted boluses containing copper oxide powder (200 mesh) mineral mix, magnesium stearate, ivermectin and 1% amberlite pressed at 13 tons psi tested in pairs in fistulated cows.

FIGURE 55. Boluses of identical composion and testing to those above, compacted at 18 tons psi.

FIG.54 1% Amberlite at 13tons psi

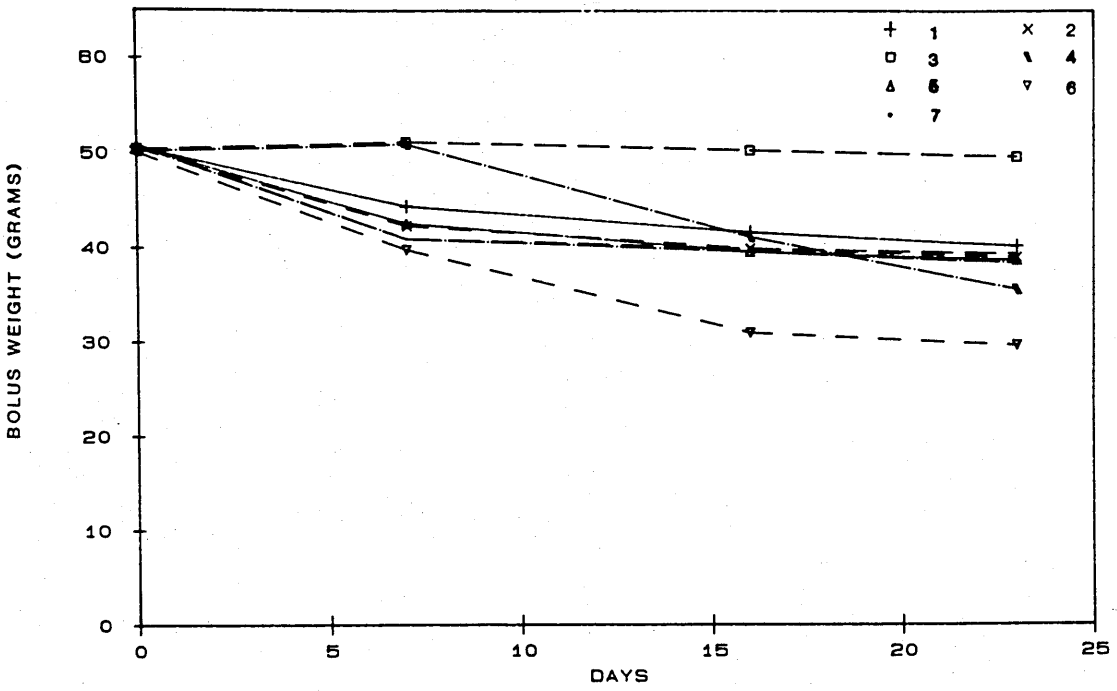
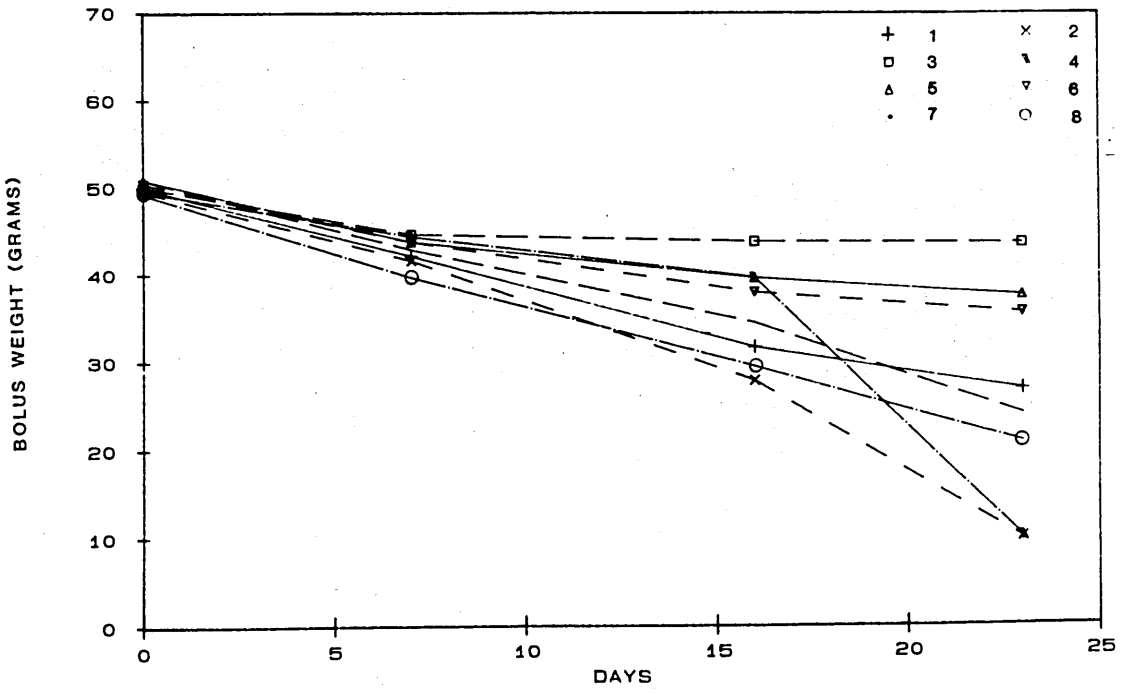


FIG.55 1% Amberlite at 18 tons psi



6. Comparison between Cylindrical Laboratory and Horizontally Compacted Mineral Boluses

INTRODUCTION

In view of previous results (Section 6:5), it was felt that the horizontally compacted bolus weighted with CuO powder had deviated too much from the original laboratory bolus design of a cylindrical bolus weighted with copper oxide needles and that some effort should be made to re-examine the original formula, and try to pinpoint the areas where the commercial formula bolus differed from those prepared in the laboratory. A study was set up with the original laboratory mixed non-granulated formulation without ivermectin added, containing copper oxide needles being compared to an identical granulated mix produced by Merck, Sharp and Dohme at Hoddesdon, Herts. At the same time, a comparison was drawn between the behaviour of 200 mesh copper oxide powder and copper oxide needles weighting in laboratory and MSD mineral formulations.

MATERIALS AND METHODS

(i) Boluses

(a) Glasgow handpressed boluses: 55% of standard mineral mix (Section 2:5), 45% copper oxide needles. Length = 56 mm, diameter = 19mm,

weight = 50 g. Coated four times with glass fibre resin (Plastic Padding Ltd.).

(b) Glasgow handpressed boluses: 55% standard mineral mix (Section 2:5), 45% copper oxide powder (200 mesh). Length = 53 mm, diameter = 19 mm, weight = 50 g. Coated four times with glass fibre resin (Plastic Padding Ltd.).

(c) Manesty compressed square cross section boluses: 1.94% magnesium stearate, 45% copper oxide needles, 53% standard mineral mix (Section 2:5). Dimensions 18 x 18 x 75 mm. Weight 60-70 g. Coated four times with glass fibre resin (Prima Glass Fibre Resins, Sumburgh).

(d) Manesty compressed square cross section boluses: 1.94% magnesium stearate, 45% copper oxide powder (200 mesh), 53% standard mineral mix (Section 2:5). Dimensions 18 x 18 x 75 mm. Weight 55-60 g. Coated four times with glass fibre resin (Prima Glass Fibre Resins, Sumburgh).

(ii) Experimental

Boluses were paired and there were three replicate

treatments of each type administered to fistulated cows indoors. All cows were on a hay and concentrate diet apart from one animal, which as part of a different experimental programme, was being fed chopped straw and concentrates. It was not expected that this animal would produce anomalous results, but the difference in its diet was noted. Boluses were examined and weighed at weekly intervals over a period of fifty-one days.

RESULTS AND DISCUSSION

Figures 56 to 59 show the results of individual pairs of boluses of each formulation tested over fifty-one days. Excluding the two boluses administered to a fistulated cow on a straw diet, which was thought to have increased the rate of erosion, the mean weight loss of Glasgow handpressed needles boluses was: 16.895 $\pm$ 3.736 g over 51 days, on a daily basis 0.331 g/day. The mean residue weight was 33 g $\pm$ 3.77 g, representing an 11% coefficient of variation. Had the bolus continued at this rate, it would have lasted another 88-111 days, giving a total life of 139-162 days. Handpressed CuO powder boluses had a negligible weight loss. Manesty CuO needles boluses eroded very swiftly, and none were recovered by fifty days. Manesty CuO powder boluses eroded very slowly at an

average rate of 0.116 g/day, with the mean over 51 days being 5.908 $\pm$ 3.418 g, a 58% coefficient of variation. At the present rate of erosion, it would last another 436 days.

Handpressed needles boluses had an erosion rate which was reasonable for covering the entire grazing season, and certainly was within the general target range. Machine pressed boluses, although only differing in shape and granulation were very much more rapid. It is obvious that CuO needles inclusion could cause the rapid wearing of square edges by projecting outwards and rupturing the coating, causing weaknesses and then total breakdown. Granulation of particles increases their size and, if needles are a component of the mix, creates an air space around the needle allowing the permeation of fluid around it and leading to relatively rapid disintegration.

CuO powder as a weighting agent has been shown by this trial to prevent sufficient erosion. Because of the smoother more homogeneous mix, the square edges of MSD boluses were not prone to wearing, so any improvement of erosion compared to that of handpressed boluses was a result of granulation. The small particle size of the mix would not encourage the incorporation of much air space, so its properties were only slightly altered by granulation.

For future development, Merck, Sharp and Dohme committed themselves to the purchase of a cylindrical die, and a re-examination of the use of CuO needles as a weighting agent. Granulation is a necessary procedure in commercial production in order to prevent the settlement of ingredients. However, there are various hydrophobic agents, such as stearic acid, which could be incorporated into the mix to overcome the effects of granulation, and perhaps reinstate the original properties of the device.

FIGURE 56 (i)-(iv) Erosion pattern of individual pairs of boluses tested in fistulated cows. Boluses were pressed vertically in a cylinder at 3 tons psi and contained mineral mix and copper oxide needles.

FIG.56(i) Glasgow handpressed boluses with CuO(n)

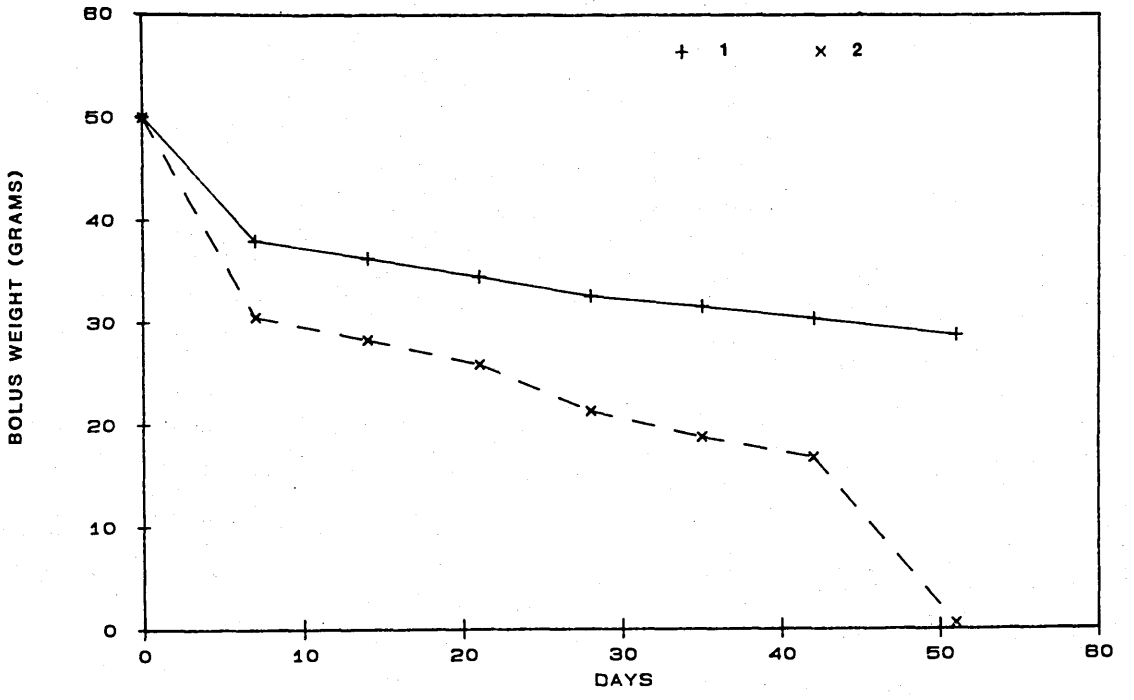


FIG.56(ii)

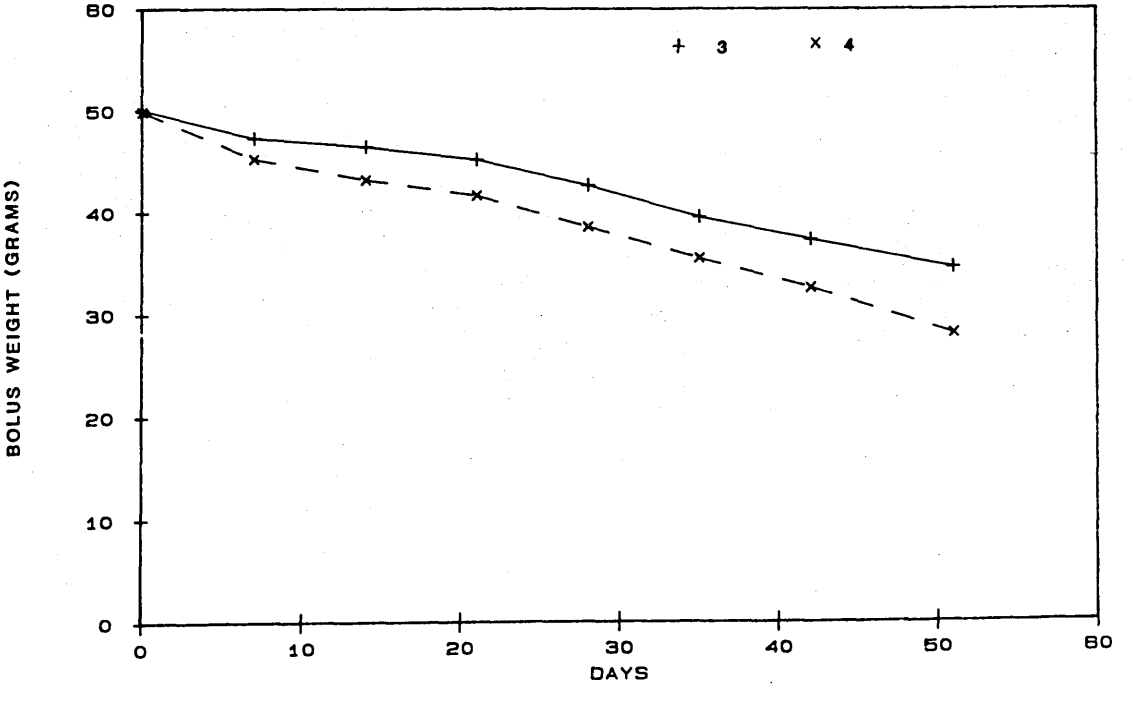


FIG.56(iii)

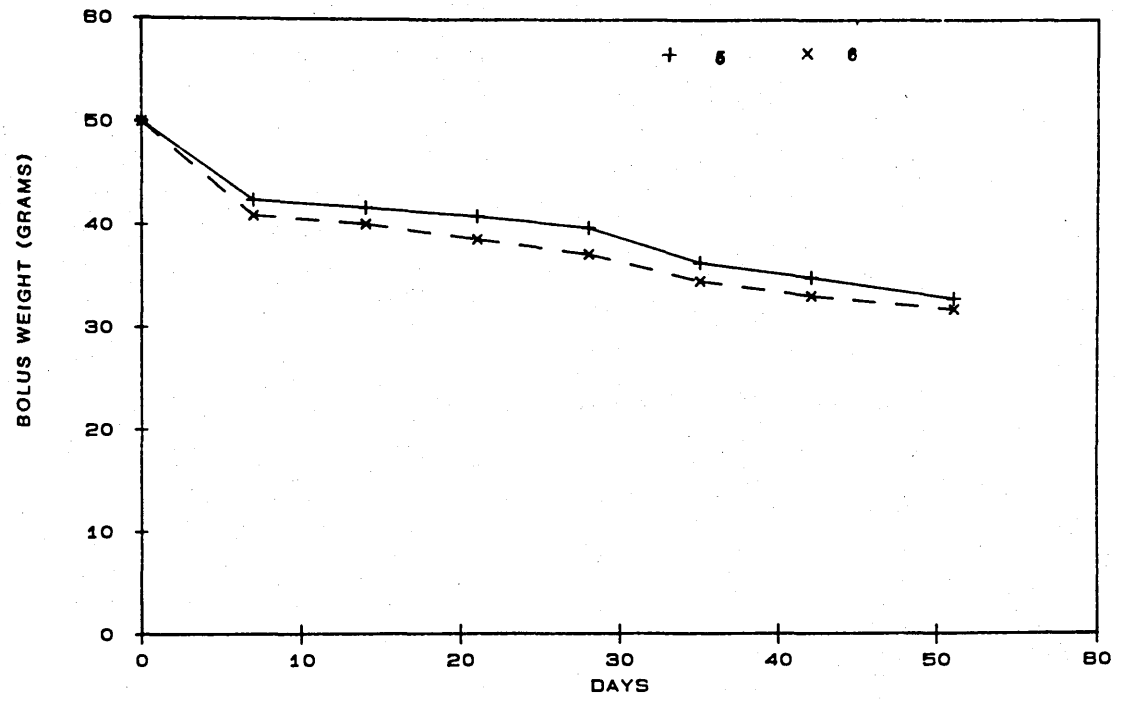


FIG.56(iv)

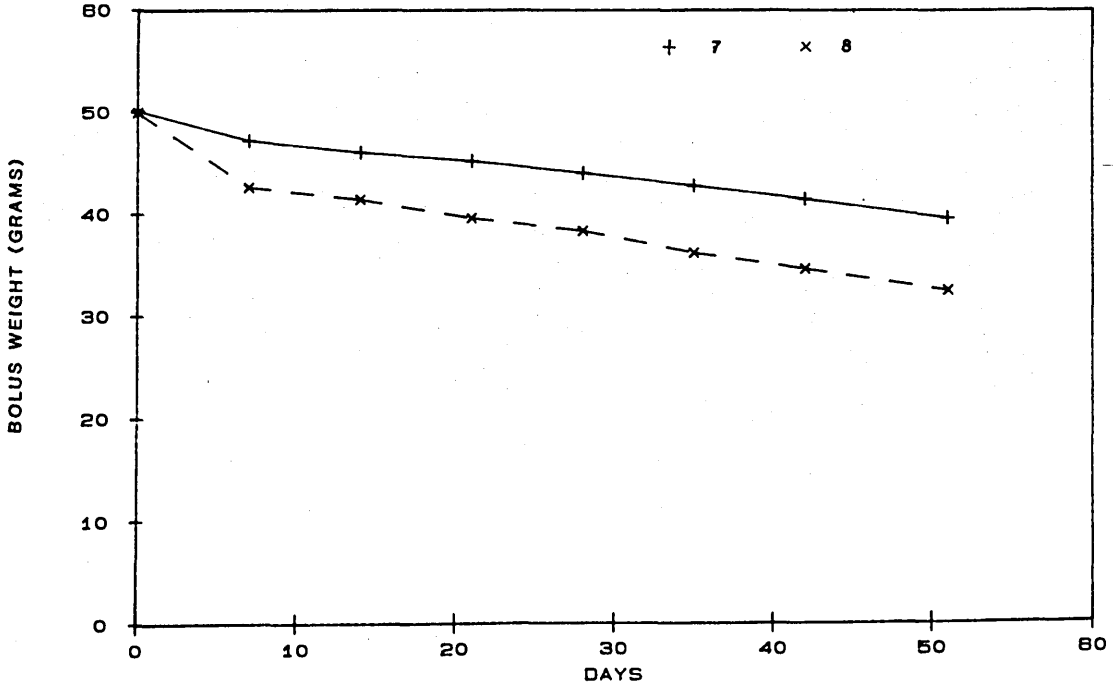


FIGURE 57 (i)-(iii) Horizontally compacted boluses containing mineral mix, copper oxide needles and magnesium stearate tested in pairs in fistulated cows. The erosion pattern shows results from individual pairs of boluses.

FIG.57(i) Manesty horizontally pressed boluses with CuO(n)

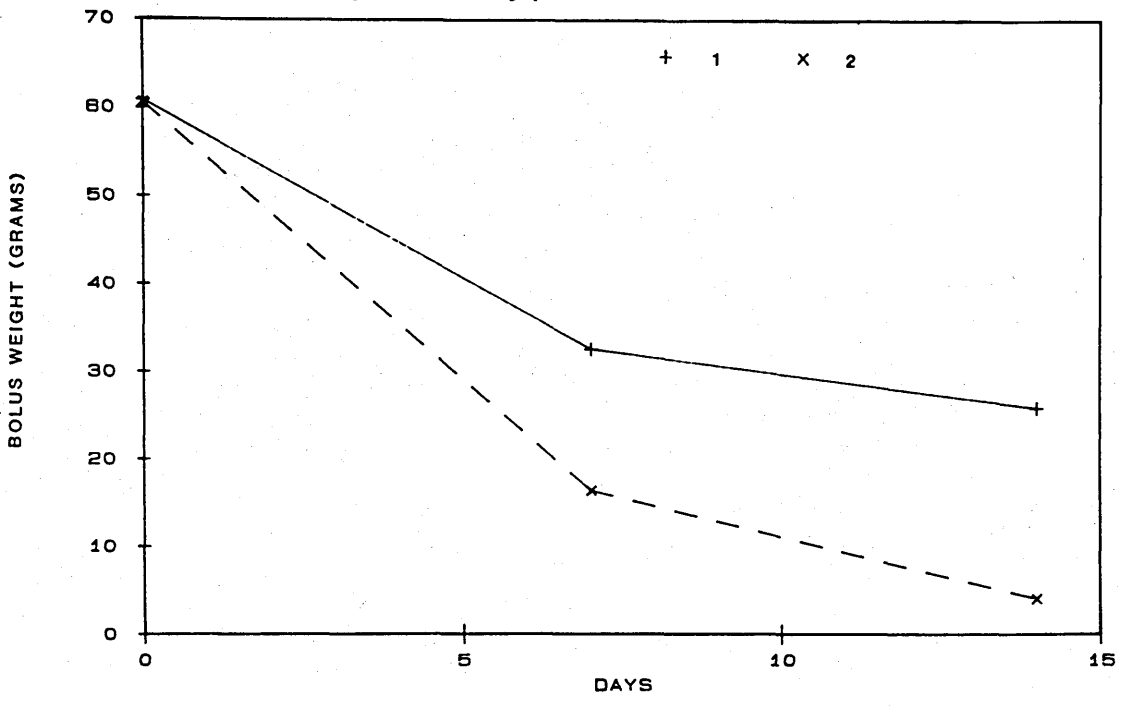


FIG.57(ii)

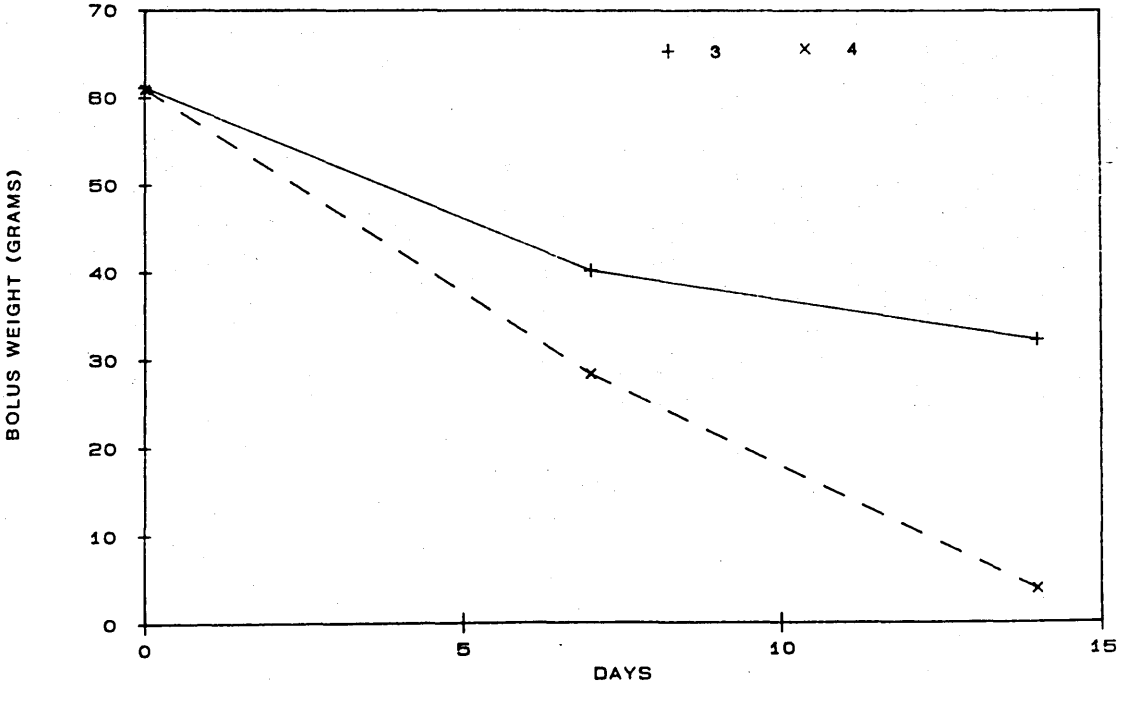


FIG.57(iii)

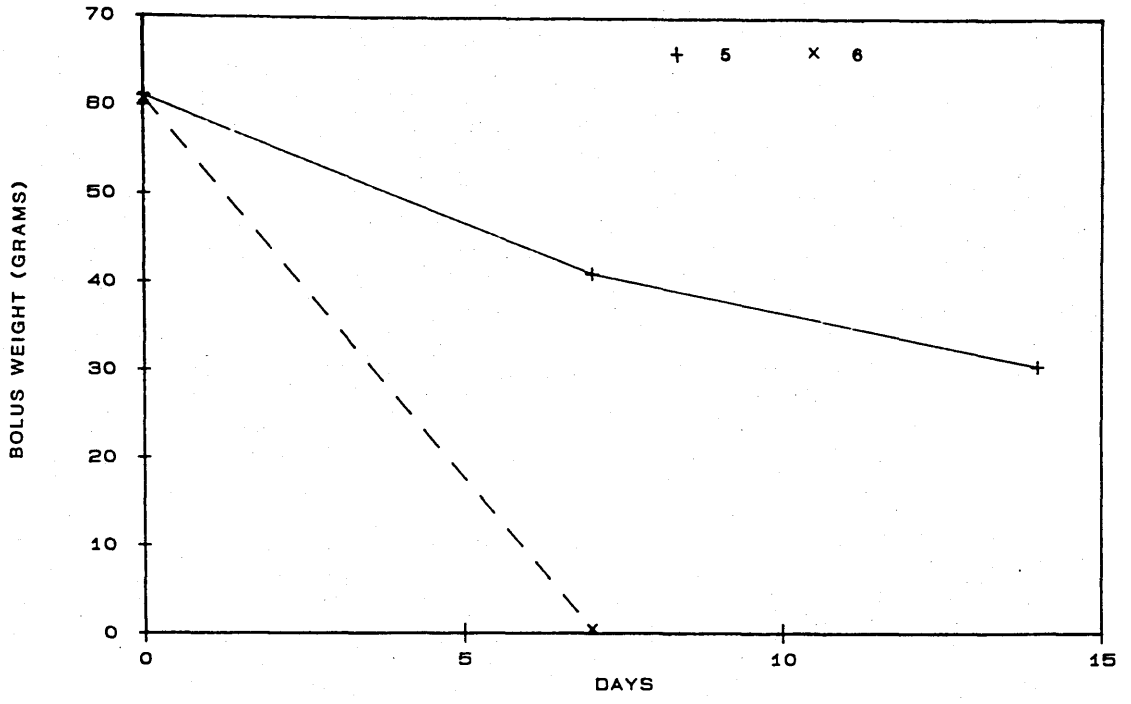


FIGURE 58 (i)-(iii) The erosion pattern of individual pairs of vertically compacted cylindrical boluses containing mineral mix and 200 mesh copper oxide powder tested in fistulated cows.

FIG.58(i) Glasgow handpressed boluses with CuO(p)

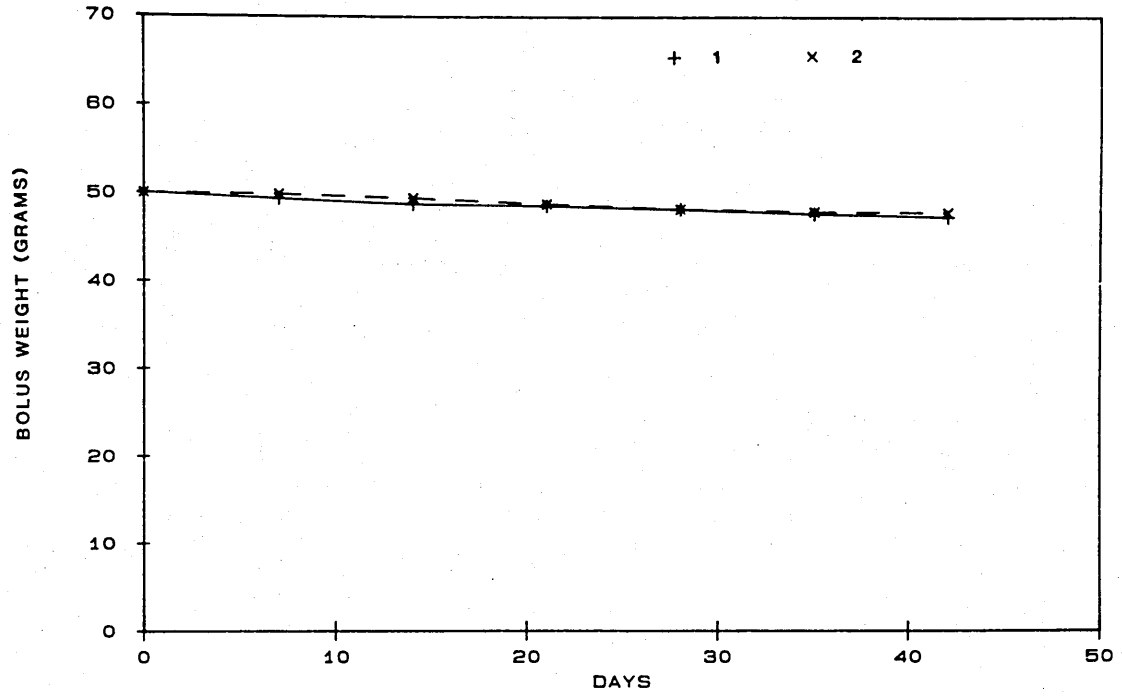


FIG.58(ii)

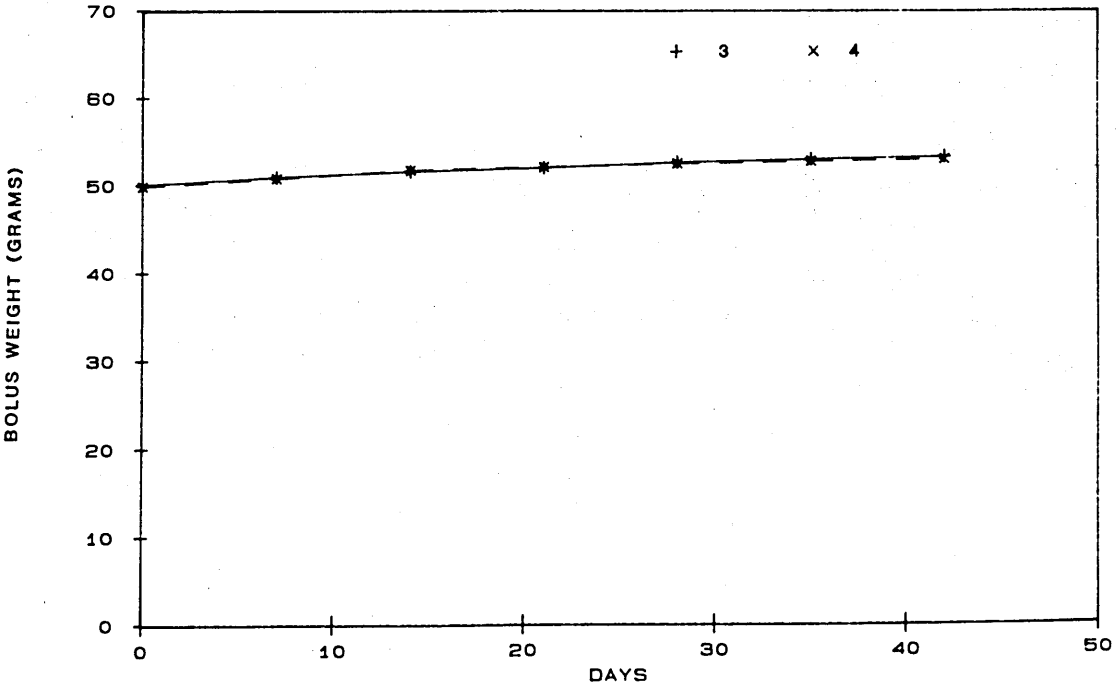


FIG.58(iii)

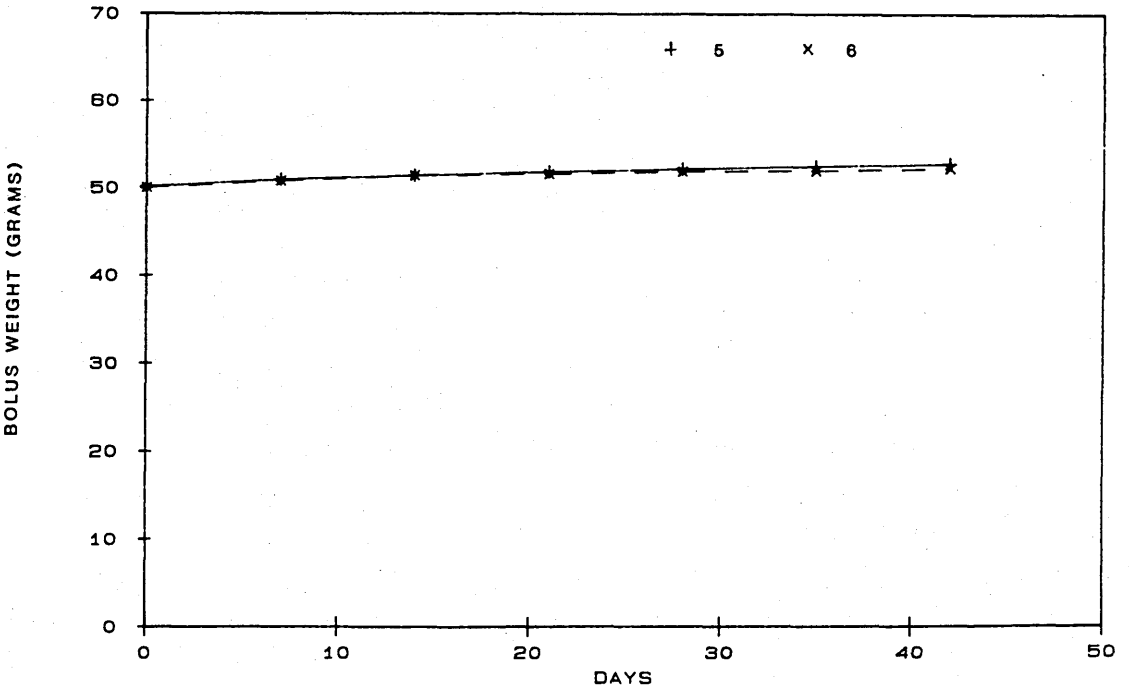


FIGURE 59 (i)-(iii) Horizontally compacted boluses which contained copper oxide powder (200 mesh), mineral mix and magnesium stearate, tested in fistulated cows. Results are plotted for individual pairs of boluses.

FIG.59(i) Horizontally pressed boluses with CuO(p)

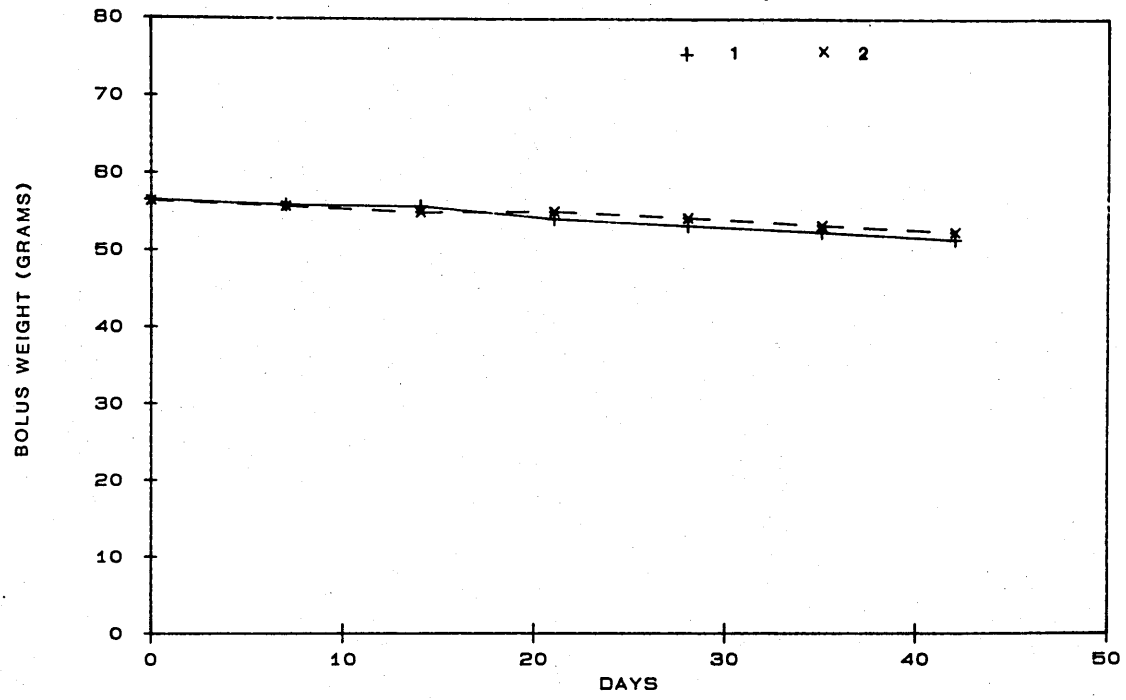


FIG.59(ii)

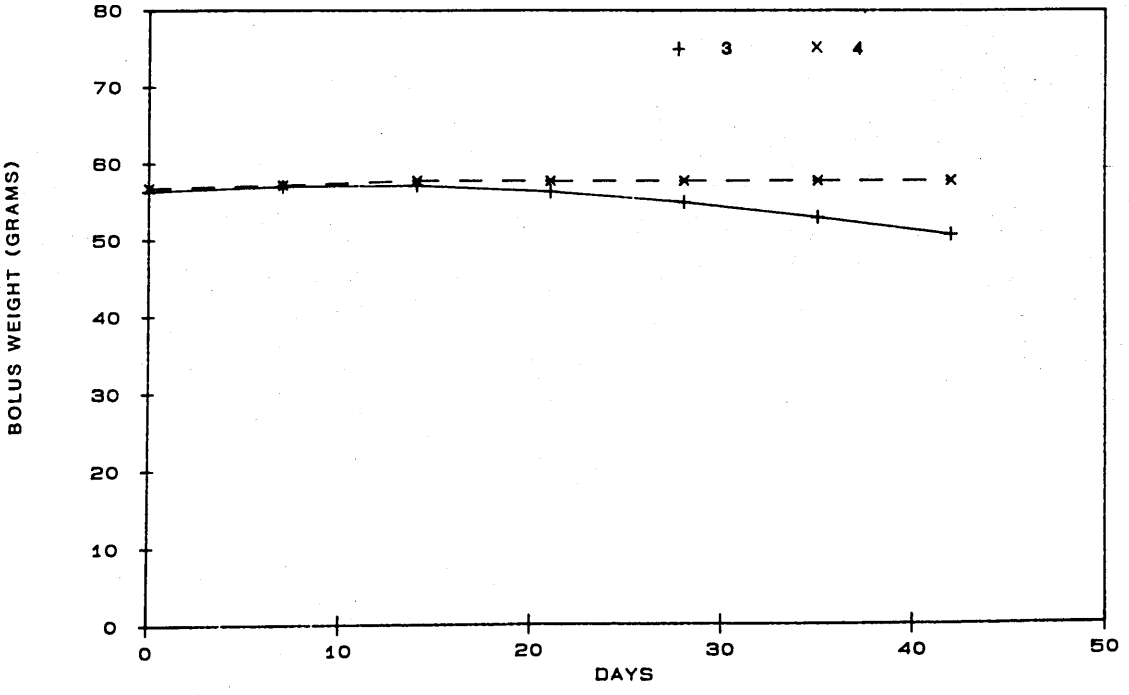
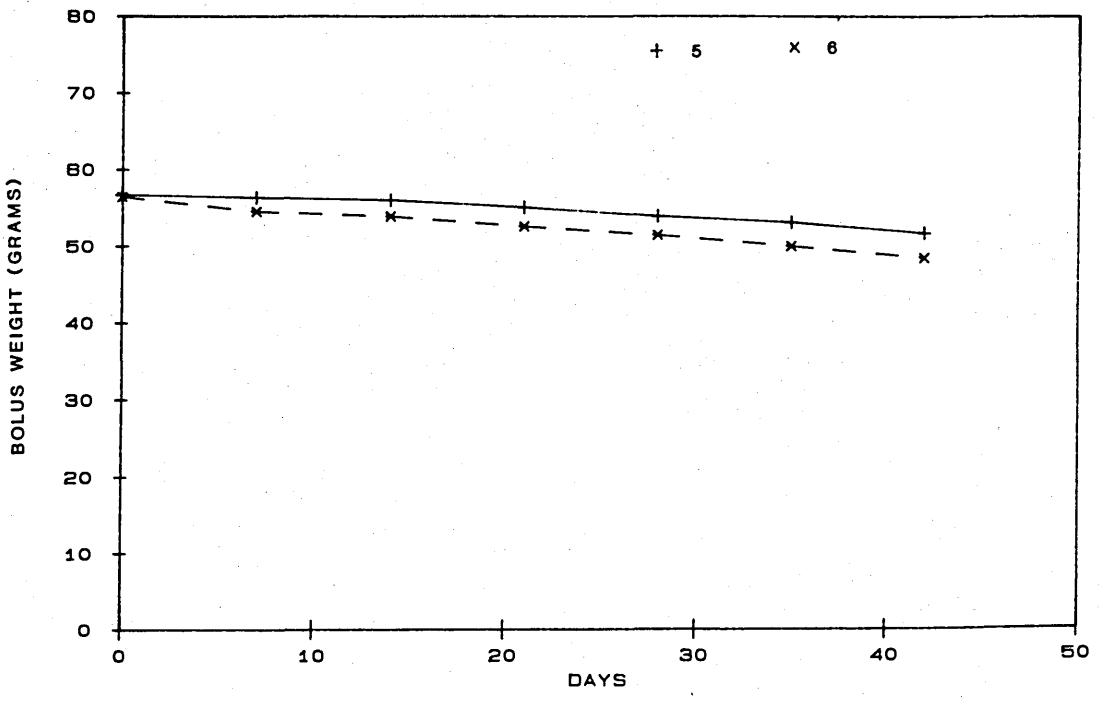


FIG.59(iii)


80
70
60
50
40
30
20
10
0
BOLUS WEIGHT (GRAMS)
+ 5
× 6
0
10
20
30
40
50
DAYS

GENERAL CONCLUSIONS

It has been demonstrated in this thesis and in other work (Kendall, 1977; Ducker and Fraser, 1975) that self help systems of administering supplements to animals at grass are not a reliable method of ensuring adequate intakes. Minerals added to the concentrate ration and trough fed, also gives rise to unacceptably high levels of variation in intake which leaves a significant number of animals open to the risk of developing deficiency diseases. Several trace elements, notably cobalt and zinc, are not readily stored by the body, and so must be consumed regularly and frequently if they are to be of physiological value.

The way forward in recent years to overcome the problem of supplementing cobalt and selenium trace elements in the diet, has been through the provision of slow release intraruminal devices for each element which last in excess of two years. It was felt however that there was a need for a shorter term combined trace element bolus device, incorporating all the major trace elements and vitamins at the ARC recommended levels.

The intraruminal bolus subsequently developed as a result was intentionally completely biodegradable

and was relatively simple to produce in the laboratory. Its erosion characteristics could be altered to a certain degree by alteration of the basic ingredients of the bolus, particularly the zinc sulphate heptahydrate and zinc oxide components. A glass fibre resin coating was found to be a suitable protection against the intraruminal environment, and allowed a reasonably fixed surface area to be continually exposed. The best erosion characteristics were observed when more than one bolus was administered per animal, therefore the definitive dose was fixed at two boluses per animal.

Fistulated cows were determined as the primary test screening animal because of the ease of bolus recovery, and later testing was applied to ewes and lambs and intact cattle.

Initially there were two sizes of bolus, 17 mm and 25 mm diameter, but later a 19 mm diameter bolus was added to the range. It was found that two 17 mm diameter boluses with two exposed ends eroded gradually over a period of 4-6 weeks in fistulated cows and in doing so supplied the full ARC (1965) mineral requirements for that period. It was found to be more difficult to control the erosion pattern of 25 mm boluses although some successful models lasting for 60-100 days were produced.

The small sized (17 mm) bolus, successful in cattle, was then tested in sheep. While the erosion characteristics of the bolus were promising, there was evidence that regurgitation had occurred in some cases since only 50% of the boluses were recovered after twenty-seven days at post mortem.

An attempt was made at this point to have the bolus pressed commercially because it was felt that the erosion characteristics of the mineral bolus were sufficiently reproducible for it to be used as a marketable mineral supplement for ruminants, particularly cattle. Two different diameters of boluses, 16 mm and 25 mm, were produced, pressed end to end. It was found on compaction that physical constraints on the length of travel of the ram on the commercial press meant that boluses had a maximum length of 32 mm, about half that required. Adaptations to existing machinery in order to increase the length of the bolus would be excessively costly, and at that time, no press was available to produce a compressed object of the required dimensions. This cast doubt on the future commercial development of the bolus in the absence of finance sufficient to alter existing machinery.

A number of boluses of varying copper oxide needles content were produced commercially and despite being smaller in size than the laboratory produced

examples, they contained sufficient material to supply the trace mineral intake of sheep for 6-8 weeks. Tested in fattening lambs, they were found to be effective in raising liver copper concentration although there were still problems with non-recovery of boluses (only 25% of boluses administered were recovered after sixty-nine days). A subsequent trial involved the use of small commercially pressed boluses with two different levels of copper oxide needles. Ewes were dosed approximately 3-6 weeks prepartum and their blood copper levels monitored at regular intervals until twelve weeks post bulletting when blood samples were collected from all ewes and their lambs. Bolused ewes showed a significant advantage in copper levels for up to three weeks, but thereafter there was no significant difference. At twelve weeks post bulletting there was no statistically significant difference between lambs born of bolused and non-bolused ewes.

As a mineral supplement, the bolus had shown a reasonable degree of success in fistulated cows. Where it had been retained in sheep, it appeared to be reasonably successful although problems of regurgitation would require to be fully investigated before the bolus could be marketted as a mineral supplement for sheep. It has been suggested by some workers (Dewey, 1958) that an intraruminal device should be 4.0 gcm^{-3} in

density before it can be fully retained in sheep. The commercial restrictions on production were, however, a barrier to further development at this stage.

Shortly after this point it was proposed that the bolus could be utilised as a carrier for an anthelmintic treatment. Various anthelmintics (morantel tartrate, morantel citrate, and levamisole hydrochloride) and the growth promoter monensin sodium had been incorporated into the basic mineral mix and pressed in the laboratory with varying degrees of success. Levamisole because of its ready solubility proved to be an excellent material for incorporating into the mineral matrix. Boluses produced from this mixture eroded at an impressively steady rate. Morantel, as either the tartrate or citrate, was unsuitable for addition to the mineral matrix. A property of morantel is that it has a tendency to swell in the presence of water. This caused the disruption of the glass fibre coat, and the rapid elution of material. Monensin, after some mineral formula manipulations, was a reasonably successful additive and produced a bolus which eroded slowly over 6-8 weeks.

A common feature of all of these additives is that they were all required in quantities of at least several grams per bolus. While the addition of levamisole improved the rate of erosion, the addition

of monensin altered the properties of the bolus such that it eroded less effectively. Therefore when it was suggested that the antiparasitic agent ivermectin might be a suitable bolus additive, there was some optimism that the basic mineral bolus release rate would be unchanged because the recommended dose of ivermectin (100 to 200 mcg/kg LW) is small. The total amount of ivermectin required for a ninety day period for protection of a 200 kg beast at grass is only 0.72 g.

Ivermectin has the added advantage of being an insecticidal agent and acaricide as well as being an anthelmintic. Therefore the potential use of a slow release intraruminal bolus containing ivermectin was much wider than that of boluses containing conventional anthelmintics. It was anticipated that should a successful bolus be developed it could also be effective against the tsetse fly (Glossina spp) and thereby have important implications on grazing in regions of Africa presently inaccessible due to the reservoir of Trypanosoma brucei brucei transmitted by tsetses and causing the disease Nagana in cattle. Ivermectin bolus administration could also reduce the necessity for frequent dipping in tropical and subtropical regions to keep various species of flies, ticks, mites, etc. at bay, and therefore would become a most useful labour saving device. Administered

as an anthelmintic treatment in Britain, the ivermectin bolus would not only act to control parasitism at grass but also have the desirable side effect of cleaning up pasture. Therefore the development of a successful slow release bolus containing ivermectin would be a commercially viable development.

After some preliminary development work in fistulated cows, ivermectin bolus treatment was tested against Ostertagia ostertagi and Cooperia oncophora in a controlled experimental situation. One bolus containing ivermectin plus one mineral only bolus was administered to each experimental animal. The treatment proved to be highly effective against these helminths although the ivermectin boluses eroded at approximately one quarter of the desired rate. The same bolus treatment was later tested in intact calves at grass where the infection was naturally acquired, in order to constitute a more complete bolus study. The evidence suggested in this case that boluses containing ivermectin had eroded more quickly than before, perhaps as quickly as within 50-70 days although at post mortem several mineral only bolus residues were recovered. Boluses were effective in controlling parasites during the early part of the season but there was evidence of parasitism in the later part of the season, apparently corresponding with the complete erosion of the bolus.

These two trials demonstrated the efficacy of this system of anthelmintic administration, although considerable improvements to the reproducibility of the release rate would be necessary.

Renewed attempts were then made to produce a bolus commercially with a view to administering ivermectin by this method.

Because of the size restrictions on the bolus imposed by commercially available tabletting machines, it was decided that the only way to manufacture a bolus of the appropriate dimensions would be to compact horizontally and produce a pellet pressed side on.

A horizontally pressed bolus was developed which was square in cross section. Pregranulation of the mineral mix and weighting agent was necessary for commercial tabletting procedures, but this was shown to alter the properties of the mineral mix. Horizontally pressed boluses containing pregranulated mix and copper oxide needles eroded at a much faster rate than the equivalent hand pressed laboratory mixed mineral bolus, with a much wider degree of variability in erosion between boluses of the same batch. It was postulated that the relatively large size of the copper oxide needle in a commercial mix contributed to a lack of homogeneity, and that should a smaller

particle be used for weighting, the mix would become more uniform and therefore produce more uniform erosion characteristics. Two sizes of copper oxide particles were tested and increasing uniformity was found with the smaller particle - 200 mesh copper oxide powder. However boluses containing copper oxide powder eroded very slowly and attempts were therefore made to increase the rate of erosion by incorporating disintegrant materials which promoted a linear release rate in vitro in small test boluses. It was found that these materials in general were effective in raising the release rate to start with, but ultimately tended to plateau out.

Towards the end of the experimental programme when the bolus was proceeding along the lines of a horizontally pressed pellet weighted with copper oxide powder, it was felt that perhaps part of the failure of the commercial enterprise to produce a workable bolus was because too many of the original features of the bolus on which the basic research was carried out had been altered.

In the last experiment described in this thesis, it was decided to make a comparison between the original laboratory design of the bolus and the equivalent horizontally pressed bolus. Results of this trial highlighted the main area of difficulty.

Handpressed needles boluses of the type tested eroded fairly consistently and were expected to have a life of 139-162 days whereas similar pregranulated horizontally pressed boluses eroded completely in fifty days. Boluses which contained powder eroded very slowly in both handpressed and machine manufactured examples. Although machine pressed were faster, they were expected to last in excess of 450 days.

The development work in the laboratory described in this thesis has led to the production of a simple mineral vehicle intraruminal device which could be altered to effectively carry a growth promoter such as monensin or an anthelmintic such as levamisole or ivermectin. When carrying ivermectin, it has been shown to be effective against parasite challenge. Because of its relative simplicity in manufacture and the inexpensive components from which it is formulated, this bolus appeared to be ideal for commercial development. Inconsistencies in erosion pattern during fistulated cow testing were occasionally a feature of apparently identical hand manufactured boluses, but it was assumed that commercial manufacture would iron out this type of variability.

Unfortunately, it has not proved possible to manufacture commercially a bolus of the same design as the laboratory bolus, and consequently the machine

tabletted boluses behave differently from what was hoped to be developed. Instead of transferring one design into commercial manufacture, a whole new type of bolus was developed to fit existing machinery and production processes.

The main question relevant to the results of this project tends to be one of economics. It has to be asked if this intraruminal device has enough potential to justify the expense of extensive modifications to existing tooling to facilitate the automated production of a bolus similar to that produced in the laboratory. There is no guarantee that a cylindrical bolus of the correct dimensions would behave any more desirably than those horizontally pressed, given that the prior granulation of mineral and weighting is an essential part of the industrial process which appears markedly to affect erosion.

If the mineral bolus was commercially developed, its use would most probably be limited to relatively short term application (2-3 months) in cattle, which could be a useful tool for cattle during the grazing season. They would perhaps require two dosing sessions throughout the season which would be superior to most oral treatments. A mineral bolus for sheep would require further research on improved retention before any claims to its efficacy could be made.

As an anthelmintic treatment, should the bolus be commercially developed and predictable in its behaviour, it would be a most useful tool for controlling parasitism at grass, with the added benefit of contributing to cleaner pasture for the subsequent grazing season. Since ivermectin is the suitable drug of choice for such a development, it has the much wider application as an insecticide and acaricide. To this end it would be of enormous advantage in areas of Africa and South America where dipping of cattle and sheep is necessary at weekly or fortnightly intervals and in areas of Africa where grazing of cattle is thwarted by the risk of Nagana.

Problems of commercial production could be substantially overcome if an alternative granulation procedure could be found which, using copper oxide needles produced a mix more similar to the original laboratory mix with more similar erosion properties. Alternatively, a hydrophobic additive such as stearic acid could be added to the mineral plus needles granulated material, to perhaps offset the normally rapid erosion with granulated mix and needles. Plans could be drawn up to extend an existing end to end tabletting machine to produce a bolus more similar in appearance to that produced in the laboratory. It is not considered to be outwith the bounds of possibility to produce the same bolus commercially as

was manufactured by hand.

It must be concluded that there was success in the laboratory in producing an essentially simple device with potential application as a mineral supplement for cattle, but more particularly as a carrier for the antiparasitic agent ivermectin. To date, attempts at commercial exploitation of the device have been unsuccessful, due to limitations in available tabletting machinery and the interference of production processes, mainly pregranulation, causing an alteration of the characteristics of the mix. It is thought however, that if copper oxide needles were to be restored to the mix in the presence of judicious choice of hydrophobic additive, there could be a restoration of some of the former properties of the laboratory manufactured bolus.

APPENDIX 1

1. Experimental Techniques

(i) Dry matter

The dry matter (DM) in the food and faecal samples was determined by heating in a hot air oven at 90°C for 24-48 hours, depending on sample size until a constant weight was attained.

(ii) Grinding of samples

All faeces, liver and feed samples were milled after drying, in preparation for analysis. Samples were ground to a size which passed through a 0.5 mm mesh sieve. The dried sample was collected from the mill and mixed thoroughly before storing in an airtight plastic tub which was then kept in a darkened room until the commencement of analysis.

(iii) Copper in faeces and food

The copper content of faeces, liver and food samples was determined by atomic absorption spectrophotometry (Perkin-Elmer, 1976). Prior to analysis, samples were digested in a 3:2:1 mixture of nitric, perchloric and sulphuric acids and diluted as appropriate.

(iv) Copper in whole blood

Copper in whole blood was determined by a zinc dibenzyl dithiocarbonate spectrophotometric method (Brown and Hemingway, 1962).

(v) Copper in blood plasma

Copper in blood plasma was determined by an atomic absorption method as follows:

a. Solutions:

(1) 1000 µg/ml copper solution -

Dissolve 0.3928 g of $CuSO_4.5H_2O$ in deionised water, add 5 ml of concentrated (36 N) H_2SO_4 and dilute to 1000 ml.

(2) Saline solution -

Dissolve 0.7608 NaCl in deionised water and dilute to 1000 ml.

b. Standards:

1 µg/ml, 3 µg/ml and 6 µg/ml copper solutions. Diluted as follows: Take 10 ml of 1000 µg/ml copper solution and dilute to 100 ml. Take 1 ml, 3 ml, and 6 ml, of 100 µg/ml standard and dilute to 100 ml. Take 2 ml of each standard of 1, 3 and 6 µg/ml standards and dilute with 8 ml of saline solution.

Set O with saline solution

Set S1 with 1 µg/ml

Set S2 with 3 µg/ml

Set S3 with 6 µg/ml

c. Method:

Centrifuge whole blood samples at 3000 rpm for 15 minutes. Pipette off the plasma into Bijou bottles.

Take 2 ml of plasma and dilute with 8 ml glass distilled water. Read on atomic absorption spectrophotometer at 324.8 nm against standards (as Perkin-Elmer, 1976).

(vi) 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.

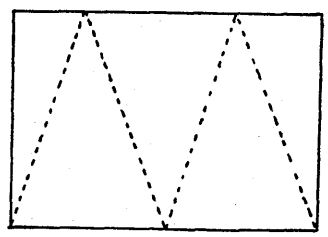
2. Blood Sampling

Venous blood samples were taken in heparinized Vacutainer tubes by jugular puncture from sheep and cattle. Whole blood samples were centrifuged immediately and the plasma removed for analysis when required.

3. Analytical Techniques for Parasitological Evaluation

(i) Pasture samples

Pasture samples were collected by traversing the experimental plots as shown in the following diagram.



Fifty evenly spaced stops were made along the route, and at each stop, four plucks of grass (the amount that could be grasped between a thumb and forefinger) were taken, giving a total of 200 plucks per plot. The grass was then processed by a method similar to that used by Parfitt (1955).

After weighing, the grass was transferred to a plastic bag in a plastic drum which could be rotated with a crank handle. Six litres of warm water were poured into the bag, the neck tied, the lid put onto the plastic drum, and the drum rotated 100 times; 50 turns in a clockwise direction; 50 in an anticlockwise direction. A further 10 turns in each direction were made before opening the plastic bag and pouring the

washings into a bucket through a wide mesh sieve.

The contents of the bucket were poured through a 57 µm sieve and the filtrate recovered placed onto a Maxa milk filter in a Baermann funnel. The larvae migrating through the temperature gradient were recovered by running off 10 ml of water from the Baermann apparatus after 12 hours. The larvae in a 1 ml aliquot were then microscopically differentiated and counted. The criteria taken for larval identification were those detailed in Technical Bulletin No. 18 (Ministry of Agriculture, Fisheries & Food, 1971)

(ii) Ivermectin assay

The procedure and multistep extraction by solvent partition followed by fluorogenic derivatization with acetic anhydride/1-methyl imidazole DMF, and finally high performance liquid chromatography. Lowest detectable concentration of ivermectin by this method varied between 0.2-0.5 ppb.

(iii) Plasma pepsinogen estimation

Blood samples for pepsinogen estimation (as for all other biochemical analyses) were taken directly from the jugular vein into heparinized Vacutainer tubes (Becton Dickinson Ltd.) and centrifuged at 2,000 rpm for 20 minutes after which clear plasma was carefully removed. The method used was essentially that described by Edwards, Jepson and Wood (1960), Br.Med.J.(1) 30-32.

Reaction

Plasma was incubated with bovine serum albumin (BSA) at pH2 for 24 hours and the phenolic amino acids liberated (tyrosine-like) were estimated using Folin-Ciocalteau Reaction. Corrections were made for the normal (i.e. non-incubated) content of tyrosine-like substances and also for the release of these substances from BSA when incubated alone.

(iv) Faecal egg counts

Faecal samples were collected directly from the rectum. The samples were examined by a modified McMaster technique (Gordon and Whitlock, 1939).

In this method 3 g faeces were homogenised with 42 ml of water and the resultant suspension

passed through a 250 µm sieve (Endecotts Test Sieves Ltd., Morden, London). After a thorough mixing of the filtrate, 15 ml were withdrawn into each of two flat bottomed centrifuge tubes (capacity 15 ml) and centrifuged at 2,000 rpm for 2 minutes. The supernatants from both tubes were then discarded and the remaining faecal mass broken up by rotary agitation. One tube was then filled to its former level with saturated salt solution and, after inverted 6 times, a volume of suspension, sufficient to fill both chambers, was quickly transferred by pipette to a 'McMaster' slide. The number of eggs under the etched areas of the slide were counted and the result multiplied by 50 to give an estimation of the number of eggs per gram of faeces according to the following calculation.

1 g of faeces in 42 ml gives 1 g in 15 ml

Volume under 1 square equals 0.15 ml

No. of eggs seen in 2 squares x 50 =
No. of eggs/gram

(v) Weights

Weights of Cochno supplied animals were recorded using a modern handling crate with an accurate

balance. As far as possible weights were recorded at the same time of day on successive weeks to minimise the possible effect of gut fill discrepancies.

(vi) Necropsy techniques

Cattle were euthanised by captive bolt and immediate exsanguination. After opening the abdomen, the pyloric sphincter was ligatured and the gastrointestinal tract removed from the body cavity. At this stage the rumen and reticulum were detached, incised and searched for bolus residue. The abomasum was removed intact with the duodenum being tied off at the abomasal-duodenal junction. After removal of any fatty surrounds, the abomasum was opened by incision along the greater curvature. A small (c 200 ml) sample of abomasal fluid was immediately collected into a glass jar and stoppered for the earliest possible determination of pH using a Radiometer pH meter type PHM 26c (Electronic Measuring Instruments Ltd., Copenhagan, Denmark). The period of time from collection to measurement of pH was generally no greater than about one hour.

(vii) Estimation of worm burdens at necropsy

The abomasal contents were collected into

graduated buckets and the volume made up with tap water to a standard 4 litres, except where an unusually large amount of material was present requiring a greater volume of water. Two samples each of 200 ml were withdrawn after thorough mixing and, following addition of 10 ml of 40% formalin, were stored in jars for subsequent microscopic examination. The abomasum was then laid out on a board, cut in half longitudinally and the mucosa from each half scraped off with a sharp post-mortem knife. The mucosal scrapings were digested in three times its own volume of pepsin-hydrochloric acid mixture (Herlich, 1956) at 42^{o}C for 6 hours. The digested mixture was then made up to 4 litres and formalinized 200 ml subsamples withdrawn as described before.

Parasites present in 10 separate 4 ml aliquots were counted and classified as adult or developing 4th stage larvae, depending on bursal or vulvular development, the presence of sheath projection and size respectively.

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